EAST WEST UNIVERSITY

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF THE LEAVES OF *CARICA PAPAYA* LINN.

Submitted By:

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACY, EASTWEST UNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF PHARMACY.

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled **"Phytochemical and Pharmacological Investigation of the leaves of *Carica papaya* Linn."** is an authentic and genuine research work carried out by me under the guidance of **Nazia Hoque,** Senior lecturer, Department of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE INVIGILATOR

This is to certify that the dissertation, entitled **"Phytochemical and Pharmacological Investigation of the leaves of *Carica papaya* Linn."** is a bonafide research work done by **Tasdik Farooq**, in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

…….

Nazia Hoque

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ENDORSEMENT BY THE CHAIRPERSON

"Phytochemical and Pharmacological Investigation of the leaves of *Carica papaya* Linn.'' is a bonafide research work done by Tasdik Farooq is a bonafide research work under the guidance of Nazia Hoque, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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Dr. Chowdhury Faiz Hossain

Chairperson and Professor

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Tasdik Farooq

ID: 2009-3-70-007

Dedication

DEDICATED TO MY PARENTS

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ABSTRACT

Carica papaya Linn belonging to the family *Caricaceae* is a well known medicinal plant in the world. It has also got good reputation in Bangladesh having the local name papay. It is used as a traditional medicine for the treatment of various diseases like cancer, malaria, dengue fever, viral infection such as common cold, eczema, warts etc. Studies conducted in some countries have shown that there is a significant antibacterial activity in organic extracts of different parts of *C. papaya*.

The aim of the present study was to evaluate the phytochemical screening and antimicrobial activity of methanolic extract of *C. papaya*.

The powdered leaf of *C. papaya* was extracted with methanol. The concentrated crude methanolic extract was then evaluated for phytochemical screening. Phytochemical screening was performed to determine the presence of carbohydrate, and various secondary metabolites such as alkaloids, flavonoids, steroids, tannins and saponins. Phytochemical screening revealed the presence of carbohydrate, tannin and saponin.

The antimicrobial activities of methanolic solvent extract of *Carica papaya* leaf were tested against the gram-positive and gram-negative bacterial strains and fungus by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The gram-positive bacteria used in the test were *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *β-hemolytic streptococcus*, *Bacillus megaterium* and the gram-negative bacteria were *Escherichia coli*, *Shigella dysenteriae*, *Salmonella paratyphi*, *Shigella boydii* and fungus like *Asperllius niger* and *Candida albicans* were also used.

The crude methanolic extract of *Carica papaya* leaf showed mild to moderate antimicrobial activities against the microorganisms at different concentrations of 50 µg/disc, 100 µg/disc and 150 µg/disc. However, no activity was found against S*treptococcus pyogenes* and *Shigella boydii*.

CHAPTER 1
INTRODUCTION

1.1 The Plants Role in Human

A modern dictionary defines health as soundness of physical, mental or moral condition especially freedom from pain or diseases. But true health is more than that- it includes the joy of living, the power and ability to lead a satisfying and purposeful life.

Modern drugs or conventional medicine is often viewed as impersonal, emphasizing crisis intervention. It is not only expensive also many of them bring about side effect, which are sometimes more dangerous than the disease itself.

Plants contain natural substances that can promote health and alleviate illness. They are source of medicines directly. The origins of medicine are tied up with plants. Alcohol was produced from starch early in civilization for use as a beverage preservative, but also as a surface sterilizing agent. Quinine from plant bark prevents malaria. Morphine, codeine, and cocaine are useful as local anesthetics and serve other purposes through side effects too. Digitoxin from foxglove has been used to regulate heartbeat. Caffeine from plants is an important daily stimulant for many humans. Nicotine and A-9-THC are important recreational drugs. There are probably many more important medicines to be found in the tropical forests of the world where countless species remain unknown. Medicinal drugs now used in the developed world come from only about 95 of the 250,000 known species of flowering plants on earth. Digitalis is used in heart patients. The list goes on... The study of new plants can and has led to the creation of medicines that can save lives and cure illnesses.

As the tropical rainforest shrinks day by day, the potential to discover new plants shrinks as well, yet few researchers are actively seeking new plant species in the jungles of South America. [Verun hey wood hug-1991]

1.2 Objective of the Research Study

The objective of this study was to assess chemical and biological activity of *Carica papaya* leaf using selected bench top bioassays, including antibacterial and antioxidant tests.

In addition to identifying phytomedicines, it can offer solutions to modern day diseases like AIDS and certain cancers.

Increased knowledge about phytomedicines can:

- Serve as alternative solutions where orthodox medicines have limitations, for examples antibiotics (in case of antibacterial-drug resistance), anticancer drugs from plants, like tubulin polymerization inhibitors (which is less toxic than current anti-cancer drugs such as Actinomycin D).
- Provide man with necessary knowledge to avoid or minimize unwanted side effects from toxicities resulting from use of herbal medicines.
- To determine plants extraordinary ability to synthesize secondary metabolites:
  Plants defense mechanisms are sophisticated which allow them to survive. They do this with an enormous variety of secondary metabolites that they synthesize. Several types of thousands of secondary metabolites have already been isolated and their structures were elucidated

The main roles of secondary metabolites have been identified to be:

- Defense against herbivores (insects, vertebrates),
- Defense against fungi and bacteria,
- Defense against viruses,
- Defense against other plants competing for light, water and nutrients,
- Signal compounds to attract pollinating and seed dispersing animals,
- Signals for communication between plants and symbiotic microorganism (N-fixing Rhizobia or mycorrhizal fungi) and
- Protection against UV-light or physical stress. [Wink, 1999]

Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. (E.g. *Dutura* has long been associated with the worship of Shiva, the Indian god). Plants are directly used as medicines by a majority

of cultures around the world, for example Chinese medicine and Indian medicine. Many foods crops have medicinal effects, for example garlic.

With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant.

1.3 Bioactive properties derived from Plants

Large numbers of surveys have been conducted in which plant extracts have been evaluated for various biological activities. Only a small sample of species are listed are in **Table 1.1:**

Table 1.1: Plants with medicinal uses indicating Biological activity, Drug name, Type of extract or plant part, Plant species

Biological activity	**Drug/extract/plant part**	**Plant name**	**Reference**
Antineoplastic	Combretastatin A-4 and B-1	*Combretum caffrum* *Combretum kraussi*	Pettit et al. (1987) Pettit et al. (1995)
Antibacterial	Juice	*Vacccinium spp.* (cranberry)	Ofek et al. (1996)
Antifungal	Grapefruit peel	*Citrus paradisa*	Stange et al. (1993)
Antiviral - AIDS	Glycyrrhizin (Flavonoid)	*Glycyrrhiza rhiza*	Watanbe et.al (1996)
Antimalarial (plasmodium)	Solvent extract	*Mahonia aquifolia*	Omulokoli et al. (1997)
Insecticide	Phenantrenes	*Combretum* apiculatum	Malan and Swinny (1993)
Molluscicide	Mollic acid	*Combretum molle*	Rogers (1989)

Schistisomiasis			
Hypoglycemic	Leurosine sulphate (alkaloid)	*Catharanthus roseus*	Svoboda et al. (1964)
Cardiotonic activity	Extract	*Carissa sp.*	Thorpe and Watson (1953)
Andro-or estrogenic	Extract	*Butea superba*	Schoeller et al. (1940)
CNS	Morphine	*Papaver somniferum*	Schmitz (1985)
Antihelminthic	Dried nuts	*Quisqualis indica* *Combretum molle*	Ladion (1985) Rogers and Verotta (1997)

1.4 Traditional Medicine Practice In Bangladesh

Traditional Medicine is the medicine or treatment based on traditional uses of plants, animals or their products, other natural substances (including some inorganic chemicals). Bangladesh possesses a rich flora of medicinal plants. Most probably 5000 species of different plants growing in this country in which more than a thousand are regarded as having medicinal properties. Although the use of traditional medicine is so deeply rooted in the cultural heritage of Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts. Among the largest ethnic group, the ***Bangalees*** on the main land, there are two distinct forms of Traditional medicine practice:

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

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

   - **Religious medicine**, which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods, etc. and

   - **Spiritual medicine,** which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.

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

   - **Unani-Tibb or Graeco-Arab system,** which has been developed by the Arab and Muslim scholars from the ancient Greek system, and

   - **Ayurvedic system,** which is the old Indian system, based on the *Vedas* the oldest scriptures of the Hindu saints of the Aryan age.

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. Apparently the recipients of these systems of medicine appear to be the rural people, but practically a good proportion of the urban population still continues to use these traditional medicines, although organized modern health care facilities are available to them.

Plant materials are used in these preparations in a variety of forms, such as small pieces, coarse powders, as their extracts, infusions, decoctions or distillates. They are dispensed as broken pieces, coarse and fine powders, pills of different sizes, in the form of compressed tablets, as liquid preparations, as semi-solid masses and in the form of ointments and creams, neatly packed in appropriate sachets, packets, aluminium foils, plastic or metallic containers and glass bottles. The containers are fully labeled with indications/contra-indications, doses and directions for use and storage, just like modern allopathic medicinal preparations.

The plant *Carica papaya* (*Linn.*) belongs to the family *Caricaceae*, is used a medicinal agent in Bangladesh. Medicinal plants are an important therapeutic aid for various ailments. Today, there is widespread interest in drugs deriving from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Medicinal plants are potential sources of medicine in developing countries to treat serious diseases. About 80% people of the rural areas of underdeveloped countries still depend on medicinal plants. Studies revealed that there are more traditional medicine providers than the allopathic practitioners especially in the rural areas (WHO, 2002). Bangladesh has a good number of medicinal plants and these plants have many biologically active compounds.

Researchers now become concerned about natural products of higher plants due to novel source of antimicrobial agents. Plants have developed sophisticated active defense mechanisms against infectious agents [Barz *et al*., 1990]. The main aim of these reactions appears to be inhibition of microorganisms with antibiotic compounds, hydrolytic enzymes, and inactivation of microbial exoenzymes with specific inhibitors and isolation of lesions. These defense mechanisms operate at different stages of infection [Kuc, 1990a]. The external plant surfaces are often covered with biopolymers (fatty acid esters) that are difficult to penetrate. In addition, external surfaces can be rich in compounds (phenolic compounds, alkaloids and steroid glycoalkaloids) that will inhibit the development of fungi and bacteria [Reuveni *et al*., 1987]. Once pathogens have passed the external barriers, they may encounter plant cells that contain sequestered glycosides [Kuc, 1990b]. The glycosides may be antimicrobial *per se* or may be hydrolyzed to yield antimicrobial phenols; these in turn may be oxidized to highly reactive quinones and free radicals [Noveroske *et al*., 1964].

Damage to a few cells may rapidly create an extremely hostile environment for a developing pathogen. This rapid, but restricted disruption of a few cells after infection can also result in the biosynthesis and accumulation of low molecular weight antimicrobial, liphophylic compounds, called phytoalexins. Phytoalexins differ in structure, with some structural similarities within plant families [Carr and Klessig, 1989]. Some are synthesized by the malonate pathway, others by the mevalonate or shikimate pathways, whereas still others require participation of two or all three of the pathways [Kuć, 1990b]. Phytoalexins can induce constitutive or other secondary metabolite pathways and link to various metabolic pathways [Barz *et al*., 1990]. Since phytoalexins are not translocated, their protective effect is limited to the area of the infection, and their synthesis and regulation are accordingly restricted. Phytoalexins are degraded by some pathogens and by the plant; thus, they are transient constituents and their accumulation is a reflection of both synthesis and degradation. Often associated with phytoalexin accumulation is the deposition around sites of injury or infection of biopolymers, which both mechanically and chemically restrict further development of pathogens [Hammerschmidt and Kuć, 1982]. These biopolymers include: lignin, a polymer of oxidized phenolic compounds; callose, a polymer of β-1, 3-linked glucopyranose; hydroxy-proline-rich glycoproteins, and suberin. The macromolecules produced after infection or some forms of physiological stress include enzymes, which can hydrolyze the walls of some pathogens [Carr and Klessig, 1989], including chitinases, β-1, 3-glucanases and proteases.

Unlike the phytoalexins and structural biopolymers, the amounts of these enzymes increase systemically in infected plants even in response to localized infection. They are often found intercellular where they would contact fungi and bacteria. These enzymes are part of a group of stress or infection-related proteins commonly referred to as pathogenesis-related (PR) proteins. The function of many of these proteins is unknown. Some may be defense compounds; others may regulate the response to infection [Tuzun *et al.*, 1989]. Another group of systemically produced biopolymer defense compounds comprises the peroxidases and phenoloxidases [Hammerschmidt *et al.*, 1982]. Both can oxidize phenols to generate protective barriers to infection, including lignin. Phenolic oxidation products can also cross-link to carbohydrates and proteins in the cell walls of plants and fungi to restrict further microbial development [Stermer and Hammerschmidt, 1987]. Peroxidases also generate hydrogen peroxide, which is strongly antimicrobial. Association with peroxidative reactions after infection is the transient localized accumulation of hydroxyl radicals and superoxide

anion, both of which are highly reactive and toxic to cells. Both plant and microbial compounds regulate the expression of genes that encode products that contribute to disease resistance. The speed and degree of gene expression and the activity of the gene products (and not the presence or absence of genes for resistance mechanisms) determine disease resistance in plants [Kuć, 1990b]. The future will probably see the restriction of pesticide use and a greater reliance on resistant plants generated using immunization and other biological control technologies, genetic engineering and classical plant breeding. However, as with past and current technology, we may create unique problems. The survival of our planet may significantly depend upon anticipating these problems and meeting the challenge of their solution.

1.5 The Medicinal Plants contribution in the New World

Just Before Modern Medicine: At the early of modern medicine the Muslim physicians were done a great job. The Arabian Muslim physicians, like Al-Razi and Ibn Sina (9^{th} to 12^{th} century AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use. Al Razi's important books are: Qitab-al-Mansuri, Al-Hawai, Qitab-al-Muluki, Qitab-al-Judari-wal-Hasabah, Maan La Yahoduruho Tibb etc. The famous medical book, Al-Kanun, of Ibn Sina was the prescribed book of medicine in the schools of western medicine for several centuries [Mian & Ghani., 1990].

The use of medicinal plants in Europe in the 13^{th} and 14^{th} centuries was based on the Doctrine of Signatures or Similar developed by Paracelsus (1490-1541 AD), a swiss alchemist and physician [Murray, 1995]. The South American countries have provided the world with many useful medicinal plants, grown naturally in their forests and planted in the medicinal plant gardens. Use of medicinal plants like coca (*Erythroxylum* species) and tobacco (*Nicotiana tabacum*) was common in these countries in the 14^{th} and 15^{th} centuries. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the *Rig Veda* (4500-1600 BC). It supplies various information of the medicinal use of plants in the Indian subcontinent [Hill, 1972]. Medicinal plants used by the Australian aborigines many centuries ago tremendously enriched the stock of medicinal plants of the world. The current list of the medicinal plants growing around the world includes more than a thousand items [Sofowora, A., 1982].

1.6 Modern Prescription Drugs

To make prescriptions easily understandable by the patients, Paracelsus (1493 AD) started to use German instead of traditional Latin language used in medicine. His book On Diseases of Miners was very important at that time. Nuremburg Pharmacopoeia was published in 1546. First London Pharmacopoeia published in 1618. Later on its name became the British Pharmacopoeia. Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi synthetic or wholly synthetic ingredients originally isolated from plants [Hill, A., 1972].

1.7 Plant Medicines, Safer and Time Tested

Plant medicines are far and away safer, gentler and better for human health than synthetic drugs. This is so because human beings have co-evolved with plants over the past few million years. We eat plants, drink their juices, ferment and distill libations from them, and consume them in a thousand forms. Ingredients in plants, from carbohydrates, fats and protein to vitamins and minerals, are part of our body composition and chemistry.

1.8 Drug Discovery from Medicinal Plants

Drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs

of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials. As part of our National Cooperative Drug Discovery Group (NCDDG) research project, numerous compounds from tropical rainforest plant species with potential anticancer activity have been identified. Our group has also isolated several compounds, mainly from edible plant species or plants used as dietary supplements that may act as chemo preventive agents. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compound. [Hill, A., 1972]

1.9 Description on Carica papaya

Carica papaya is an evergreen shrub or small tree that grows best in full sun to light shade. The papaya plant has been described with a large variety of adjectives, which acknowledge the structural and functional complexity of this giant tropical herb. *Carica papaya*, with a somatic chromosome number of 18, is the sole species of this genus of the Caricaceae, a family well represented in the Neotropics, which includes six genera with at least 35 species [Fisher, 1980; Ming et al, 2008; Carvalho and Renner, 2013)].

Papayas have always held an attraction for people. It is a power house of nutrients and is available throughout the year. Papaya as well the leaf is a good source of Vitamin A (Catotene), Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin C (Ascorbic acid), Vitamin E, Niacin, Minerals such as Calcium, Iron, Phosphorous, Potassium, Proteins, Fats, Calories, Carbohydrates, β-carotene, Fibers and Folate that helps to boost the number of platelets present on the blood.

Papayas may be very helpful for the prevention of atherosclerosis and diabetic heart disease. Papayas are an excellent source of vitamin C as well as a good source of vitamin E and vitamin A (through their concentration of pro-vitamin A carotenoid phytonutrients), three very powerful antioxidants. These nutrients help prevent the oxidation of cholesterol. Only when cholesterol becomes oxidized is it able to stick to and build up in blood vessel walls, forming dangerous plaques that can eventually cause heart attacks or strokes. One way in which dietary vitamin E and vitamin C may exert this effect is through their suggested

association with a compound called paraoxonase, an enzyme that inhibits LDL cholesterol and HDL cholesterol oxidation. Papayas are also a good source of fiber, which has been shown to lower high cholesterol levels. The folic acid found in papayas is needed for the conversion of a substance called homocysteine into benign amino acids such as cysteine or methionine. If unconverted, homocysteine can directly damage blood vessel walls and, if levels get too high, is considered a significant risk factor for a heart attack or stroke. [Whfoods, 2013]

The nutrients in papaya have also been shown to be helpful in the prevention of colon cancer. Papaya's fiber is able to bind to cancer-causing toxins in the colon and keep them away from the healthy colon cells. In addition, papaya's folate, vitamin C, beta-carotene, and vitamin E have each been associated with a reduced risk of colon cancer. These nutrients provide synergistic protection for colon cells from free radical damage to their DNA. Increasing the intake of these nutrients by enjoying papaya is an especially good idea for individuals at risk of colon cancer. [Aravind et al, 2013]

Papaya effectively treats and improves all types of digestive and abdominal disorders. It is a medicine for dyspepsia, hyperacidity, dysentery and constipation. Papaya helps in the digestion of proteins as it is a rich source of proteolytic enzymes. Even papain-a digestive enzyme found in papaya is extracted, dried as a powder and used as aid indigestion. Ripe fruit consumed regularly helps in habitual constipation. [Aravind et al, 2013]

The enzymes papain and chymopapain and antioxidant nutrients found in papaya have been found helpful in lowering inflammation and healing burns. That is why people with diseases (such as asthma, rheumatoid arthritis, and osteoarthritis) that are worsened by inflammation, find relief as the severity of the condition reduces after taking all these nutrients. [Aravind et al, 2013]

It has been reported that papaya helps in the prevention of diabetic heart disease and also prevents premature aging. The skin of papaya works as a best medicine for wounds.

The milky juice of *Carica papaya* when extracted and dried, is used as chewing gum, medication for digestion problems, toothpaste and meat tenderizers. It has been used to treat digestive problems and intestinal worms as well as warts, sinusitis, eczema, cutaneous tubercles and hardness of the skin. [Aravind et al, 2013]

Green fruits are used to treat high blood pressure, round worm infection, dyspepsia, constipation, amenorrhoea, skin disease, general debility and genitor-urinary disorders [Burkhill, 1985].

Carica papaya plants produce natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. *Carica papaya L*. leaf tea or extract has a reputation as a tumour-destroying agent. [Last, 2008]

The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence. [Elizabeth, 1994)]

Fresh, green papaya leaf is an antiseptic, whilst the brown, dried papaya leaf is the best as a tonic and blood purifier [Atta, 1999]. Chewing the seeds of ripe papaya fruit also helps to clear nasal congestion, [Elizabeth, 1994]. The green unripe pawpaw has a therapeutic value due to its antiseptic quality. It cleans the intestines from bacteria, more so that (only a healthy intestine is able to absorb vitamin and minerals, especially vitamin B12).
Because of all the abundant nutrients, papaya was reputably called “The Fruit of Angels” by Christopher Columbus in the 20th century. Today, papaya is considered one of the famous fruits in the world. [Morton, J., 1987]

1.10 General characteristics of Carica papaya

Scientific Names: *Carica papaya Linn, Carica hermaphrodita* Blanco, *Carica cubensis Solms, Carica sativa* Tussac, *Carica mamaja* Vellero, *Carica papaya* Karsten [StuartXchange, 2013]

Family: *Caricaceae*

Bengali Name/Vernacular Name: Pepe, Papeya; Koiya (Chittagong). [Dr. Uddin, S. B., 2013]

Tribal Name: Pepo, Cokia (Tipra), Ptega (Rakhaing), Somphula (Khumi), Kamco (Bawm). [Dr. Uddin, S. B., 2013]

English Name: Papaya, Papaya tree, Melon tree, Papaia, Pawpaw, Papaw, Papau

Other Vernacular Names:

- Arabic: Fafay, Babaya
- Assamese: Omita
- Burmese: Thimbaw
- Czech: Papaja
- French: Papaye, Papayer
- German: Melonenbaum, Papayabaum
- Hindi: Papeeta, Papiitaa
- Italian: Papaia
- Japanese: Motukuwa, Papaia, Popoo
- Korean: Pa pa ya
- Portugese: Ababaia, Mamao, Papaia, Fruto de Mamoeiro, Papaeira
- Russian: Papaia
- Spanish: Fruta bomba, Lechosa, Melon zapote, Papayero, Papayo, Papaya
- Thai: Loko, Malako
- Urdu: Papiitaa, Pappeeta
- Vietnamese: Du Du

  [StuartXchange, 2013]

Type: Broad leaf evergreen

Zone: 10 to 12

Height: 6.00 to 20.00 feet

Spread: 3.00 to 15.00 feet

Bloom Time: Seasonal bloomer

Bloom Description: Yellowish-white

Sun: Full sun; **Water:** Medium

Parts Used: Leaves, fruit, roots and latex of trunk

[Missouri botanical garden]

1.11 Botanical description of the plant

Carica papaya is an evergreen, tree-like herb, 2-10 m tall, usually unbranched, although sometimes branched due to injury, containing white latex in all parts.

Leaves: Leaves spirally arranged, clustered near apex of trunk; petiole up to 1 m long, hollow, greenish or purplish-green; lamina orbicular, 25-75 cm in diameter, palmate, deeply 7-lobed, glabrous, prominently veined; lobes deeply and broadly toothed.

The leaf contains beta-carotene, calcium, carpaine, fats, flavonols, niacin, papain, tannins, and vitamin C (in higher concentration in the leaf than in the fruit). The leaf, unlike the fruit, is not a source of the protein-dissolving enzyme papain, but the latex (sap) in the leaf stem is [Orwa et al. 2009]

Stem: The stem is cylindrical, 10-30 cm in diameter at the base to 5-10 cm at the crown, hollow with prominent leaf scars and spongy fibrous tissue. It has an extensive rooting system. Stem density is only 0.13 g cm^{-3}. The single stem provides structural support, body mass, storage capacity, defense substances, height, and competitive ability, and carries a bidirectional flow of water, nutrients, various organic compounds, and chemical and physical signals that regulate root and shoot relations. [Morton, J., 1987]

Fruits: The papaya fruit is pear-shaped with a bright golden-yellow skin. The flesh of the fruit is a brighter orange-yellow, juicy and silky smooth, with a sweet and sour flavor. The shiny gray or black seeds in the interior of the fruit have a peppery taste and are edible, although they are usually discarded.

The fruit yields an enzyme, papain, best known as a digestive aid but most commonly used to "clear" freshly brewed beer. This enzyme is especially concentrated in the fruit when it is unripe. [Morton, J., 1987]

Flowers: The 5-petalled flowers are fleshy, waxy and slightly fragrant. Some plants bear only short-stalked pistillate (female) flowers, waxy and ivory-white; or hermaprodite (perfect) flowers (having female and male organs), ivory-white with bright-yellow anthers and borne on short stalks; while others may bear only staminate (male) flowers, clustered on panicles to 5 or 6 ft (1.5-1.8 m) long.
Hermaphrodite (bisexual) papaya flowers are slender and thin and they are attached close to the stem. Male flowers will not be able to become a papaya fruit. Female flowers that are not

pollinated will drop off from the tree. Hermaphrodite flowers are most sought after by growers. They are self pollinating and can give you a papaya fruit. [Morton, J., 1987]

Seeds: Seeds are numerous in central cavities, rounded, blackish, about 0.6 cm in diameter, each enclosed in a gelatinous membrane (aril). [Morton, J., 1987]

Roots: The papaya root is predominately a non-axial, fibrous system, composed of one or two 0.5–1.0 m long tap roots. Secondary roots emerge from the upper sections and branch profusely. [Jimenez et al, 2013]

1.12 Taxonomic hierarchy of the investigated Plant

Kingdom: Plantae

Subkingdom: Viridaeplantae

Infrakingdom: Streptophyta

Division: Tracheophyta

Subdivision: Spermatophytina

Infradivision: Angiospermae

Class: Magnoliopsida

Superorder: Rosanae

Order: Brassicales

Family: Caricaceae

Genus: Carica L.

Species: Carica papaya L.

[ITIS Report, 2013]

1.13 The Plant Family: *Caricaceae*

The **Caricaceae** are a family of flowering plants in the order Brassicales, found primarily in tropical regions of Central and South America and Africa. They are short-lived evergreen pachycaul shrubs or small trees growing to 5–10 m tall. Many bear edible fruit.

The family comprises six genera and about 34-35 species:

- *Carica* – one species, *Carica papaya* (Papaya), Americas
- *Cylicomorpha* – two species, Africa
- *Horovitzia* – one species, Mexico
- *Jacaratia* – eight species, Americas
- *Jarilla* – three species, Americas
- *Vasconcellea* – twenty species, Americas

[Carvalho, F.A., 2013]

1.14 Origin and Distribution of *Carica papaya*

Though the exact area of origin is unknown, the papaya is believed native to tropical America, perhaps in southern Mexico and neighboring Central America. It is recorded that seeds were taken to Panama and then the Dominican Republic before 1525 and cultivation spread to warm elevations throughout South and Central America, southern Mexico, the West Indies and Bahamas and to Bermuda in 1616. Spaniards carried seeds to the Philippines about 1550 and the papaya traveled from there to Malacca and India. Seeds were sent from India to Naples in 1626. Now the papaya is familiar in nearly all tropical regions of the Old World and the Pacific Islands and has become naturalized in many areas. Seeds were probably brought to Florida from the Bahamas. Up to about 1959, the papaya was commonly grown in southern and central Florida in home gardens and on a small commercial scale.

In the 1950's an Italian entrepreneur, Albert Santo, imported papayas into Miami by air from Santa Marta, Colombia, Puerto Rico and Cuba for sale locally as well as shipping fresh to New York, and he also processed quantities into juice or preserves in his own Miami factory.

Successful commercial production today is primarily in Hawaii, tropical Africa, the Philippines, India, Ceylon, Malaya and Australia, apart from the widespread but smaller scale

production in South Africa, and Latin America. It is also widely cultivated throughout Bangladesh. [Morton, J., 1987]

Table 1.2: Documented Species Distribution [Orwa et al. 2009]

Native	Costa Rica, Mexico, US
Exotic	Antigua and Barbuda, Australia, Bahamas, Barbados, Brazil, Cambodia, Cameroon, Chile, Colombia, Cuba, Democratic Republic of Congo, Dominica, Dominican Republic, Ecuador, Eritrea, Fiji, Grenada, Guadeloupe, Haiti, India, Indonesia, Jamaica, Kenya, Laos, Malaysia, Martinique, Montserrat, Myanmar, Netherlands Antilles, New Zealand, Nicaragua, Nigeria, Papua New Guinea, Peru, Philippines, Puerto Rico, Samoa, Singapore, Solomon Islands, South Africa, Sri Lanka, St Kitts and Nevis, St Lucia, St Vincent and the Grenadines, Sudan, Tanzania, Thailand, Tonga, Trinidad and Tobago, Uganda, Venezuela, Vietnam, Virgin Islands (US), Zanzibar.

1.15 Ecology/ Cultivation of *Carica papaya*

Carica papaya thrives in warm areas with adequate rainfall and temperature range of 21-33^{o}C. Its altitude range is similar to that of the banana, from sea level up to elevations at which frost occurs (often around 1600 m). Frost can kill the plant, and cool and overcast weather delays fruit ripening and depresses fruit quality. Fruit tastes much better when grown during warm sunny season, but yield can be very high at elevations around 1000 m, which is the altitude for papaya production in East Africa in the 1960$_{S.}$ Evenly distributed annual rainfall of 1200 mm is sufficient if water conservation practices are employed. Plantations should be in sheltered locations or surrounded by windbreaks; strong winds are detrimental, particularly on soils, which cannot make up for large transpiration losses. *Carica papaya* grows best in light, well-drained soils rich in organic matter with soil p^{H} 6.0-6.5. It can tolerate any kind of soil provided it is well-drained and not too dry. The roots are very

sensitive to water logging and even short periods of flooding can kill the plants [globinmed, 2013]

1.16 Plant Growth and Development

Under appropriate conditions of water availability, light, oxygen, air temperature, and humidity, papaya seeds undergo epigeal germination (Fig. 1.1a); emergence is typically completed in 2–3 weeks [Fisher 1980]. Primary leaves of young seedlings are not lobed (Fig. 1.1b) but become so after the appearance of the second leaf (Fig. 1.1c). Papaya leaves of adult plants are simple, large, and palmate (Fig. 1.1b). In tropical conditions, approximately two leaves emerge at the apex of the plant in a 3/8 spiral phyllotaxy every week (Fisher 1980). Leaf life commonly spans for 3–6 months under tropical conditions and persistent scars remain on the trunk as they abscise (Fig. 1.1e). The loss of leaves on the lower section of the plant and the continuous emergence of new ones at the apex give the canopy a sort of umbrella shape that casts a considerable amount of shade. The papaya plant develops very fast, taking 3–8 months from seed germination to flowering (juvenile phase) and 9–15 months for harvest [Paterson et al. 2008]. The plant can live up to 20 years; however, due to excessive plant height and pathological constraints, the commercial life of a papaya orchard is normally 2–3 years. [Jimenez et al. 2013]

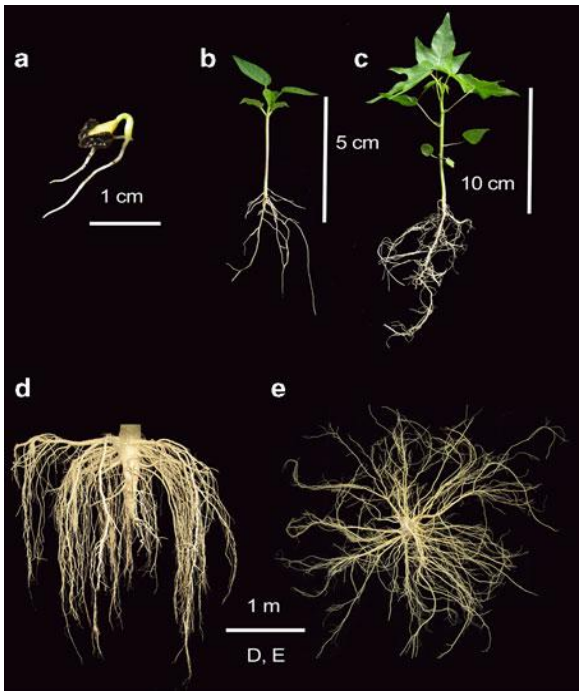


Fig 1.1: Papaya seedlings and root system. (a) Germinating papaya seed. **(b)** Ten-day-old papaya seedling showing cotyledonary leaves and first true leaves. **(c)** Three-week-old papaya seedling with six true leaves. **(d)** Side view of an excavated 5-month-old papaya root system, showing the main and secondary roots. **(e)** Upper view of the same root system, showing horizontal distribution of secondary roots. [Jimenez et al. 2013]

1.17 Morphology, Architecture, and Anatomy of the Adult Plant

Papaya is usually a single-stemmed, semi-woody giant herb with fast, indeterminate growth (1-3 m during the first year). The plants may attain up to 10 m, although under modern cultivation height vegetative growth may induce axillary bud break and branching at the lower portions seldom surpasses 5–6 m. Occasionally, vigorous vegetative growth may induce axillary bud break and branching at the lower portions of the plant, which rarely exceeds a few centimeters in length. Some branching may also occur if apical dominance is lost due to tip damage, and, in tall plants, "distance" may release the lower buds from the dominant effect of the apex [Morton, J., 1987].

The plant produces large palmate leaves (~0.6 m^2), with five to nine pinnate lobes (Fig. 1.2b) of various widths (40–60 cm), arranged in a spiral pattern (Fig. 1.2e) and clustered in the upper section of adult individuals [Morton 1987; Ming et al. 2008]. Leaf blades are dorsiventral and subtended by 30–105 cm long, hollow petioles that grow nearly horizontal, endowed with a starch-rich endodermis, perhaps important for cavitation repair [Bucci et al. 2003; Posse et al. 2009; Leal-Costa et al. 2010]. The leaf epidermis and the palisade parenchyma are composed of a single cell layer, while the spongy mesophyll consists of four to six layers of tissue. Reflective grains and druses are abundant throughout the leaf [Fisher, 1980]. Papaya leaves are hypostomatic, with anomocytic (no subsidiary cells) or anisocytic (asymmetric guard cells) stomata [Carneiro and Cruz 2009; Leal-Costa et al. 2010]. Stomatal density of sunlit leaves is approximately 400/ mm^2, which can adjust readily to environmental conditions of light, water, and heat. Important biologically active compounds have been identified in papaya leaves [Canini et al. 2007 ; Zunjar et al. 2011], where they function in metabolism, defense, signaling, and protection from excess light, among others [El Moussaoui et al. 2001 ; Konno et al. 2004]. Adult plants may have three possible sexual forms: female, male, and hermaphroditic (Figs. 1.2a–d and 1.3a–f).

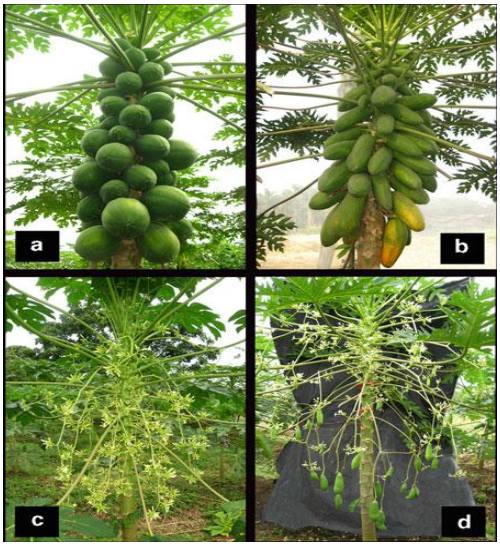


Fig. 1.2: Types of papaya plants according to sex forms. (a) Female. **(b)** Hermaphroditic. **(c)** Male. **(d)** Male fruit- bearing plant.

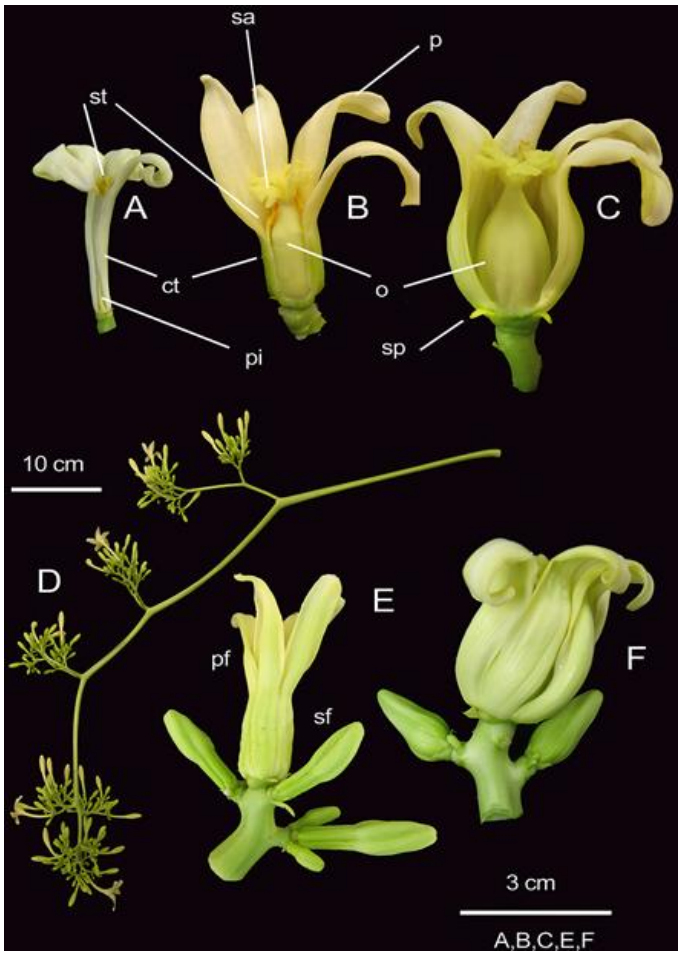


Fig. 1.3: Papaya flowers with one petal removed to show internal parts (a – c) and inflorescences (d-f). (a) Staminate flower showing stamens (st), pistillode (pi) and corolla tube (ct). **(b)** Perfect flower showing st, ct, stigmata (sa), petal (p) and an elongated ovary (o). **(c)** Pistillate flowers showing sepals (sp), petals and round ovary (o). **(d)** Long male inflorescence with dozens of staminate flowers. **(e)** Andromonoecious cyme showing one dominant perfect (pf) and five secondary staminate flowers (sf). **(f)** Female cyme with three pistillate flowers. [Jimenez et al. 2013]

1.18 Sex Expression

Papaya has three sex forms (female, male, and hermaphrodite), regulated by an incipient X–Y chromosome system. Papayas can be either dioecious (with male and female plants) or gynodioecious (with hermaphrodite and female plants). Several studies suggest that the Y chromosome contains a small specific region that controls expression of male (Y) or hermaphrodite (Y^h) types. Female plants are of the XX form. All combinations among the Y and/or Y^h chromosomes are lethal; therefore, the male and hermaphrodite types are heterozygous (XY and XY^h, respectively) [Jimenez et al. 2013].

1.19 Pests and Diseases

- A white scale, Pseudaulacspis pentagona, thickly encrusts young trees.
- Xyleborus beetles bore into weak stems and kill the plant.
- Fruit flies (Diptera) lay eggs in ripening fruits, causing them to rot.
- Several mites attack C. papaya; the mites, Hemitarsoneumus latus and several species of Tetranychus, cause leaves to yellow and shed and damage the fruit.
- Root-knot nematodes Meloidogyne spp. and Rotylenchulus reniformis may be serious pests.
- Beetles (Coleoptera) make holes on the trunk.
- Polyphagous grasshoppers and mole crickets cut seedlings at ground level.
- Numerous fungi cause diseases on C. papaya. The disease anthracnose is caused by pathogens Colletotrichum gloeosporioides and Glomerella cingulata.
- Pythium spp., Rhizoctonia spp. and Fusarium spp. cause damping-off in seedlings.

- Phytophthora spp. cause root, foot and trunk rots.
- Aphids transmit a virus that causes ring spots; symptoms include chlorosis in younger leaves, vein clearing, mottling of laminae and shortened petioles.
- A virus related to the cucurbit mosaic and transmitted from cucumbers and watermelons by the green peach aphid (Myzus persicae) causes a bitter flavour in the fruit.
- A tree infected by the pathogen Cercospora papayae shows symptoms of round, grey-white lesions on leaves and black, sunken lesions on the fruit.
- The disease, known as cercospora leaf spot attacks leaves of seedlings under humid, poorly ventilated conditions.
- Bunchy-top, a mycoplasma disease, is transmitted by a hopper (Empoasca spp.).
- Other diseases cause seed rot, premature shedding of leaves and dropping of flowers and young fruit. [Orwa et al. 2009]

1.20 Tree Management

Weeds must be controlled, especially during the initial stages of establishment. Herbicides, hand weeding, mulching and use of cover crops are some of the practical methods used in the control of weeds. Even though fairly resistant to drought, C. papaya requires a constant water supply. C. papaya is very responsive to fertilizers, and yield can be significantly improved by proper fertilization. Control of pH is also very important. Fruit production begins within a year of planting and is continuous thereafter. C. papaya produces 30-150 fruits/year. As the fruit is formed in the leaf axils, plants must be kept growing continuously for maximum yield. Mature trees may be rejuvenated by cutting back to 30 cm above the ground. The latex should be tapped at least once a week. [Orwa et al. 2009]

1.21 The Chemistry of *Carica papaya*

Carica papaya contains many biologically active compounds; the level of compounds varies in fruit, latex, seed, leaf and root. In addition, plant parts from male and female tree, or the cultivation can also cause a variation in quantity of compounds [Morton, 1977]. Biochemical

information of *C. papaya* is shown in **Table: 1.3**. Apart from nutritional substances, many biologically active compounds in Carica papaya have been reported.

Table 1.3: The Biochemistry of various parts of *Carica papaya* [Sharma and Ogbeide, 1982]

Per 100 gm				
Biochemistry	**Green fruit**	**Ripe fruit**	**Seeds**	**Leaves**
Calories	26	32-45	-	74
H_2O (g)	92.1	87.1-90.8	-	77.5
Protein (g)	1.0	0.4-0.6	24.3	7.0
Fat (g)	0.1	0.1	25.3	2.0
Total Carbohydrate(g)	6.2	8.3-11.8	32.5	11.3
Fiber (g)	0.9	0.5-0.9	17.0	1.8
Ash (g)	0.6	0.4-0.6	8.8	2.2
Mineral (mg)				
Ca	38	20-24	-	344
Fe	0.3	0.3-0.7	-	0.8
P	20	15-24	-	142
Na	7	3-4	-	16
K	215	221-234	-	652
Other Chemicals				
β-carotene equivalent (µg)	15	710-1,050	-	11,565
Thiamine (mg)	0.02	0.03-0.04	-	0.09
Riboflavin (mg)	0.03	0.03-0.05	-	0.48
Niacin (mg)	0.3	0.3-0.4	-	2.1
Ascorbic acid (mg)	40	52-73	-	140
Vitamin E (mg)	-	-	-	136

Table 1.4: Nutritional value of *Carica Papaya* [Nutrition data, 2013]

Vitamins in papaya	**Measure units**	**Value per 100 grams**
Vitamin C (ascorbic acid)	mg	60.9
Thiamin	mg	0.023
Riboflavin	mg	0.027
Niacin	mg	0.357
Pantothenic acid	mg	0.191
Vitamin B-6	mg	0.038
Folate (total)	µg	37
Folate (food)	µg	37
Folate (DFE)	µg	37
Vitamin A (RAE)	µg	47
Carotene (beta)	µg	274
Carotene (alpha)	µg	2
Cryptoxanthin (beta)	µg	589
Vitamin A, fat soluble (IU, µg)	IU	950
Lycopene	µg	1828
Lutein plus zeaxanthin	µg	89
Vitamin E (alpha tocopherol)	mg	0.30
Tocopherol (beta)	mg	0.02
Tocopherol (gamma)	mg	0.09
Tocopherol (delta)	mg	0.01
Vitamin K, Phylloquinone	µg	2.6
Fatty Acids in papaya		
Saturated fatty acids (total)	g	0.081
Dodecanoic acid	g	0.002
Tetradecanoic acid	g	0.013
Hexadecanoic acid	g	0.060
Octadecanoic acid (Lauric acid)	g	0.004

Monounsaturated fatty acids (total)	g	0.072
Octadecenoic acid (Omega-9)	g	0.034
Polyunsaturated fatty acids (total)	g	0.058
Octadecadienoic acid (Omega-6)	g	0.011
Octadecatrienoic acid (Omega-6)	g	0.047
Amino acids in papaya		
Tryptophan	g	0.008
Threonine	g	0.011
Isoleucine	g	0.008
Leucine	g	0.016
Lysine	g	0.025
Methionine	g	0.002
Phenylalanine	g	0.009
Tyrosine	g	0.005
Valine	g	0.010
Arginine	g	0.010
Histidine	g	0.005
Alanine	g	0.014
Aspartic acid	g	0.049
Glutamic acid	g	0.033
Glycine	g	0.018
Proline	g	0.010
Serine	g	0.015

Table 1.5: Active ingredients present in various parts of *Carica papaya* [Krishna, Paridhavi et al, 2008]

Part	Constituent
Fruit	**Carotenoids:** β-carotene, Lycopene, Lutein, Crytoxanthin, Violaxanthin, Zeaxanthin **Terpenes:** α-terpinene, γ-terpinene **Monoterpenoids:** Linalool, 4-terpinol, α-phellandrene, Cis-trans-linalool oxide **Antibacterial:** Chitinase **Phytoalexine:** Danielone **Glucoside:** Benzyl-β-D glucoside, 2-phenyl ethyl-β-D glucoside, 4-hydroxy-phenyl-2 ethyl-β-D-glucoside, and Four isomeric malonated benzyl-β-D-glucosides. **Alkaloid:** Carpaine **Volatile Compound:** Cis and trans 2, 6-dimethyl-3,6 epoxy-7 octen-2-ol **Isothiocyanate:** Benzyl isothiocyanate **Minerals:** Calcium, Phosphorus, Iron **Vitamins:** Vitamin C, Thiamine, Riboflavin, Niacin **Protein, Carbohydrates, Fat, Amino acid, Citric acid, Malic acid** **Other Compounds:** α-linolenic acid, Butanoic acid, Methyl butanoate
Juice	N-butyric, n-hexanoic and n-octanoic acids, Lipids; Myristic, Palmitic, Stearic, Linoleic, Linolenic and Cis -vaccenic and Oleic acids.
Seed	**Enzyme:** Myrosin **Alkaloid:** Carpaine **Glycoside:** Sinigrin, Caricin **Isothiocyanate:** Benzyl isothiocyanate **Glucosinolate:** Benzyl glucosinolate **Sterol:** β-sitosterol **Fatty acid, Crude protein, Crude fiber, Papaya oil** **Benzyl thiourea:** Carpasemine **Other Compounds:** Glucotropecolin, Hentriaconate
Root	**Alkaliod:** Nicotine, **Glycoside:** Carposide, **Enzyme:** Myrosine
Leaf	**Enzyme:** Papain, Chymopapain, Caricain, Glycylendopeptidase **Alkaloids:** Carpaine, Carpinine, Pseudocarpaine, Dehydrocarpaine I and II,

	Nicotine, Choline, Bispiperidine **Flavonoids:** Myricetin, Kaemferol, Quercetin **Phenolic Compounds:** Caffeic acid, P-coumaric acid, Chlorogenic acid **Carotenoids:** β-carotene, Lycopene, Lutein, Cryptoxanthin, Violaxanthin and Zeaxanthin **Glycoside:** Carposide **Cardiac Glycoside:** Cardenolides **Glucoside:** Cyanogenic glucoside, Benzyl glucosinolate **Vitamins:** Thiamine, Riboflavin, Niacin, Ascorbic acid, α-tocopherol **Minerals:** Ca, P, K, Mg, Zn, Mn, Fe **Amino acids:** Tryptophan, Methionine, Lysine **Other Compounds:** Cystatin, Saponins, Tannins, Anthraquinolones, Reducing sugars, Steroids
Bark	**Alkaloid:** Carpaine **Glycoside:** Carposide **Sterol:** β-sitosterol **Poly alcohol:** Xylitol **Glucose, Fructose, Sucrose, Galactose**
Latex	**Enzymes:** Proteolytic enzymes, Papain, Chemopapain, Chymopapain A, B, and C, Peptidase A and B, Glutamine cyclotransferase, Lysozymes.
Stem	**Enzyme:** Papain **Flavonoids:** Myricetin, Kaemferol, Quercetin **Minerals:** Ca, P, K, Mg, Zn, Mn, Fe

1.22 Biochemistry of *Carica papaya* Leaf

The leaves of papaya have been shown to contain many active components:

- **Enzymes:** Papain, Chymopapain, Caricain, Glycyl endopeptidase
- **Alkaloids:** Carpaine, Pseudocarpaine, Dehydrocarpaine I & II, Nicotine, Choline, Bispiperidine, Carpinine
- **Flavonoid:** Myricetin, Kaemferol, Quercetin
- **Phenolic compounds:** Caffeic acid, P-coumaric acid, Chlorogenic acid

- **Glycoside:** Carposide
- **Cardiac glycoside:** Cardenolids
- **Glucoside:** Cyanogenic glucoside, Benzyl glucosinolate
- **Carotenoids:** β-carotene, Lycopene, Lutein, Cryptoxanthin, Violaxanthin, Zeaxanthin
- **Vitamins:** Thiamine, Riboflavin, Niacin, Ascorbic acid and α-tocopherol
- **Amino acids:** Tryptophan, Methionine, Lysine
- **Electrolytes and Minerals:** Ca, P, K, Mg, Zn, Mn, Fe
- **Other compounds:** Cystatin, Saponins, Tannins, Anthraquinolones, Reducing sugars, Steroids.
  [Krishna, Paridhavi et al, 2008]

1.23 Health Benefits of Papaya Fruit

Some of the important benefits of papaya fruit are as follows:

- **Increase the body's immunity:** Eating papaya fruit is believed to increase your body's immunity against disease. That is because the content of vitamin A and vitamin B are needed to improve the immunity contained in papaya. Thus, diseases caused by decreased immunity such as coughs, colds, flu and infections can be prevented by eating papaya fruit.

- **Protects against free radicals:** Papaya fruit also contains vitamin C, vitamin E, pantothenic acid, folic acid, flavonoids, magnesium, minerals, fiber and potassium, which act as antioxidants. As is known, serves as a protective antioxidant the body from free radicals.

- **Assist wound healing:** Papaya fruit can assist the body in the healing process of wounds, especially burns, because it contains the enzyme papain and chymopapain enzyme that is useful to reduce inflammation and get rid of dead skin tissue from the injured skin. In addition, several other diseases also can be more severe when the body becomes inflamed. For this reason, papaya fruit is also recommended to be consumed by people who are sick.

- **Preventing Cancer:** One of the important properties of papaya fruit for the body is able to prevent cancer, especially colon cancer. This is possible because the papaya contain antioxidants and fiber which is pretty much so that it can help those who are experiencing difficulty in bowel movements. In addition, a study also mentions that the extract dried papaya can resist the growth of cancer cells.

- **Enhance Male Virility:** Papaya is able to increase virility because they contain an enzyme called arginine. In the medical world, arginine is known as a substance that can increase blood flow in the penis of men. This substance also serves to increase the nitric acid in the body that relaxes the muscles around blood vessels that supply the genitals. Thus, these blood vessels will dilate and blood flow to the penis will increase.

- **Preventing Lung Disease:** Papaya fruit is very useful for people who have weak lungs because they contain vitamin A in a considerable degree. Thus, eating papaya will make their bodies more resistant to transmission of diseases such as bronchitis caused by weak lungs.

- **Controlling premature aging:** Papaya fruit is believed by experts to control premature aging by helping the body to digest food properly. When the body properly digests all the nutrients needed by the body, then the body will be healthier and organ functions will run smoothly.

- **Prevent Stroke and Heart Disease:** The papaya fruit contains lots of fiber and antioxidants, which can help prevent the oxidation of cholesterol in the liver and lower levels automatically. With lower levels of cholesterol in the blood, then you will also avoid the risk of stroke and heart disease. In addition, the efficacy of papaya fruit also to maintain a healthy circulatory system and heart because they contain folic acid which can eradicate these harmful substances damaging the walls of blood vessels that cause heart attacks.

- **Laxative:** Ripe papaya fruit is laxative which assures of regular bowel movement.

- **Antifungal Activity:** Danielone is a phytoalexin found in the papaya fruit. This compound showed high antifungal activity against Colletotrichum gloesporioides, a pathogenic fungus of papaya.
  [Broke, 2013]

1.24 Health Benefits of Papaya Juice

Here are some of the effective benefits which can be gained from drinking papaya juice:

- **Papaya juice is rich in antioxidants:** Papaya is one of the top fruit sources of antioxidants, which are substances that help to protect the body's cells against the harmful effects of free radicals such as damaged cells and heart disease. Antioxidants include substances like Vitamins A, C, and E; lycopene, selenium, lutein, and beta-carotene.
  As an antioxidant rich food, papaya juice is known to help prevent cancer- both for the young and old- as antioxidants help the body to fight those cells that cause cancer.

- **Papaya juice aids in the body's digestion:** Aside from antioxidants, papaya is also a rich source of enzymes. These enzymes are proteins that help to perform a chemical change in the body, such as the breaking down of foods and blood clotting. Drinking papaya juice helps the body to have enough enzymes to aid in the prevention of digestive problems such as heart burn, acid reflux, and indigestion. Aside from this, papaya juice also works as a natural laxative by preventing constipation and helping in the elimination of waste products through its high fiber content.

- **A natural defense against Heart disease:** The rich amount of antioxidants in a papaya juice prevents the oxidation of cholesterol, which means that plaque found in the blood vessel will not build up (thus preventing heart diseases or stroke). Plaque buildup significantly reduces the flow of blood to the brain or heart, which could be a very dangerous situation.
  High levels of fiber in this healthy fruit drink also lower a person's cholesterol levels. Papain, an enzyme also found in papayas, is known for its effect in the prevention of heart diseases.

- **Papaya juice works efficiently as an anti-inflammatory drink:** Enzymes found in papaya juice, specifically papain and chymopapain, are known to lower inflammation in the body. It also speeds up the healing from burns through this enzymes by breaking down the skin's inactive proteins and reducing the dead tissue from the burns.

- **Effective Detoxifying agent:** In addition to its numerous health benefits to the body, papaya juice can also help the body clean by removing all unwanted toxins; thus working as a natural cleanser. It is rich in fiber which helps to remove the toxins, and prevents colon cancer as fiber binds itself to the colon.

- **Weight Loss:** Papaya juice has been found effective for those who wish to lose weight because papaya is said to be lower in calories as compared to other fruits.

- **Liver Cancer:** Owing to the presence of Lycopene in papaya, drinking its juice has been found useful in liver cancer. It has an antiproliferative effect on liver cancer.

- **Colon Cancer:** As papaya juice contains various nutrients like folate, vitamin C and E it is effective in preventing colon cancer.

- **Arthritis:** Drinking papaya juice has been found beneficial for those with osteoarthritis and rheumatoid arthritis because it is anti-inflammatory.

- **Treatment of Cystic fibrosis:** Papaya juice has been found to be very effective for treating those with cystic fibrosis which is basically characterized by breathing problems.

- **Ease Constipation:** It is one of the best sources of fiber so it helps in easing out constipation because it contains a protease called as papain.

- **Treatment of High Blood Pressure:** Papaya juice works well in the treatment of high blood pressure.

- **Prevention of Hypertension and Stroke:** It is also said to be quite effective in preventing stroke and hypertension and hence recommended most of the times by doctors.
  [Apsara, 2013]

1.25 Health Benefits of Papaya Leaf

Papaya leaf has a numberless of benefits. Some of them are mentioned below:

1) **Treatment of Dengue fever:** Papaya leaf juice is a traditional method of curing dengue fever without posing any side effects. Scientific research and several case studies have indicated that papaya leaf juice contains the enzymes chymopapin and papain that boost platelets, also known as thrombocytes and also relieve symptoms. [Saba, 2013]

2) **Treatment of Malaria:** Papaya leaf has been found to possess anti- malarial properties as well. Thus, papaya leaf juice is often used in some parts of the world as a prophylactic for preventing malaria in certain endemic regions. [Saba, 2013]

3) **Treatment of Viral infection:** Papaya leaf juice is its ability to fight viral infection such as the common cold virus. It is a natural way of regenerating white blood cells and platelets. Papaya leaves contain over 50 ingredients including the vitamins A, C and E that support the immune system. [Saba, 2013]

4) **Treatment of Typhoid fever:** Papaya leaf juice is often used in herbal medicines to remove intestinal worms as it contains tannins that protect the intestine from re-infection from tanning proteins in the lining of the intestinal wall. This way the worms cannot attach themselves. Thus, they are effective in suppressing the cause of typhoid fever. [Saba, 2013]

5) **Anti-cancer properties:** The greatest anti-cancer properties of papaya are concentrated in its leaf extract. Papaya Leaves have a milky sap that's great for preventing and killing cancer cells because it contains acetogenin. Papaya leaf juice contains certain enzymes that have dramatic cancer fighting properties against a wide

range of tumors such as cervix cancer, breast cancer, liver cancer, lung cancer and pancreatic cancer without any toxic effects on the body. As a result, papaya leaf extract is often recommended as part of chemotherapy in some parts of the world. By regulating the T-cells, papaya leaf extract increases the immune system's response to cancer. [Saba, 2013]

6) **Prostate Enlargement:** Papaya Leaf tea can help with benign prostate enlargement and also help with rectal lesions problems associated with prostate enlargement. [Haider, 2013]

7) **Provides important Micronutrients:** Micronutrient analysis of *Carica papaya* leaves indicate that they are good source of vitamin A, folic acid, magnesium as well as vitamin B12. [Rochway, 2013]

8) **Aids in Red Blood Cell production:** The presence of important antioxidants such as vitamin A, C and the micronutrients folic acid and vitamin B12 play an important role in red blood cell production. This indicates that papaya leaf extracts may aid in synthesis of red blood cells in the body. [Rochway, 2013]

9) **Immune Booster:** Papaya leaf extracts were found to exhibit positive effects on the immune system by a mechanism called immunomodulation. A study showed that there was an increase in production of Th2 type cytokines with papaya leaf extracts. Th2 type cytokines are a group of lymphocytes produced to boost the immune response of the body. [Rochway, 2013]

10) **Potent Antioxidant:** *Carica papaya* leaf water extract is effective against gastric ulcer and oxidative stress. The papaya leaf extracts exhibits antioxidant effect by reducing the oxidative damage and boosting the activity of endogenous antioxidant glutathione peroxidase. The active components in papaya leaf extract namely papain, ascorbic acid, flavonoids, chymopapain, cyanogenic glucosides, cystatin, and glucosinolates were found to increase the total antioxidant power in the blood and reduce the oxidative damage. [Rochway, 2013]

11) **Weight Loss:** It is low in calories and high in nutritional values. Hence, it is a good food for those who want to lose some weight. [Saba, 2013]

12) **Void the Heart Attack or Stroke:** The folic acid found in papaya leaf is needed for the conversion of homocysteine into amino acids such as cysteine or methionine. If unconverted, homocysteine can directly damage blood vessel walls, is considered a significant risk factor for a heart attack or stroke. [Aravind et al. 2013]

13) **Increase Appetite:** Papaya Leaf tea increases appetite bringing vigor and vitality back to the body. [Haider, 2013]

14) **Increase Platelet Count:** Papaya Leaf Tea cures thrombocytopenia or low platelet counts which keep a person from clotting. There have been many studies showing that Papaya Leaf Tea can increase platelet counts in cases of vitamin deficiencies, chemotherapy, dengue fever and more. [Haider, 2013]

15) **Laxative:** Papaya Leaves made into a strong tea also work well as a good laxative for those who suffer from constipation. [Haider, 2013]

16) **Anti-aging:** Papaya Leaf also contains 50 or more different amino acids such as: threonine, glutamate acid, glycine, valine, leucine, phenylalanine, lysine, tritophan, cysteine, histidine, tyrosine, alanine, proline, aspartate, and more... and some of these agents are used in cosmetics for creating young healthy looking skin, and have been used as anti-aging compounds. [Haider, 2013]

17) **Ease Menstrual Pain**: Papaya leaf juice helps in controlling the menstrual irregularities in women. Papaya helps to ease menstrual cramps and helps in promoting regular flow of menstruation. [Haider, 2013]

18) **Anti-inflammatory Properties:** Papaya leaf contains anti-inflammatory enzymes that help in relieving pain for those who are suffering from arthritis, edema and osteoporosis. [Aravind et al. 2013]

19) **Anti-bacterial effect:** Papaya leaf extracts contained anti-bacterial compounds that were effective against both gram positive and gram negative bacteria. [Rochway, 2013]

20) **Prevention of Indigestion and Heartburn:** Papaya Leaves are great for getting rid of invading bacteria that cause upset stomach problems because they contain karpain which kills bacteria. And it contains lots of papain, protease enzyme, and amylase enzyme which help to breaks down proteins, carbohydrates, and sooth the GI tract and helps with acid reflux. [Haider, 2013]

21) **Healing of GI tract disorders:** Papaya Leaves reduce inflammation of the stomach lining and heal gastric ulcers by killing H. pylori bacteria. And Papaya Leaf tea soothes away colon inflammation from IBS and other inflammatory bowel diseases. [Haider, 2013]

22) **Gluten digestion:** Papaya leaf tea may also help digest the wheat protein gluten, which is difficult for some people to digest and causes an autoimmune condition known as celiac disease. [Haider, 2013]

23) **Cataracts:** Papaya Leaf tea can prevent cataracts. [Haider, 2013]

24) **Emphysema:** Papaya Leaves contain lots of Vitamin D which prevents emphysema. [Haider, 2013]

25) **Skin Benefits:**

- Papaya leaf juice has a higher content of vitamins A and C in comparison to the fruit, which promote skin health. [Saba, 2013]

- Papaya leaf juice acts as a skin cleansing agent. This can be attributed to the presence of karpain compounds that inhibit microorganisms and other toxins, thus giving you a clear skin and providing protection against skin problems like acne, pimples, freckles and blemishes. [Saba, 2013]

- Juice of papaya leaves is effective in treating eczema. [Saba, 2013]

- Fresh papaya leaf juice helps in healing open wounds and sores. [Saba, 2013]

- The milky juice extracted from the tip of papaya leaves can soften the hardened skin or the corn/wart on the feet. [Saba, 2013]

26) **Hair Benefits:** Due to the abundance of vitamins, minerals and enzymes, papaya leaf extract is often used in a number of hair care formulations like shampoos and conditioners to promote hair growth, strengthen the hair shaft and ward off problems like balding, thinning, dandruff etc. Papaya leaf juice has the following benefits for hair. [Saba, 2013]

- Papaya leaf juice is often used by health care stores or salons for hair treatment. When used as a conditioner along with other ingredients like coconut milk and honey, it adds shine to dull, lifeless hair and softens coarse and unruly hair. [Saba, 2013]

- Papaya leaf extract is particularly used as an ingredient in anti-dandruff shampoos meant to control flakes and prevent dandruff. Due to the presence of karpain compounds, it is effective in removing dirt and oil, as well as chemical build up from hair without any side effects. [Saba, 2013]

27) **Treatment of Diabetic Patients:** Diabetics have three good reasons to try papaya leaf extracts.

- **Enhances insulin sensitivity:** Poor insulin sensitivity is the cause for type-2 diabetes, which causes inefficient glucose uptake by the cells. Several animal studies and some human studies have shown that papaya leaf extracts were able to demonstrate glucose lowering effect.

  A study on Mauritian population showed that papaya leaf extract supplementation decreased the enzyme levels of ALT and AST (bio-markers of type 2 diabetes) among diabetic patients and improved insulin sensitivity. [Marks, 2013]

- **Decreases diabetes complications:** The mix of antioxidants in papaya leaf extracts is helpful in decreasing the secondary complications of diabetes such as fatty liver, kidney damage and oxidative stress. [Marks, 2013]

- **Accelerates wound healing:** The process of wound healing is delayed or hindered in a diabetic patient and can cause other complications. Many studies have confirmed that consuming papaya leaves enhanced the process of wound healing- due to its anti-bacterial and remarkable antioxidant action. [Marks, 2013]

28) **Other uses:** The leaves of papaya tree are used for treating nervous pains, nausea, and elephantoid growths. [Haider, 2013]

1.26 Benefits of Papaya Root

- Juice from papaya roots is used to in some countries of Asia to ease urinary troubles.
- A decoction formed by boiling the outer part of the roots of papaya tree is used to cure dyspepsia.
  [Aravind et al. 2013]

1.27 Benefits of Papaya Peel

Papaya peel is often used in cosmetics. The papaya peel can also be used in many home remedies:

- Sunscreen and soothing slave- The presence of vitamin A helps to restore and rebuild damaged skin. Applied papaya peel is used as skin lightening agent. When peel mixed with honey and applied, it can soothe and moisturizes the skin.
- Fight dandruff
- Muscle relaxant
  [Aravind et al. 2013]

1.28 Healing properties of Papaya Seeds

The papaya seeds are very pungent and peppery, making them almost unpalatable. However, the seeds seem to have more potent medicinal values than the flesh.

- Papaya seeds are have antibacterial properties and are effective against E.coli, Salmonella and Staphylococcus infections.
- Papaya seeds may protect the kidneys from toxin-induced kidney failure.
- Papaya seeds can eliminate intestinal parasites.
- Papaya seeds help to detoxify the liver.
- Acts as skin irritant to lower fever.
- Cures piles and typhoid.
- Possesses anti-helminthic and anti-amoebic properties.
  [Aravind et al. 2013]

1.29 Medicinal and Pharmacological properties of Papaya

Various Pharmacological actions and medicinal uses of different parts of the papaya are reported with the crude extracts and different fractions from crude extracts of different parts of papaya. They have been used as traditional medicine for the treatment of various diseases.

Many biologically active phytochemical from different parts of papaya tree (latex, seed, leaf, root, stem, bark and fruit) have been isolated from papaya and studied for their potency, **Table: 1.6**. Antifungal chitinase has been genetically cloned and characterized from papaya fruit. Classified a class IV chitinase based on amino acid sequence homology with other plant chitinase, the recombinant chitinase also exhibit antibacterial activity.

Commercially available spray dried latex of papaya fruits has isolatable chamopapain which exhibit immunological properties. Papaya fruits are used as topical ulcer dressing in some hospitals including the Spanish town hospital, Kingston public hospital and university hospital in the West Indies, Jamara. The dressing preparation from papaya promotes desloughing, granulation and healing and also reduces odor in chronic skin ulcer. Some hospitals use it as burn dressing which is tolerable by children and is economical and widely available [Krishna, Paridhavi et al. 2008]

Table 1.6: Constituents of different parts of the papaya tree [Krishna et al. 2008]

Part	**Medicinal uses**
Latex	Anathematic, relieves dyspepsia, cures diarrhea, pain of burn and topical use, bleeding hemorrhoids, stomachic , whooping cough
Ripe Fruit	Stomachic, digestive, carminative diuretic, dysentery and chronic diarrhea, expectorant, sedative and tonic ,relieves obesity, bleeding piles, wound of urinary tract, ringworm and skin disease psoriasis
Unripe Fruit	Laxative ,diuretic, dried fruit reduces enlarged spleen and liver, use snake bit to remove poison, abortifaciant, anti- implantation activity and antibacterial activity
Seeds	Carminative , emmenagogue , vermifuge, abortifaciant, counter irritant, as paste in the treatment of ringworm and psoriasis ,anti-fertility agent in malic
Seed Juice	Bleeding piles and enlarged liver and pectoral properties
Root	Abortifacient, diuretic, checking irregular bleeding from the uterus, piles, antifungal activity
Leaves	Young leaves as vegetable , Jaundice(fine paste), urinary complaints &gonorrhea (infusion) dressing wound fresh leave, antibacterial
Flower	Jaundice, emmenagogue, febrifuge and pectoral properties
Steam Bark	Jaundice, anti-hemolytic activity, STD , store teeth(inner bark) ,anti-fungal activity

1.30 Industrial Usage of Papaya

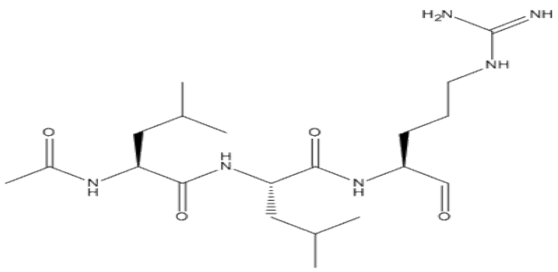
- Papaya is primarily a fresh-market fruit, and is used in drinks, jams, pectin, candies and as crystallized fruit [Aravind et al. 2013].
- Green fruit may be cooked as a vegetable, as may the leaves, flowers and roots.
- Among the purified plant proteins used commercially, important plant-derived enzymes include papain and chymopapain (enzymes derived from papaya that are used medicinally and as meat tenderizers) [Tyler et al.1981].

- Papain is also used as hair conditioner.
- Leaves of *Carica papaya* is used as soap substitute which are able to remove stains.
- Papain has milk clotting (rennet) and protein digesting properties.
- Among many other myriad of uses papain among others as; beer chill-haze removal; degumming natural silk; cleaning silks and wools before dying; removing hair from hides during tanning; meat- tenderizer added into chewing gums; extracting oil from tuna liver; added in dentifrices, shampoos and face-lifting preparations [James and McCaskill 1983]; or used in the manufacture of rubber [Anibijuwon and Udeze 2009].

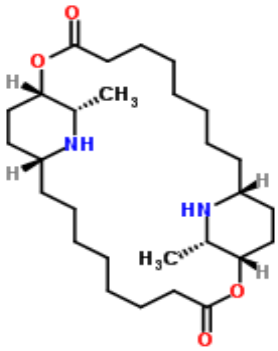
1.31 Allergies, Side effects and Toxicity of Papaya

- Papaya is frequently used as a hair conditioner, but should be used in small amounts. Papaya releases a latex fluid when not quite ripe, which can cause irritation and provoke allergic reaction in some people.
- Externally the papaya latex is an irritant to the skin and internally it causes severe gastritis.
- The latex concentration of unripe papayas is speculated to cause uterine contractions, which may lead to a miscarriage.
- Papaya seed extracts in large doses have a contraceptive effect on rats and monkeys, but in small doses have no effect on the unborn animals.
- Excessive consumption of papaya can cause carotenemia, the yellowing of soles and palms, which is otherwise harmless. However, a very large dose would need to be consumed; papaya contains about 6% of the level of beta carotene found in carrots (the most common cause of carotenemia).
- Cyangenic glucosides present in the leaves and roots, which can form cyanide which can subsequently introduce undesirable effects on human health.
- Papain is also a potential allergen. People who eat too much papaya and ingest high levels of papain may develop symptoms consistent with hay fever or asthma, including wheezing, breathing difficulties and nasal congestion.
  [Aravind et al. 2013]

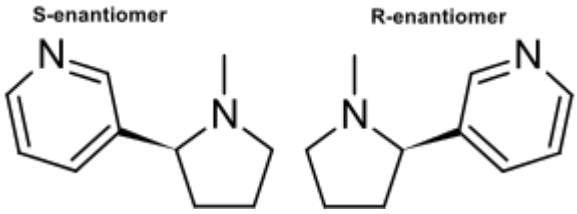
1.32 Structure of Phytochemical constituents identified in *Carica papaya*



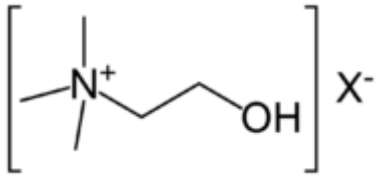
Papain



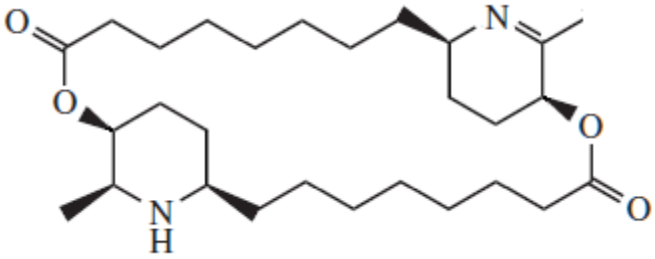
Carpaine



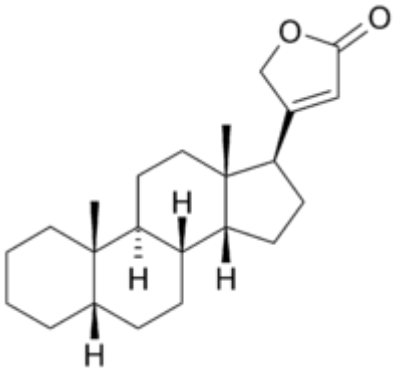
Nicotine



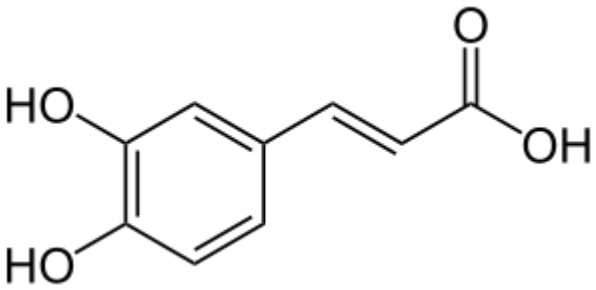
Choline



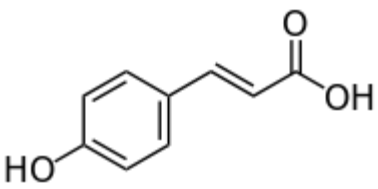
Dehydrocarpaine



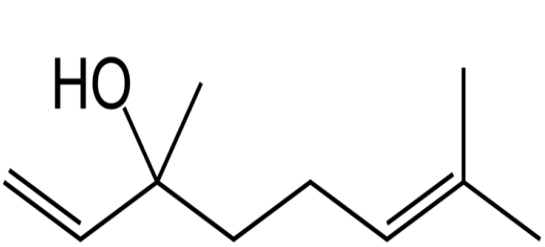
Cardenolide



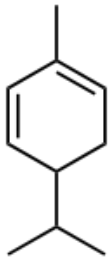
Caffeic acid



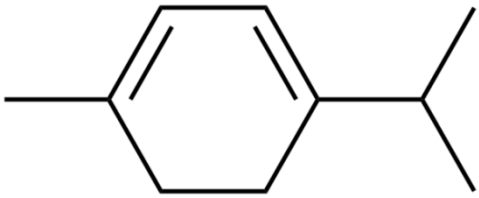
P-coumaric acid



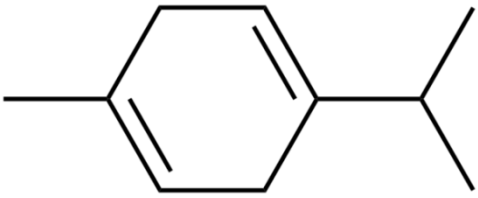
Linalool



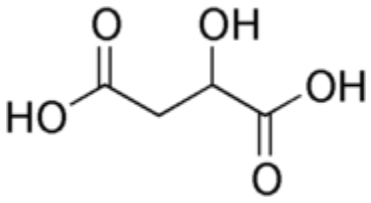
α-phellandrene



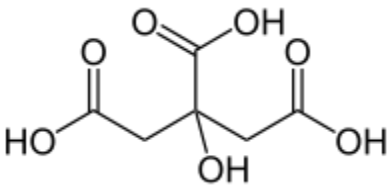
α-terpinene



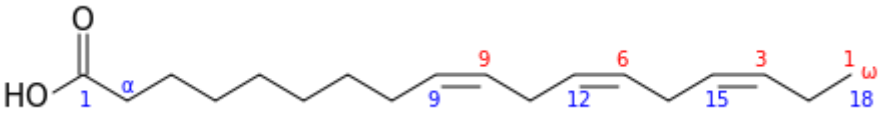
γ-terpinene



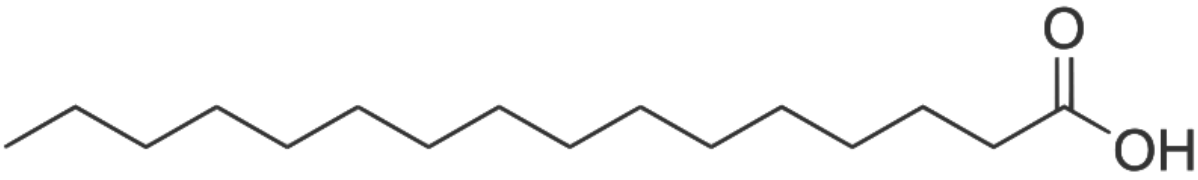
Malic acid



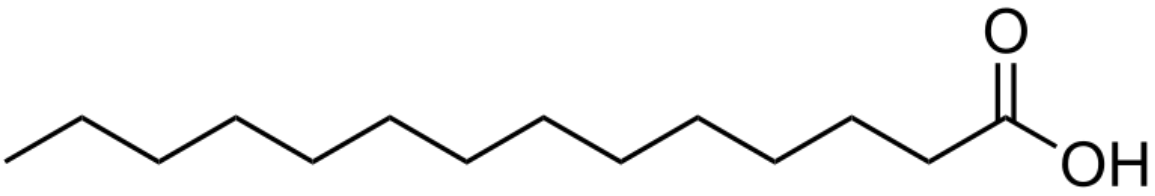
Citric acid



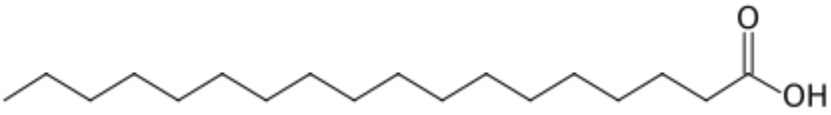
α-linolenic acid



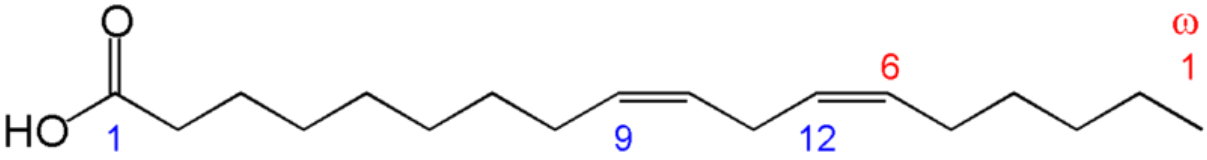
Palmitic acid



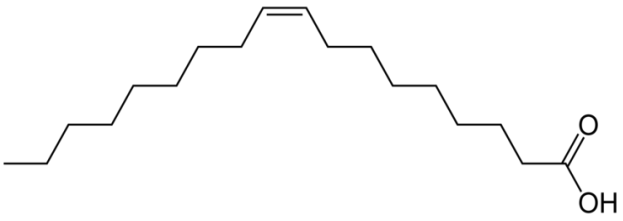
Myristic acid



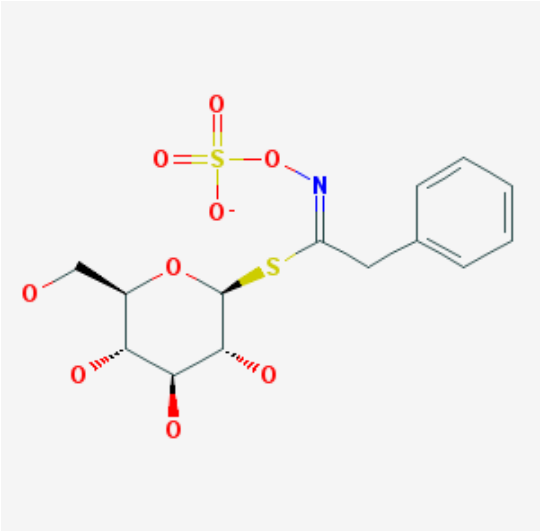
Stearic acid



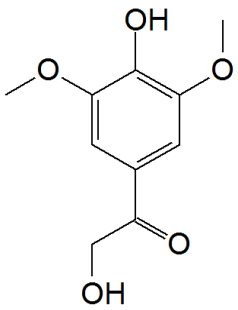
Linoleic acid



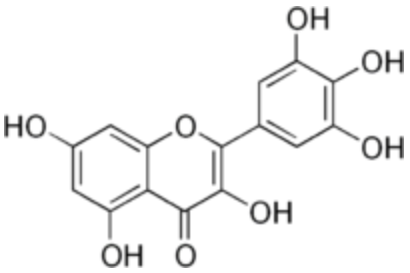
Oleic acid



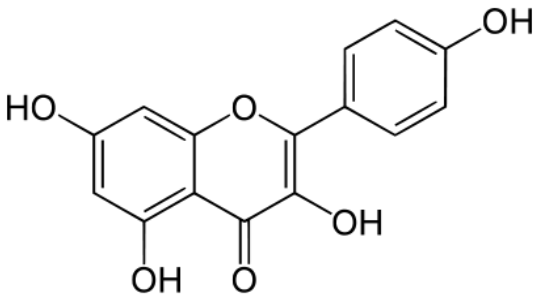
Benzyl glucosinolate



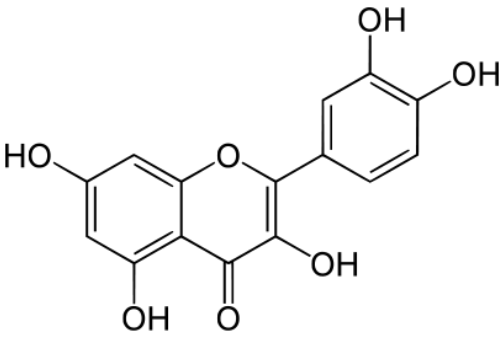
Danielone



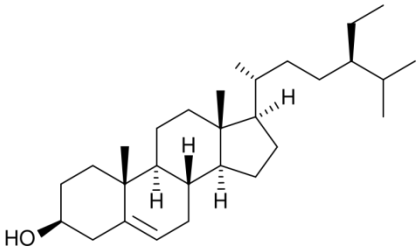
Myricetin



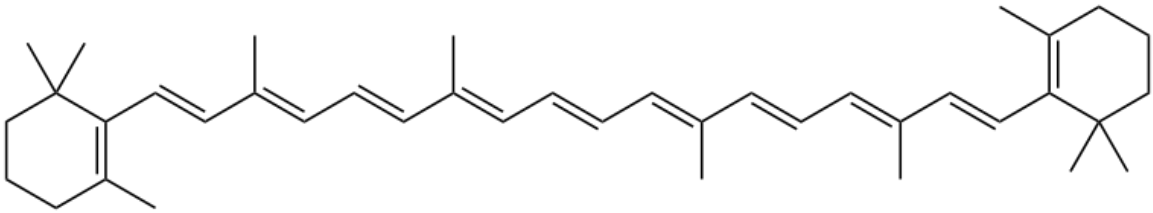
Kaemferol



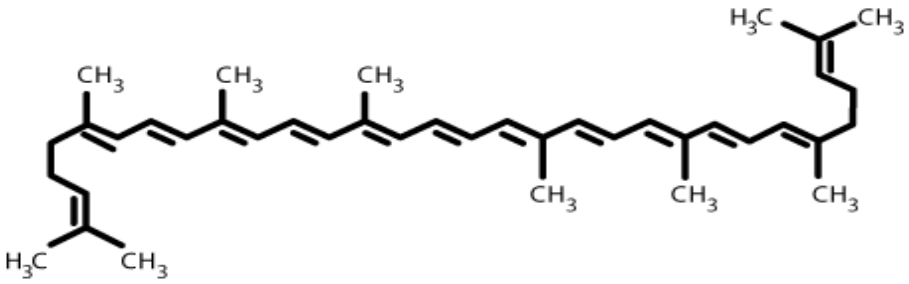
Quercetin



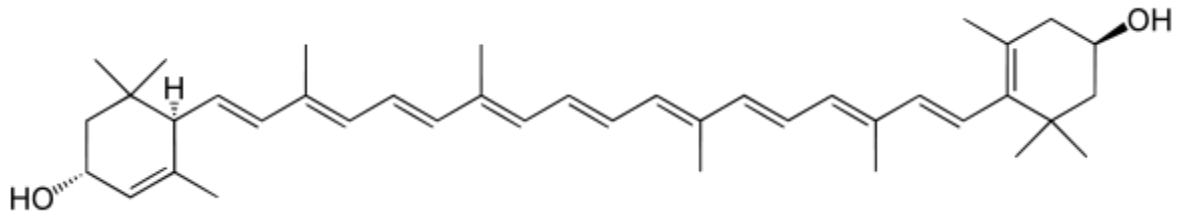
β-sitosterol



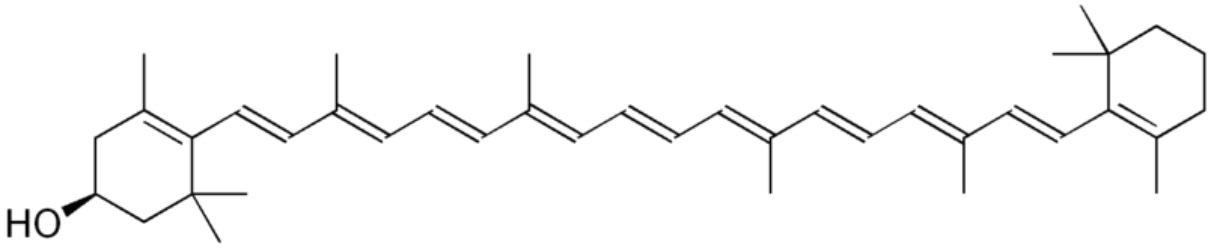
β-carotene



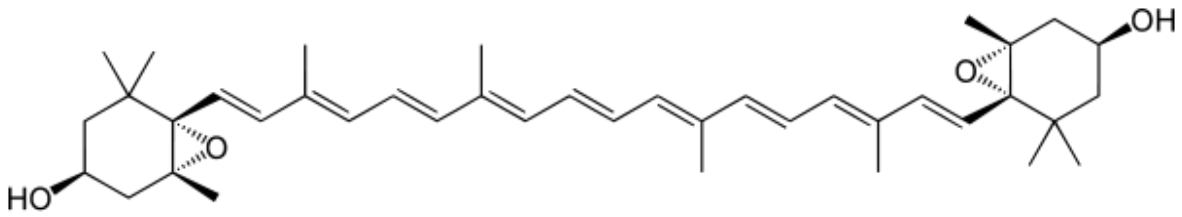
Lycopene



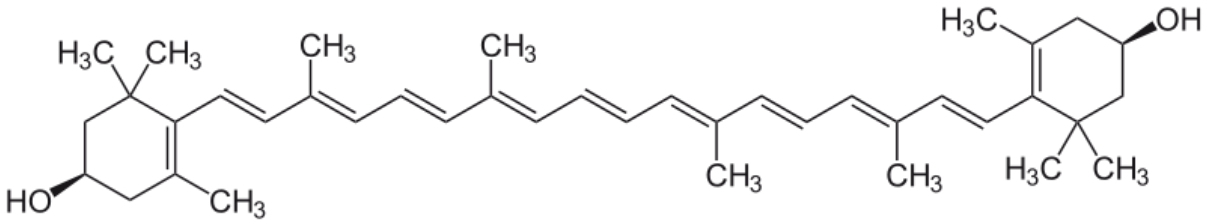
Lutein



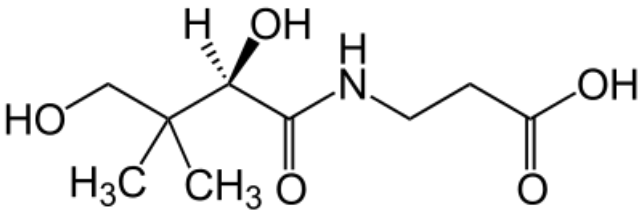
Cryptoxanthin



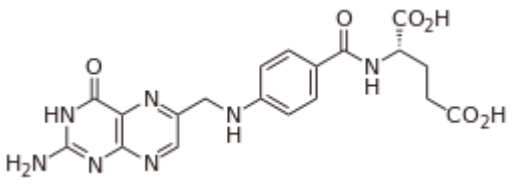
Violaxanthin



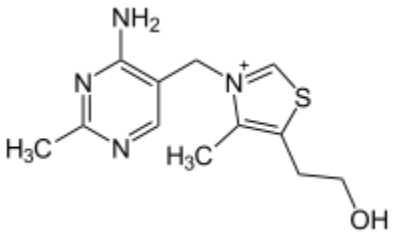
Zeaxanthin



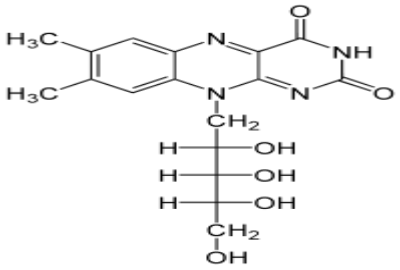
Pantothenic acid



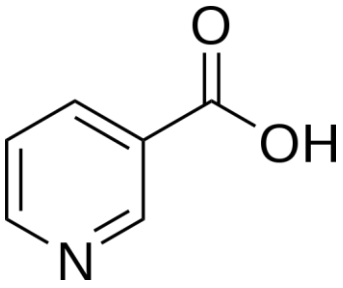
Folic acid



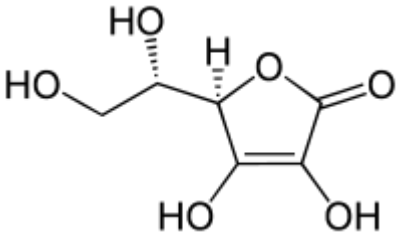
Thiamine



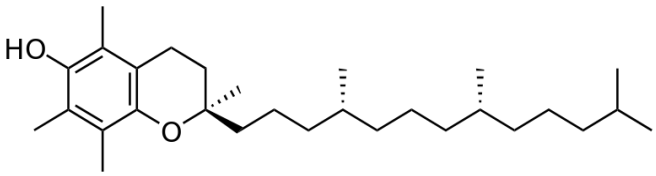
Riboflavin



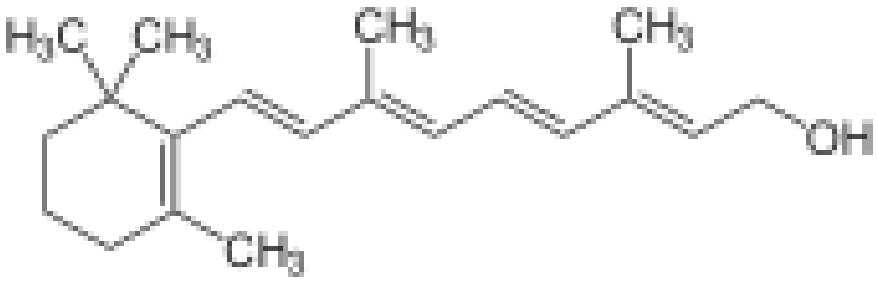
Niacin



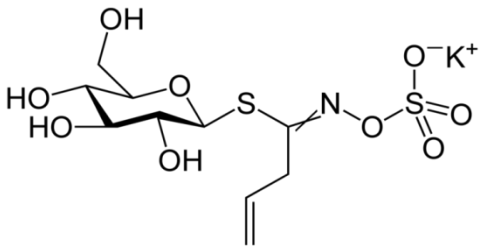
Ascorbic acid



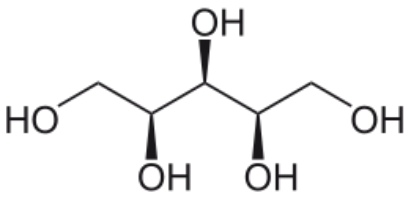
α-tocopherol



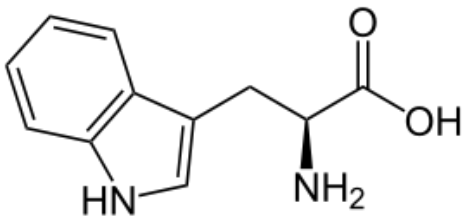
Vitamin A



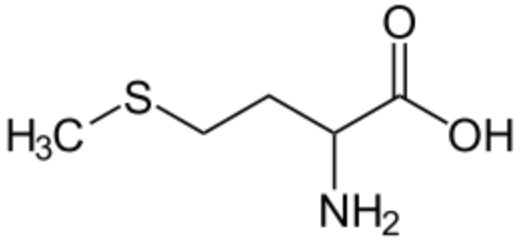
Sinigrin



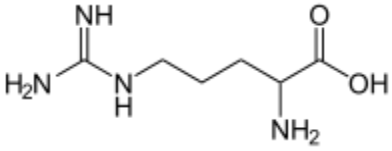
Xylitol



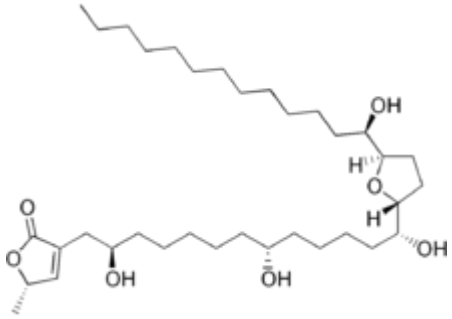
L-Tryptophan



Methionine



Arginine



Acetogenin

Table 1.7: Summary of Compounds/Chemical groups identified in *Carica papaya* and their Bioactivities/Use

Compound/Group	Reported Bioactivity/Use	References
Proteolytic Enzymes (Papain, Chymopapain)	Antioxidant Analgesic Anti-inflammatory Antibacterial Antiviral Antifungal Anticancer Anti-allergic Aid in digestion Laxative Assist wound healing Boost platelets	[Apsara, 2013; Aravind et al., 2013; Broke, 2013; Saba, 2013; Rochway, 2013]
Alkaloids (Carpaine,	Cytotoxic	[Aravind et al., 2013;

Pseudocarpaine, Dehydrocarpaine I & II)	Antitumor Anticancer Analgesic Anti-inflammatory Diuretic Amoebicide Antibacterial Antisplasmodic Antimalarial Heart depressant Antihelmintic	Baskaran et al., 2012; Burdick, 1971; Krishna et al., 2008; Mulkijanyan et al., 1991; Tuffley and Williams, 1951]
Cardiac glycoside (Cardenolide)	Increase force of contraction of heart muscle	[Baskaran et al., 2012]
Phenolic Compounds (Caffeic acid, P-coumaric acid, Chlorogenic acid)	Antioxidant	[Aravind et al., 2013]
Danielone	Antifungal	[Aravind et al., 2013]
Cystatin	Antioxidant	[Rochway, 2013]
Glucoside (Cyanogenic glucoside, Benzyl glucosinolate)	Antioxidant	[Rochway, 2013]
Terpenoids (Linalool, α-phellandrene, 4-terpinol, Cis-trans-linalool oxide)	Cytotoxic Antibacterial Antifungal Anti-inflammatory Hypoglycemic	[Baskaran et al., 1991; Krishna et al., 2008]
Flavonoids (Myricetin, Kaemferol, Quercetin)	Antioxidant Analgesic Antipyretic Hypoglycemic	[Broke, 2013; Baskaran et al., 2013; Krishna et al., 2008]
Carotenoids (β-carotene, Lycopene, Lutein, Crytoxanthin, Violaxanthin,	Antioxidant Anticancer Anti-aging	[Apsara, 2013; Aravind et al., 2013]

Zeaxanthin)		
Vitamin-A	Antioxidant Antiviral Anti-aging Immune booster Aid in Red blood cell production	[Broke, 2013; Rochway, 2013; Saba, 2013]
Vitamin-C	Antioxidant Anticancer Antiviral Anti-aging Immune booster	[Broke, 2013; Rochway, 2013; Saba, 2013]
Vitamin-E	Antioxidant Anti-inflammatory Anticancer Anti-aging Immune booster	[Apsara, 2013; Broke, 2013; Saba, 2013]
Vitamin-B12	Aid in RBC production	[Rochway, 2013]
Folic acid	Antioxidant Aid in RBC production Prevent heart attack & Stroke	[Broke, 2013; Rochway, 2013]
Pantothenic acid	Antioxidant Anticancer Anti-aging Heal wound	[Broke, 2013; Rochway, 2013]
Chitinase	Antibacterial	[Rochway, 2013]
Arginine	Enhance male virility	[Broke, 2013]
Acetogenin	Anticancer	[Haider, 2013]
Benzyl isothiocyanate	Anthelmintic	[Nakamura Y, et al, 2007]

CHAPTER 2
LITERATURE REVIEW

2.1 Antimalarial activity of Carica papaya (Family: Caricaceae) leaf extract against Plasmodium falciparum

The objective of this experiment was to determine the antimalarial activity of the ethanol leaf extract of Carica papaya *(C. papaya)*, blood stages of CQ-sensitive and CQ resistant strains against *Plasmodium falcipurum (P.falcipurum)* as target species.

C. papaya leaf was collected in and around Kalveerampalyam village, Coimbatore, Tamil Nadu, India. C. papaya leaf was washed with tap water and shade dried at room temperature. An electrical blender powdered the dried plant materials (leaves). The powder 500g of the leaf was extracted with 1.5 L of organic solvents of ethanol for 8 h using a Soxhlet apparatus. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 2%, 4%, 6%, 8% and 10%, respectively.

The highest larval mortality in the ethanol leaf extract of C. papaya against the 1st to 4th instars larvae and pupae values of LC50= 3.65%, 4.28%, 5.41%, 6.70%, and 7.50%, respectively. The LC90 values of 9.61%, 11.75%, 13.53%, 16.36%, and 16.92%, respectively. Plant extracts showed moderate to good antiparasitic effects. These four concentrations (25, 50,100 and 150 µg/mL) of ethanol leaf extracts exhibited promising inhibitory activity against the CQ sensitive strain with (IC50) values 40.75%, 36.54%, 25.30%, and 18.0% and in CQ resistant 50.23%, 32.50%, 21.45%, and 23.12% against P. falciparum.

In conclusion, the results indicate the effective plant extracts have the potential to be used as an ideal eco-friendly approach for the control of vector mosquitoes [Kovendan K., et al, 2012].

2.2 Anti-fertility Effects of *Carica papaya* Linn: Methanol Leaf Extracts in Male Wistar Rats

Carica papaya methanol leaf extract (CPMLE) is used widely in West Africa as anti-malarial and antimicrobial. The aim of this experiment was to study the effects of (CPMLE) on fertility in male Wistar rats using sperm counts and percentage of defective sperm cells as markers. Acute toxicity test was performed. Thirty-two male Wistar rats were divided into 4 groups and treated orally with 100, 200 and 400 mg kg^{-1} weight respectively with the extract. Group 4 served as control (10 mL kg^{-1} distilled water). After 28 days of treatment, serum biochemical parameters including Aspartate aminotransferase (AST), Blood Urea Nitrogen (BUN), Total Bilirubin (TB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), triglycerides, total protein and albumin and histopathological study of the testes, kidney, heart and liver were assayed. Acute toxicity results showed that CPMLE produced no mortalities at the dose of 1500 mg kg^{-1}. However, there were CNS signs as well as diuresis. CPMLE significantly ($p<0.01$) produced dose dependent decreases in sperm counts and increased the percentage of defective sperm cells. There were significant ($p<0.05$) increases in AST and BUN. Histopathological studies showed mild kidney and cardiac hyperaemia, slight hepatic degeneration and severe necrosis of the germinal epithelium of the testes. These show that CPMLE can produce some defects to fertility and may be used to control birth rate [Udeh E. Nkeiruka and Nwaehujor O. Chinaka, 2013].

2.3 Central and Cardiovascular Effects of the Alcoholic Extract of the Leaves of *Carica papaya*

The central effects of an alcoholic extract of *Carica papaya* leaf were investigated in male rats. The extract (≥ 10 mg kg^{-1}, i.p.) induced a dose-dependent sedative effect. The extract (≥ 5 mg kg, i.p.) also induced central muscle relaxation. The behavioral effects of the extract were associated with an initial desynchronization of the electroencephalogram (EEG) and an increased activity of the electromyogram (EMG). This was followed by a deactivating pattern in the optic chiasma while the EMG activity was diminished. The extract at doses ≥50 mg kg^{-1} (i.p.) completely protected the rats against pentylenetetrazol-induced seizures, while doses of 5 mg kg^{-1} (i.p.) gave 50% protection. The extract at doses of 100 and 200 mg kg^{-1}

(i.p.) also gave 100% protection against maximal electroshock-induced convulsions [A. Gupta, Co. Wambebe and D. L. Parsons, 1990].

2.4 Hepatoprotective and in vivo Antioxidant Activity of Methanol Extract of Carica papaya Linn. Leaves on Paracetamol Induced Liver Damage in Rats

The present study was performed to investigate the hepatoprotective activity of methanol extract of Carica papaya Linn leaves against paracetamol induced hepatic damage in rats. The substantially elevated serum levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, Total protein and Total bilirubin were significantly restored by the leaf extract. The methanol extract at an oral dose of 400mg/kg exhibited a significant protection against paracetamol induced liver damage. Silymarin was used as standard reference which exhibited significant hepatoprotective activity. The histopathological examination of liver provided supportive evidence. Paracetamol intoxication markedly decreased the level of reduced glutathione, superoxide dismutase, catalase and glutathione peroxidase, which was significantly enhanced by Carica papaya Linn leaf extract. Lipid peroxidation level, which was increased in paracetamol intoxication, was significantly reduced in liver by methanol extract of Carica papaya Linn extract. Furthermore, the acute toxicity of the extract showed no signs of toxicity up to a dose level of 2000mg/kg. The results suggest that the hepatoprotective effect of Carica papaya Linn leaves might be contributed to its modulation on detoxification enzymes and its antioxidant and free radical scavenger effects [Rajesh, Venugopalan; Perumal, Perumal; Rajesh, Gaddam; Rajavel, Rajagoundan, 2012].

2.5. Antioxidant analysis of different parts of Carica papaya

This study was conducted to compare the total antioxidant activity (TAA), total phenolic content (TPC) and total flavonoid content (TFC) from the different parts of papaya tree including their ripe and unripe fruit, seeds and the young leaves. Two methods namely DPPH radical scavenging activity and β-carotene bleaching assay were used to determine the TAA, whereas TPC was determined by Folin-Ciocalteu's method while TFC by aluminium trichloride (AlCl3). For these purposes, methanolic extracts (80%) were prepared. The results showed that the highest antioxidant activity through β-carotene bleaching assay was observed

in unripe fruit (90.67 ± 0.29%) followed by young leave, ripe fruit and the seed. In other hand, young leaves exhibited a significant higher scavenging effect compared to others and the dose required in reducing the absorbance of DPPH control solution by 50% (EC50) was calculated at 1.0 ± 0.08mg/ml. The EC50 values were 4.3 ± 0.01mg/ml, 6.5 ± 0.01mg/ml and7.8 ± 0.06mg/ml for unripe fruit, ripe fruit and seeds respectively. Interestingly, both TPC and TFC also showed that young leaves had the highest antioxidant content (424.89 ± 0.22mgGAE/ 100 g dry weight and 333.14 ± 1.03mg rutin equivalent/ 100 g dry weight, respectively). Statistically, Pearson correlation showed there were positive correlations between TPC and TFC with antioxidant activity assayed by DPPH radical scavenging assay(r=0.846 and r=0.873, respectively). However there was no correlation between TPC and TFC with β-carotene bleaching activity. In brief, taken into account all the parameters measured, antioxidants were highly remarkable in the sequence of young leaves > unripe fruit > ripe fruit > seed. Nevertheless, further investigation for isolation and identification of the phytoconstituents responsible for antioxidant activity is desirable [Maisarah et al, 2013].

2.6 In-vitro sensitivity pattern of some urinary tract isolates to Carica Papaya extracts

Powdered leaves of Carica papaya (L.) were extracted with ethanol and partitioned in chloroform and distilled water. The extract and fractions were tested for antibacterial activity against clinical isolates of Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas species using disc diffusion and microbroth dilution technique. The extract and fractions were further subjected to phytochemical tests for the presence of secondary metabolites using standard procedures. Results of sensitivity test results showed that ethanol extract of the leaf was active against E. coli and K. pneumoniae (7mm each) at 1000µg/disc concentration while chloroform and water fractions of the leaf were active against Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis at 1000µg/disc concentration with zone diameter of 7mm each. Both the extract and fractions were inactive against P. aeruginosa at all concentrations used in this study. The results of phytochemical screening indicated the presence of alkaloids, flavonoids, steroids and tannins in either ethanol extract, fraction(s) or both. This indicates that the Carica papaya has the potential for the production of drugs against organisms causing urinary tract infections [Yusha'u, M., et al, 2009].

2.7 Antisickling property of *Carica papaya* leaf extract

Sickle cell disease (SCD) results from a mutation in the hemoglobin inside the red blood cells, where a glutamic acid at position 6 is replaced by a valine. Many phytomedicines have been identified as potential antisickling agents, stemming from reported usage as ethnomedicines by the local folk. This research examined methanolic leaf extracts of *Carica papaya* L. (Caricaceae) for possible *in vitro* antisickling and membrane-stabilizing activities involving the use of positive (p-hydroxybenzoic acid 5 mg/ml) and negative (normal saline) controls for the antisickling experiments and osmotic fragility test on Hbss red blood cells obtained from non-crisis state sickle cell patients. Fragiliograms indicated that the plant extract reduced hemolysis and protected erythrocyte membrane integrity under osmotic stress conditions. Pretreatment of SS cell suspensions with *C. papaya* leaf extract inhibited formation of sickle cells under severe hypoxia, with only 0 - 5% sickle cells at 40 min compared with untreated SS cell suspensions which had over 60% sickle cells. These results indicate the feasibility of *C. papaya* as an attractive potential candidate for SCD therapy [Imaga N.O. A., et al, 2009].

2.8 Carica papaya leaves Juice Significantly Accelerates the Rate of Increase in Platelet Count among Patients with Dengue Fever and Dengue Haemorrhagic Fever

The study was conducted to investigate the platelet increasing property of Carica papaya leaves juice (CPLJ) in patients with dengue fever (DF). An open labeled randomized controlled trial was carried out on 228 patients with DF and dengue hemorrhagic fever (DHF). Approximately half the patients received the juice, for 3 consecutive days while the others remained as controls and received the standard management. Their full blood count was monitored 8 hours for 48 hours. Gene expression studies were conducted on the ALOX 12 and PTAFR genes. The mean increase in platelet counts were compared in both groups using repeated measure ANCOVA. There was a significant increase in mean platelet count observed in the intervention group ($P< 0.001$) but not in the control group 40 hours since the first dose of CPLJ. Comparison of mean platelet count between intervention and control group showed that mean platelet count in intervention group was significantly higher than control group after 40 and 48 hours of admission ($P<0.01$). The ALOX 12 (FC = 15.00) and PTAFR (FC = 13.42) genes were highly expressed among those on the juice. It was

concluded that CPLJ does significantly increase the platelet count in patients with DF and DHF [Subenthiran S.,et al, 2013].

2.9 The effect of aqueous extract of Carica papaya leaves on liver enzymes and blood cell counts of normal albino rats

The effects of crude leaf extract of *Carica papaya* (Linn) on aspartate aminostransferase (AST), alanine aminotransferase (ALT), total white blood cells (WBC), lymphocytes, neutrophils, thrombocytes and on body weight of normal albino rats were investigated. Albino rats weighing between 74 g and 90.5 g, of both sexes were used for the study. 2 ml of 0.11 g/ml aqueous extract was administered daily to each of the experimented rats for the 7 and 14 days study periods respectively, using stomach canula and the parameters analyzed using standard methods. Observations indicated that extract of *C. papaya* (Pawpaw) leaves did not induce any significant changes in the levels of AST and ALT. The results also showed that the *C. papaya* leaves extract influenced the immunological pathways. There was an increase in the levels of white blood cells, thrombocytes, lymphocytes and neutrophils of the test rats, and a significant weight gain in the animals at the end of the study period. Based on the findings in this work, the *C. papaya* leaves extract tested could hardly advance any adverse effects on the liver and also have immunological effect on the animal body [BI Nwiloh, NM Nwinuka, MO Monanu, 2009].

2.10 Antibacterial Activity of Papaya leaf extracts against Pathogenic Bacteria

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. [Romasi E. F., 2011]

2.11 Phytochemical Screening of the Polar Extracts of Carica papaya Linn. and the Evaluation of their anti-HIV-1 Activity

This research work deals with the evaluation of anti-HIV-1 effect of *Carica papaya* aerial parts, polar parts and also the investigation of the chemical content from the polar extracts of the plant. The methanol and aqueous extracts of *Carica papaya* were tested for their anti-HIV-1 activity using the syncytia formation assay. Methanol and aqueous extracts of *Carica papaya* aerial parts showed activity as anti-HIV-1 agents, both of the extracts Therapeutic index (TI) of 5.51 and 7.13 compared with the standard drug. Phytochemical analysis of both the extracts proves the presence of phytocomponents as flavonoids, tannins, alkaloids, carbohydrates and triterpenes. The results have shown that *Carica papaya* methanol and aqueous extracts have drug ability as anti- HIV-1 agents. [Rashed K., et al, 2013]

2.12 Phytochemical and Nutrient Evaluation of *Carica papaya* (Pawpaw) leaves

Three samples of C*arica papaya* leaves (Green, Yellow and Brown) were collected randomly from Ogbomoso town, Oyo state, Nigeria and analyzed for the phytochemical composition, vitamins and mineral constituents.

Phytochemical screening revealed the presence of bioactive compound saponins, cardiac glycoside alkaloids and absence of tannins in the three samples. Results showed that the plant leaves contained the vitamins, (mg/100g), thiamine (B1): green leaves 0.94, yellow leaves 0.41, brown leaves 0.52; riboflavin (B2): green leaves 0.13, yellow leaves 0.04, brown leaves 0.06; ascorbic acid (C): green leaves 16.29, yellow green 9.62, brown leaves 11.26. Mineral analysis showed highest values (mg/kg) of Ca, 8612.50; Mg, 67.75; Na, 1782.00; K, 2889.00; Mn, 9.50 in the green leaves, and Fe, 147.50 in yellow leaves as compared to other elements

examined. Thus green pawpaw leaf gave a source of essential nutrients while yellow pawpaw was a source of iron. Therefore pawpaw leaves can be manipulated in the herbal treatment of various diseases and as a potential source of useful elements for drugs formulation. [Adeyeye A., et al, 2010]

2.13 Wound-healing potential of an ethanol extract of *Carica papaya* (Caricaceae) seeds

This study evaluated the wound-healing and antimicrobial activity of *C. papaya* seed extract. Ethanol extract of *C. papaya* seed (50 mg/kg/day) was evaluated for its wound-healing activity in Sprague-Dawley rats using excision wound model. Animals were randomly divided into four groups of six each (group 1 served as control, group 2 treated with papaya seed extract, group 3 treated with a standard drug mupirocin and papaya seed extract (1:1 ratio) and group 4 treated with a mupirocin ointment. Rate of wound contraction and hydroxyproline content were determined to assess the wound-healing activity of the seed extract. The group 2 animals showed a significant decrease in wound area of 89% over 13 days when compared with groups 1 (82%), 3 (86%) and 4 (84%) respectively. The hydroxyproline content was significantly higher with the granulation tissue obtained from group 2 animals which were treated with *C. papaya* seed extract. Histological analysis of granulation tissue of the group 2 animals showed the deposition of well-organized collagen. The extract exhibited antimicrobial activity against *Salmonella choleraesuis* and *Staphylococcus aureus*. The results suggest that *C. papaya* promotes significant wound healing in rats and further evaluation for this activity in humans is suggested. [Nayak BS, et al, 2012]

2.14 Traditional and Medicinal Uses of *Carica papaya*

Papaya, botanical name Carica papaya, is a lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties. The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties. *Carica papaya* can be used for treatment of a numerous diseases like warts, corns, sinuses, eczema, cutaneous tubercles, glandular

tumors, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs and many, as a result *Carica papaya* can be regarded as Neutraceutical. The present article reviews the pharmacological uses of *Carica papaya* and side/toxic effects. *Carica papaya* contains an enzyme known as papain which is present in the bark, leaves, and fruit. The milky juice is extracted, dried and used as a chewing gum for digestive problems, tooth paste and meat tenderizers. It also contains many biological active compounds including chymopapain and papain which is the ingredient that aids digestive system, and again used in treatment of arthritis. [Aravind et al, 2013]

2.15 Papaya (Carica papaya): source material for Anticancer

Papaya (*Carica papaya* Linn) is well known for its exceptional nutritional and medicinal properties through the world. The whole plant including its leaves, fruits, seed, bark, latex and their juice are used as medicine. Papaya is considering as Nutracuitical fruits due to its multifaceted properties. The whole plant of papaya contain enzyme Papain, Lycopene, Isothiocyanate, important Mineral, Vitamins, Carbohydrates, Carotenoids, Flavonoids. These important nutritious fruits feed the body and immune system. A papaya in Vitro study shows that it will treat many cancer cell line and papaya physiochemical having anticancer activities. Papaya is rich in enzyme papain which is effective against cancer. Papain breaks down the fibrin cancer cell wall and protein into amino acid form. Other than papain it also contains lycopene which is highly reactive towards oxygen and free radical. Isothiocyanate is effective against breast, lung, colon, pancreas, prostate as well as Leukemia. These enzymes are capable of inhibiting both formation and development of cancer cell. [Krishnamurthy R., et al, 2013]

2.16 Development of quality standards of *Carica papaya* Linn leaves

The present study was performed for the development of physico-chemical parameters of Carica papaya Linn leaves belonging to family Caricaceae. The study comprises physico-chemical and phytochemical evaluation to confirm purity and authenticity of Carica papaya leaf based on WHO guidelines. Microscopy of the leaf showed presence of epidermis, collenchyma, and parenchyma, scellerenchyma, xylem, phloem and pith was found to be absent. Successive extractive value is found highest in petroleum ether extract 20.44 % (on

dry weight basis). Mean ash values (%) are 16.72 (total), 3.25 (acid insoluble ash), and 6.05 (water soluble ash) and moisture content is found to be 7.77 % and the phytochemical analysis indicate the presence of carbohydrates, terpenoids, flavonoids, phenolic compounds in different extracts of C.papaya L. leaf. TLC fingerprinting profile of different extract was also developed which exhibited presence of several medium polar compounds. [Anjum V, et al, 2013]

2.17 Papaya seed represents a rich source of biologically active isothiocyanate

In the present study, papaya (Carica papaya) seed and edible pulp were carefully separated and then the contents of benzyl isothiocyanate and the corresponding glucosinolate (benzyl glucosinolate, glucotropaeolin) quantified in each part. The papaya seed with myrosinase inactivation contained >1 mmol of benzyl glucosinolate in 100 g of fresh seed. The papaya seed extract also showed a very high activity of myrosinase and, without myrosinase inactivation, produced 460 micromol of benzyl isothiocyanate in 100 g of seed. In contrast, papaya pulp contained an undetectable amount of benzyl glucosinolate and showed no significant myrosinase activity. The n-hexane extract of the papaya seed homogenate was highly effective in inhibiting superoxide generation and apoptosis induction in HL-60 cells, the activities of which are comparable to those of authentic benzyl isothiocyanate. [Nakamura Y, et al, 2007]

2.18 Antimicrobial activity evaluation of the root of *Carica papaya* Linn

Carica papaya root extracts of the plant were tested for their in vitro antimicrobial activity by Continuous Hot Extraction (Soxhelation) method. The test organisms were *P.vesicularis, sterptococcus faecalis, Aeromonas hydrophilia, Salmonela typhae, Stphylococcus cohni, Serratia ficaria and E.coli*. The Zone of inhibition was determined for concentration ranging from 12.5mg/ml to 50mg/ml. (12.5mg/ml, 25mg/ml, 37.5mg/ml, and 50mg/ml). [Sahu P K., et al, 2011]

2.19 Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L) on clinical bacterial and fungal isolates

The efficacy of treatments with C. papaya is dependent on the quantity of the different chemical substances present in the preparation. The quantity of chemical substances varies in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree. The antibacterial and antifungal ability of both fresh and dried leaves of C. papaya against bacteria and fungi of medical importance was carried out. The aqueous, ethanol and acetone extract of both the dried and fresh leaves were tested at 25, 50 and 100 mg/ml concentrations on both the bacteria and fungi isolates using the disc diffusion method. Results showed very significant broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria and fungi. The organic extracts were more effective than aqueous extracts. The result further showed that the dry sample was effective against both Gram-positive and Gram-negative bacteria while the fresh sample was more effective against Gram-negative bacteria. The dried leaf extract was potent against some of the bacteria which standard antibiotics were not able to inhibit. C. papaya leaves showed a better antibacterial activity than antifungal activity. Demonstration of antimicrobial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in this plant for the development of newer antibacterial agents. [Esan B E., et al, 2012].

2.20 Hypoglycemic effect of *Carica papaya* leaves in Streptozotocin-induced diabetic rats

The purpose of this study was to assess the hypoglycemic effect of the aqueous extract of *C. papaya* leaves in diabetic rats. Several studies have reported that some parts of the *C. papaya* plant exert hypoglycemic effects in both animals and humans.

Diabetes was induced in rats by intraperitoneal administration of 60 mg/kg of streptozotocin (STZ). The aqueous extract of *C. papaya* was administered in three different doses (0.75, 1.5 and 3 g/100 mL) as drinking water to both diabetic and non-diabetic animals during 4 weeks.

The aqueous extract of *Carica papaya* (0.75 g and 1.5 g/100 mL) significantly decreased blood glucose levels ($p<0.05$) in diabetic rats. It also decreased cholesterol, triacylglycerol and amino-transferases blood levels. Low plasma insulin levels did not change after treatment in diabetic rats, but they significantly increased in non-diabetic animals. Pancreatic islet cells

were normal in non-diabetic treated animals, whereas in diabetic treated rats, *C. papaya* could help islet regeneration manifested as preservation of cell size. In the liver of diabetic treated rats, *C. papaya* prevented hepatocyte disruption, as well as accumulation of glycogen and lipids. Finally, an antioxidant effect of *C. papaya* extract was also detected in diabetic rats.

This study showed that the aqueous extract of C. papaya exerted a hypoglycemic and antioxidant effect; it also improved the lipid profile in diabetic rats. In addition, the leaf extract positively affected integrity and function of both liver and pancreas. [Rojop I E J, et al, 2012]

2.21 Summary of Literature Review on the Traditional use/ Bioactivity in *C.papaya*

The following is a brief report of traditional use and reported bioactivity of *C. papaya*.

Table 2.1: Review of Traditional use and reported Bioactivity of *C. papaya*

Traditional use/ Bioactivity	Plant Part	References
Antimalarial	Leaf	[Kovendan K., et al, 2012]
Anti-fertility	Leaf	[Udeh E. Nkeiruka and Nwaehujor O. Chinaka, 2013]
Digestive disorders	Bark, Leaf, Fruit	[Aravind et al, 2013]
Antisickling	Leaf	[Imaga N.O. A., et al, 2007]
Immunological effect	Leaf	[BI Nwiloh, NM Nwinuka, MO Monanu, 2009]
Increase Platelet count	Leaf	[Subenthiran S., et al, 2013]
Antioxidant	Whole plant	[Maisarah et al, 2013]
Antiviral	Leaf	[Aravind et al, 2013]
Antibacterial	Leaf, Seed	[Aravind et al, 2013; Romasi E. F., 2011]
Antifungal	Leaf	[Aravind et al, 2013]
Anti-HIV-1	Aerial parts	[Rashed K., et al, 2013]
Wound-healing activity	Seed	[Nayak BS, et al, 2012]
Anticancer	Fruit, Leaf	[Krishnamurthy R., et al, 2013]
Hypoglycemic	Leaf	[Rojop I E J, et al, 2012]
Skin disorders	Peel, Leaf, fruit	[Aravind et al, 2013]
Antihypertensive	Fruit	[Aravind et al, 2013]
Anti-inflammatory	Leaf, Fruit, Bark	[Aravind et al, 2013]

CHAPTER 3
MATERIALS AND METHODS

3.1 PREPARATION OF PLANT EXTRACT FOR EXPERIMENTS

3.1.1 Collection and Identification of *Carica papaya* Leaf

Carica papaya leaf was collected in the month of April 2013 from Sobahanbag during rainy season when weeds were in their maximum densities.

3.1.2 Washing and Drying of Papaya Leaf

At first the leaves were thoroughly washed with tap water to remove dust, soil, bird's droppings etc within them. The leaves were dried under sunlight for one week. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven at 50^0C for 2 hours.

3.1.3 Grinding and Storage of Dried Samples

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered leaf was 300.18 gm which was measured using electronic balance and it was found to be 300.18 gm.

3.1.4 Extraction of the Dried Powdered Sample

The fine powder of papaya leaves was dissolved in 200 ml methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

3.1.5 Filtration of the Extract

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

3.1.6 Solvent Evaporation

The filtrate was kept in rotary evaporator for complete evaporation of the solvent. The solution was also kept in the hot plate and stirred frequently for solvent evaporation. After running this procedure, a gummy extraction was obtained which was preserved in refrigerator.

3.1.7 Principle of a Rotary Evaporator

A rotary evaporator is a specially designed instrument for the evaporation of solvent (single-stage or straight distillation) under vacuum. The evaporator consists of a heating bath with a rotating flask, in which the liquid is distributed as a thin film over the hot wall surfaces and can evaporate easily. The evaporation rate is regulated by the heating bath Materials and Method temperature, the size of flask, the pressure of distillation and the speed of rotation. The concentrated extract was then air dried to solid residue for solvent-solvent partitioning.

Fig 3.1: Rotary Evaporator (IKA HB10 Basic, Biometra)

3.2 PHYTOCHEMICAL INVESTIGATION OF *C. papaya* leaves

3.2.1 Principle of Phytochemical Screening

Plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary Phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. Qualitative phytochemical analysis for studying the presence of active compounds like Alkaloids, Carbohydrates, Phytosterols, Saponins, Glycosides, Phenols, Flavonoids, Diterpenes, Protein & amino acids. Successive isolation of phytocompounds from plant materials depended on the type of solvent used in extraction procedure. The qualitative changes in the phytochemical analysis of tested plant species are correlated to methods of preparation [Pandith, 2012].

3.2.2 Equipments

1. Test tubes
2. Watch glass
3. Holder
4. Burner

3.2.3 Reagents and Chemicals

Table 3.1: Reagents and Chemicals required for Phytochemical Screening

Tests	**Reagents**
Carbohydrate Test	Molisch's reagent
Alkaloid Test	a) Mayer's reagent (potassiomercuric iodide solution b) Wagner's reagent (solution of iodine in KI) c) Hager' reagent (saturated solution of picric acid)
Flavonoid Test	Conc. HCl
Steroid Test	Chloroform, Conc. H_2SO_4
Tannin Test	$FeCl_3$ (5%)
Saponin Test	Distilled water

3.2.4 Solvents

1. Alcohol
2. Chloroform
3. Distilled water

3.2.5 Test Compound

Methanolic extract of *Carica papaya* leaf

3.2.6 Preparation of Sample Solution

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

3.2.7 Phytochemical Tests

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

3.2.7.1 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 within.
- 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.

3.2.7.2 Alkaloid Test

- A small volume of 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.
- **Mayer's Reagent:** Formation of white and cream color precipitate indicated the presence of alkaloids.
- **Hager's Reagent:** Formation of yellow crystalline precipitate indicated the presence of alkaloids.

- **Wagner's Reagent:** Formation of brownish-black precipitate indicated the presence of alkaloids.

3.2.7.3 Flavonoid Test

- A few drops of conc. HCl were added to a small amount of an extract.
- Immediate development of a red color indicated the presence of flavonoid.

3.2.7.4 Steroid Test

- 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.

3.2.7.5 Tannin Test

- 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.

3.2.7.6 Saponin Test

- About 0.5 ml 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.

3.3 Antimicrobial Screening

3.3.1 Introduction

Bacteria and fungi are responsible for many infectious diseases. The increasing clinical implications of drug resistant fungal and bacterial pathogens have lent additional urgency to antimicrobial drug research. 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.

i) Disc diffusion method
ii) Serial dilution method
iii) Bioautographic method

But there is no standardized method for expressing the results of antimicrobial screening [Ayafor *et. al*., 1982]. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great number of factors viz., the extraction methods, inoculums volume, culture medium composition, p^H, and incubation temperature can influence the results.

Among the above-mentioned techniques the disc diffusion [Bauer *et al*., 1966] is a widely accepted *in vitro* investigation for preliminary screening of test agents, which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method [Roland, R., 1982].

3.3.2 Principle of Disc Diffusion Method

The Kirby-Bauer test for antibiotic susceptibility, called the disc diffusion test, is a standard that has been used for years. In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient.

Solutions of known concentration (ug/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) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (e.g. Kanamycin) discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). 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. As a result, there is a gradual change of test materials concentration in the media surrounding the discs. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as **zone of inhibition**. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter [Barry, 1976; Bayer *et al.,* 1966].

This test must be rigorously standardized since zone size is also dependent on inoculums size, medium composition, temperature of incubation, excess moisture and thickness of the agar. If these conditions are uniform, reproducible tests can be obtained and zone diameter is only a function of the susceptibility of the test organism.

Zone diameter can be correlated with susceptibility as measured by the dilution method. Further correlations using zone diameter allow the designation of an organism as “susceptible”, “intermediate”, or “resistant” to concentrations of an antibiotic which can be attained in the blood or other body fluids of patients requiring chemotherapy.

In the present study the crude extracts, fractions as well as some pure compounds were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required [Bayer *et al.,* 1966].

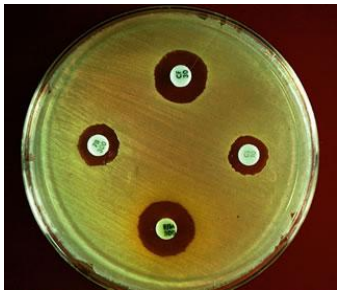


Fig 3.2: Disc diffusion method

3.3.3 Culture Medium and their Composition

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

Table 3.2: Composition of Nutrient Agar Medium

Ingredients	Amounts
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water q.s.	100 ml
p^H	7.2 ± 0.1 at 25^0C

Table 3.3: Composition of Nutrient Broth Medium

Ingredients	Amounts
Bacto beef extract	0.3 gm
Bacto peptone	0.5 gm
Distilled water q.s.	100 ml
p^H	7.2 ±0.1 at 25^0C

3.3.4 Description of microorganisms

Escherichia coli

- *Escherichia coli* is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms).
- Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans.
- The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine.
- E. coli and related bacteria constitute about 0.1% of gut flora, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease.

Shigella dysenteriae

- *Shigella dysenteriae* is a species of the rod-shaped bacterial genus Shigella.
- Shigella can cause shigellosis (bacillary dysentery).
- Shigellae are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.
- *S. dysenteriae*, spread by contaminated water and food, causes the most severe dysentery because of its potent and deadly Shiga toxin, but other species may also be dysentery agents.

- Contamination is often caused by bacteria on unwashed hands during food preparation, or soiled hands reaching the mouth.

Salmonella paratyphi

- *Salmonella paratyphi* is a gram-negative, motile, facultatively anaerobic, rod-shaped microbe that is a member of the Enterobacteriaceae family.
- The bacterium is transmitted by contact with faeces or, rarely, the urine of a carrier, or through contaminated food.

Aspergillus niger

- Aspergillus niger or A. niger is a fungus and one of the most common species of the genus Aspergillus.
- It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food.
- A. niger is less likely to cause human disease than some other Aspergillus species, but, if large amounts of spores are inhaled, A. niger can be deadly. This is due to a serious lung disease, aspergillosis.

Staphylococcus saprophyticus

- Staphylococcus saprophyticus is a Gram-positive, coagulase-negative bacterium belonging to the Staphylococcus genus.
- S. saprophyticus is often implicated in community-acquired urinary tract infections.

Streptococcus pyogenes

- Streptococcus pyogenes is a spherical, Gram-positive bacterium that is the cause of group A streptococcal infections.
- S. pyogenes displays streptococcal group A antigen on its cell wall.

Staphylococcus aureus

- Staphylococcus aureus is a Gram-positive bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin.
- Although S. aureus is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning.

Shigella boydii

- Shigella boydii is a Gram-negative bacteria of the genus Shigella.
- Like other members of the genus, S. boydii is a non-motile, non-spore forming, rod-shaped bacteria which can cause dysentery in humans through fecal-oral contamination.

Bacillus subtilis

- Bacillus subtilis is a Gram-positive, catalase-positive bacterium.
- A member of the genus Bacillus, B. subtilis is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions.
- Although this species is commonly found in soil, more evidence suggests that B. subtilis is a normal gut commensal in humans.

Candida albicans

- Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans.
- C. albicans is a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract.

Bacillus cereus

- Bacillus cereus is an endemic, soil-dwelling, Gram-positive, rod-shaped, beta hemolytic bacterium.
- Some strains are harmful to humans and cause food borne illness, while other strains can be beneficial as probiotics for animals.

β-hemolytic Streptococcus

- It is a gram positive bacterium responsible for a wide range of both invasive and non-invasive infections.

Bacillus megaterium

- Bacillus megaterium is a rod-like, Gram-positive, mainly aerobic spore forming bacterium found in widely diverse habitats.
- With a cell length of up to 4 µm and a diameter of 1.5 µm, *B. megaterium* is amongst the biggest known bacteria.
- The cells often occur in pairs and chains, where the cells are joined together by polysaccharides on the cell walls.

3.4 Materials Required

3.4.1 Sample: Concentrated Crude Methanolic Extract of *Carica papaya* (leaf)

3.4.2 Bacterial Culture: Bacterial strain

3.4.3 Reagents

- Nutrient Agar (Micromaster, Germany)
- Methanol (Merck, Germany)
- Ethanol (Merck, Germany)
- Sodium chloride (Merck, Mumbai)

3.4.4 Apparatus

- Laminar Air Flow Cabinet (Esco, Singapore)
- Incubator (Ehret KBK 4200, Germany)
- Autoclave (Hirayama, Japan)
- Hot Air Oven (Nuve FN 500, Turkey)
- Vortex Mixer (Gemmy Inc, Taiwan)
- Electronic Balance (Shimadzu, Japan)

3.4.5 Equipment

- Micropipette (Eppendorf, Germany)
- Micropipette Tips (Eppendrof, Germany)
- Reagent Bottle (Scott Duran, Germany)
- Petri dishes
- Eppendorf tube
- Vial
- Pipette and Pipette pumper
- Inoculating Loop
- Sterile Forceps
- Spreader
- Filter paper (Hangzhou Xinhua Paper Industry Co. Ltd., China)
- Spatula
- Candle

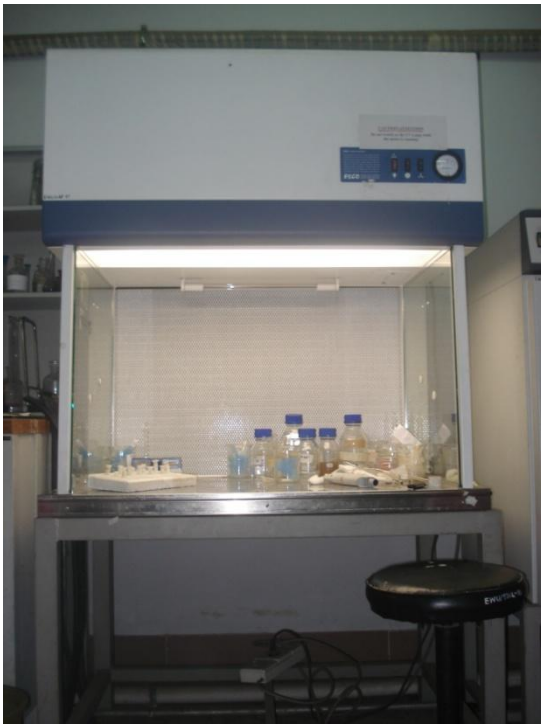


Fig 3.3: Laminar Air Flow Cabinet

3.4.6 Test Organisms

The microbial strains used for the experiment were collected as pure cultures. Both gram positive, gram-negative bacteria and fungi were taken for the test listed in the **Table 4.1**.

Table 3.4: List of Test Microorganisms:

Gram Positive Bacteria	Gram Negative Bacteria	Fungi
Staphylococcus Saprophyticus	*Escherichia coli*	*Aspergillus niger*
Streptococcus pyogenes	*Shigella dysenteriae*	*Candida albicans*
Staphylococcus aureus	*Salmonella paratyphi*	
Bacillus subtilis	*Shigella boydii*	
Bacillus cereus		
β-hemolytic streptococcus		
Bacillus megaterium		

3.5 Methods

3.5.1 Sterilization of Petri dishes

Petri dishes having 130 mm diameter were used in this test. The petri dishes were placed in the hot air oven at 150ºc temperature for 15 minutes for sterilization. After sterilization, the petri dishes were transferred inside the laminar air flow cabinet to avoid contamination.

3.5.2 Media preparation and sterilization

The composition of the supplied agar medium was 28g per 1000 ml and thus the amount required for this test was calculated by unitary method. 11.2 gm nutrient agar was weighted and taken in the reagent bottle to prepare 400 ml of agar solution. Then distilled water was added up to 400 ml and the reagent bottle was put in autoclave machine at a temperature of 121ºc for 15 minutes at about 1.30 hours for sterilization.

3.5.3 Sterilization of Tips and Eppendorf tube

The micropipette tips and eppendorf tubes were placed in autoclave machine at a temperature of 121ºc for 15 minutes at about 1.30 hours, for sterilization.

3.5.4 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.

3.5.5 Preparation of 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 and fungal suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

3.5.6 Stock Solution Preparation

To prepare the stock solution of samples of 50 µg/disc concentration, 0.025 gm sample was dissolved in 10 ml methanol in the test tube. Then the solution was shaken to dissolve the sample properly. Similarly, to prepare the stock solution of samples of 100 µg/disc and 150 µg/disc concentrations, 0.05 gm and 0.075 gm samples were dissolved in 10 ml methanol in two different test tubes respectively. Then the solutions in the test tubes were shaken to dissolve the sample properly.

3.5.7 Preparation of the Isotonic Solution

A 0.9% isotonic solution had to be prepared. This was prepared by weighing 0.9 g of Sodium chloride (Nacl) and by dissolving the measured Sodium chloride in 100 ml of distilled water. The isotonic solution was also autoclaved at 121^{o}c for 15 minutes.

3.5.8 Dilution of the Test micro-organisms

Previously cultured petri dishes of the test microorganisms were assembled. At first, an inoculating loop was sterilized in a bunsen burner. Then it was used to scrape a small colony of a specific species of microorganism from its culture. Now, the microorganism on the loop was transferred to a sterilized eppendorf tube, already containing 1 ml of isotonic solution. Then, the inoculating loop was resterilized and used to transfer another species of microorganism to a fresh eppendorf tube already filled with isotonic solution. In this way all

the microorganisms were transferred to fresh eppendorf tubes and thus were made ready for the test. The eppendorf tubes were then applied on a vortex mixer for proper mixing of microorganism with the isotonic solution.

3.5.9 Preparation of Discs

Two types of discs were used for antimicrobial screening.

3.5.9.1 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) standard disc was used as the reference.

3.5.9.2 Preparation of Paper Discs

Filter paper disc (6 mm diameter) was prepared from filter paper by using punch machine. Then it was sterilized in autoclave machine. After sterilization, each disc was impregnated with 6 µl sample solution by using micropipette (20 µl) and residual solvents were completely evaporated in air.

3.5.10 Preparation of Agar Plate

According to the name of bacteria Petri dishes were marked. Agar medium was dispensed into each Petri dish to get 3-4 mm depth of agar media each. After pouring the agar medium, all Petri dishes were kept in room temperature so that the medium can properly solidify.

3.5.11 Inoculation of microorganisms

1ml diluted bacterial suspension in the eppendorf tube was transferred on agar plate by micropipette (100-1000 µl) after solidification of the agar medium. By using spreader the bacterial suspension was spread on agar medium. Paper discs containing samples of three different concentrations were placed on to nutrient agar medium. Standard disc (Kanamycin, 30µg) were used as positive and placed on to the agar medium.

3.5.12 Diffusion and Incubation

The sample discs, the standard antibiotic discs and 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 at 37^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium.

3.5.13 Determination of Antimicrobial activity by measuring the Zone of Inhibition

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

CHAPTER 4
RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Phytochemical Screening

Preliminary phytochemical screening results of crude C. papaya leaf are mentioned in **Table 4.1** which revealed the presence of only Tannin and saponin.

Table 4.1: Result of chemical group test of the crude methanolic extract of *C. papaya* leaf

Tests	Reagents	Result
1.Carbohydrate	Molisch's	(+)
2.Alkaloid	Mayer, Hager, Wagner	(-)
3.Flavonoid	Conc. HCl	(-)
4.Steroid	Conc. H_2SO_4, Chloroform	(-)
5.Tannin	$FeCl_3$ (5%)	(+)
6.Saponin	Water	(+)

(+) Present; (-) Absent

4.1.2 In vitro Antimicrobial Screening

The antimicrobial activities of methanolic extract of *C. papaya* were examined in the present study. The results of antibacterial activity are given in the **Table 4.2**, which clearly show that the methanolic extract of *C.papaya* possesses some antibacterial activity against the entire tested organisms except *Streptococcus pyogenes* and *Shigella boydii*.

Table 4.2: Results showing Antimicrobial activity of *Carica papaya* leaf

Microorganisms	Zone of inhibition (mm)			
	50 (µg/disc)	**100 (µg/disc)**	**150 (µg/disc)**	**Kanamycin (30 µg/disc)**
1. *Escherichia coli*	10.5 mm	10 mm	9 mm	36 mm
2. *Shigella dysenteriae*	9.5 mm	8.5 mm	8 mm	33 mm
3. *Salmonella paratyphi*	(-)	(-)	8 mm	33 mm
4. *Aspergillus niger*	9.5 mm	6 mm	6.5 mm	32 mm
5. *Staphylococcus Saprophyticus*	8.5 mm	5.5 mm	6 mm	33 mm
6. *Streptococcus pyogenes*	(-)	(-)	(-)	26 mm
7. *Staphylococcus aureus*	12.5 mm	14.5 mm	5.5 mm	34 mm
8. *Shigella boydii*	(-)	(-)	(-)	25 mm
9. *Bacillus subtilis*	8.5 mm	12.5 mm	5.5 mm	35 mm
10. *Candida albicans*	8.8 mm	8.8 mm	9.9 mm	25 mm
11. *Bacillus cereus*	5.5 mm	5.5 mm	8.8 mm	30 mm
12. *β-hemolytic streptococcus*	6.5 mm	7.8 mm	8.9 mm	25 mm
13. *Bacillus megaterium*	5.5 mm	8.8 mm	7.8 mm	26 mm

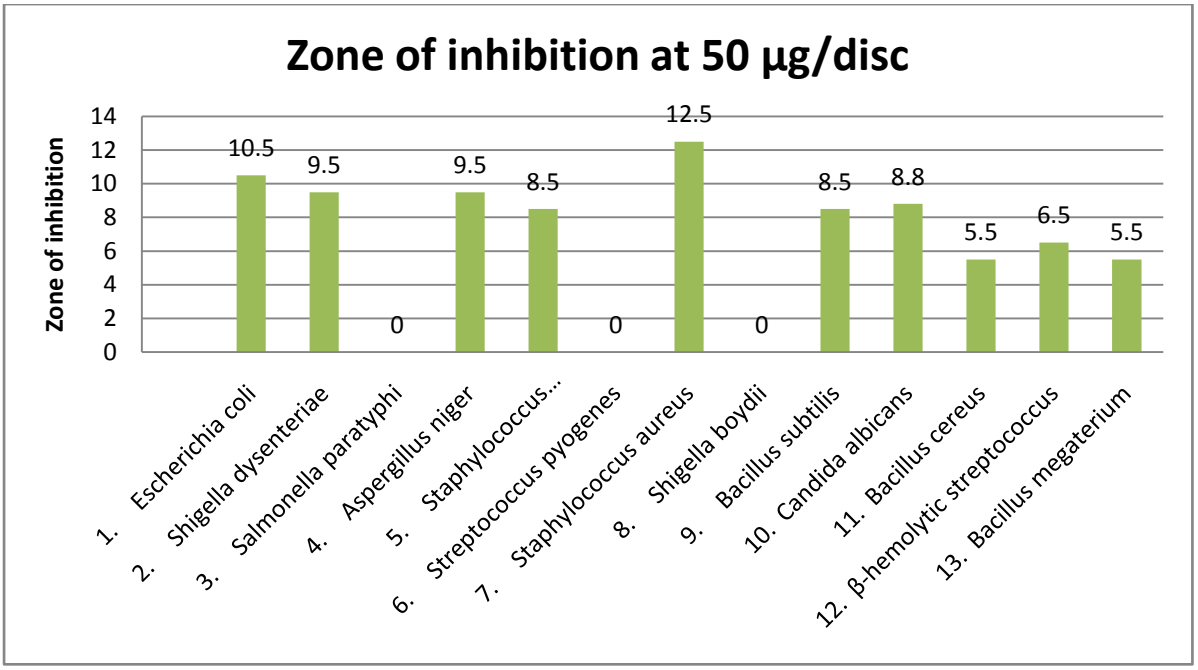


Fig 4.1: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms at a concentration of 50 µg/disc

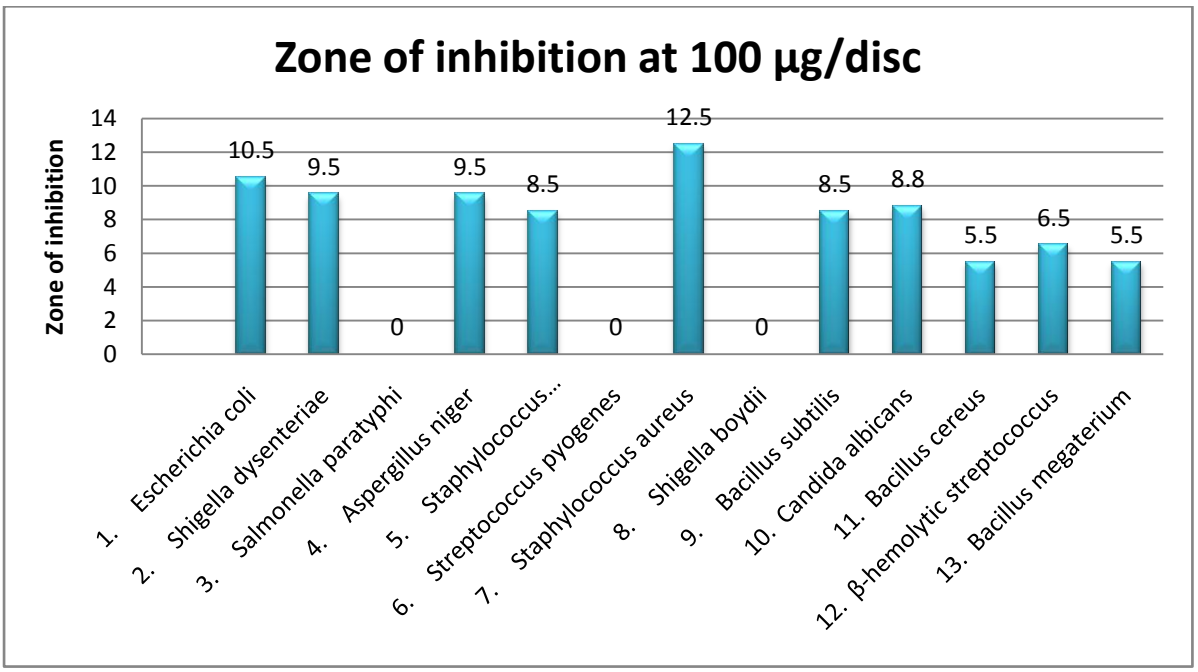


Fig 4.2: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms at a concentration of 100 µg/disc

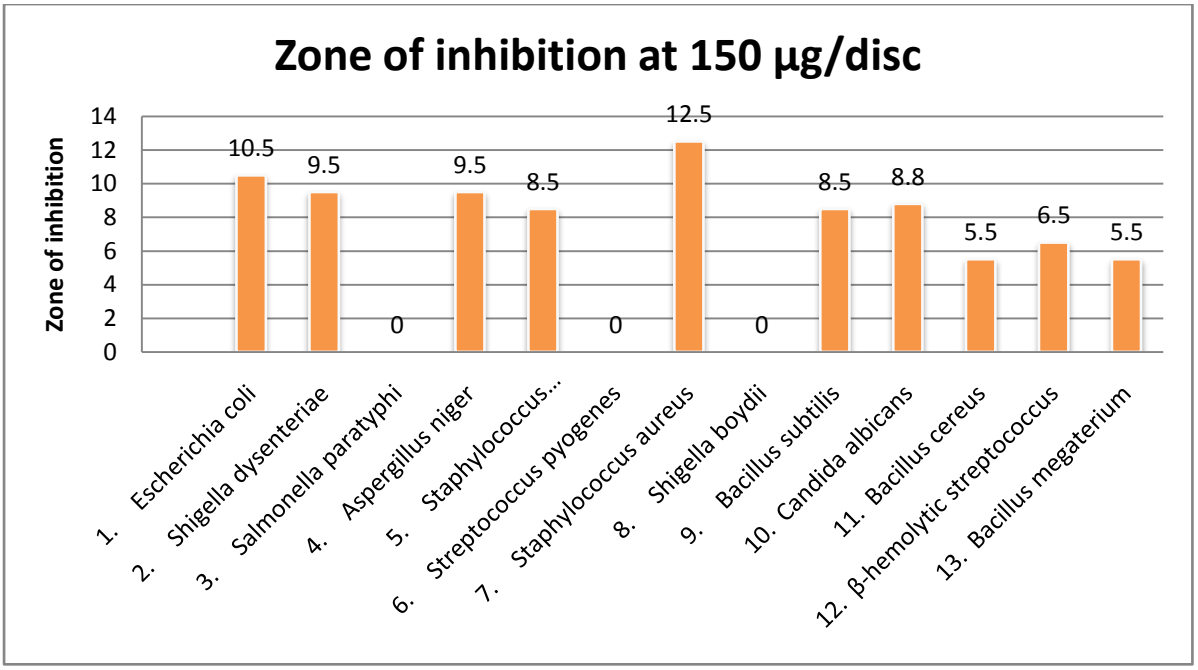


Fig 4.3: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms at a concentration of 150 µg/disc

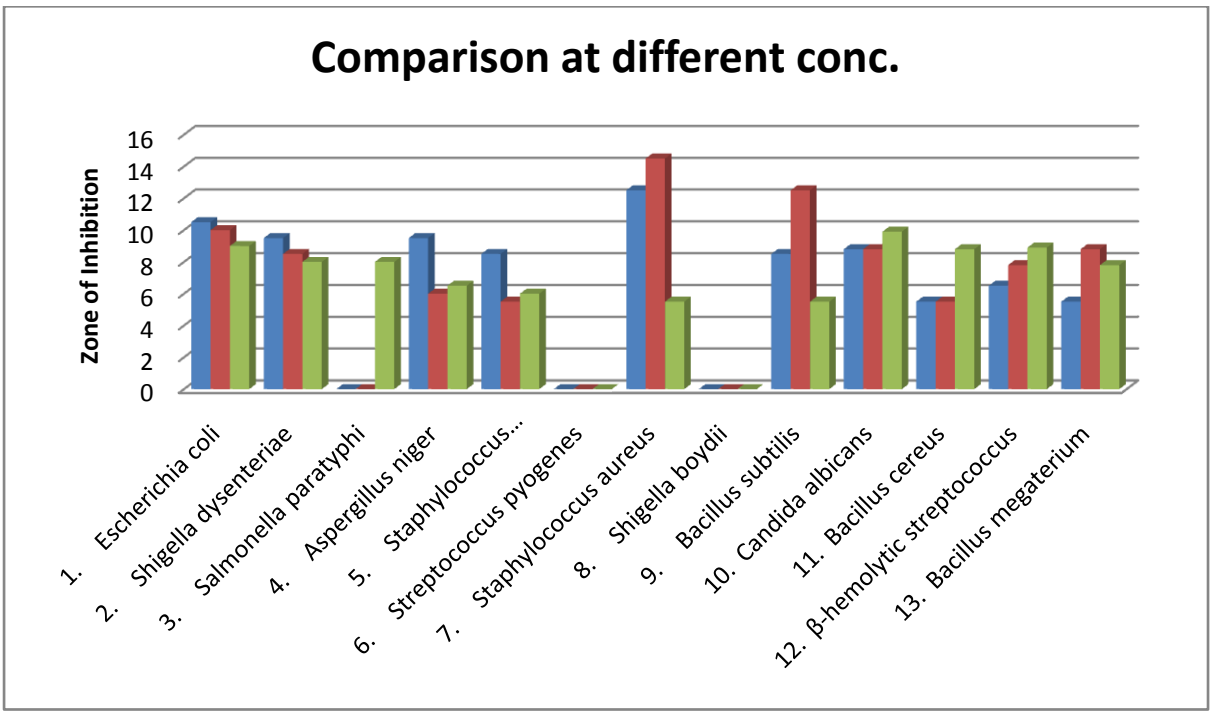


Fig 4.4: Comparison of antimicrobial activity (zone of inhibition) of different microorganisms at different concentrations

4.2 DISCUSSION

The therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. The presence of saponins supports the fact that papaya leaf has cytotoxic effects such as permealization of the intestine as saponins are Cytotoxic. It also gives the leaves the bitter taste. Saponin has relationship with sex hormone involved in controlling the onset of labor in women and the subsequent release of milk. Another important action of saponins is their expectorant action through the stimulation of a reflex of the upper digestive tract. Alkaloids are the most efficient therapeutically significant plant substance. Carpaine is the major alkaloid found in *C. papaya* and occurs in all green parts of the tree and in the seeds. The alkaloid, carpaine, slows the heart rate in humans and thus reduces blood pressure. Its action is similar to the drug prescribed for heart patients, digitalis. The alkaloid also possesses analgesic, antibacterial, anti-inflammatory and anticancer properties. The presence of alkaloids in the leaves show that *C. papaya* can be used as an effective antimalarial agent, since alkaloids consist of quinine, which is antimalarial. The cardiac glycosides therapeutically have the ability to increase the force and power of heart beat without increasing the amount of oxygen needed by the heart muscle. They can thus increase the efficiency of the heart and at the same time steady excess heart beats without strain to the organ. Deficiency of ascorbic acid is associated with pains in the joint and defect in skeletal calcification, anemia, and manifestation of scurvy hemorrhage from mucous membrane of the mouth and GI tract. The function of ascorbic acid accounts for its demand for normal wound healing. There is also an interesting ability of ascorbic acid as antioxidant, to prevent or at least minimize the formation of carcinogenic substances from dietary material. As a result, presence of ascorbic acid in *Carica papaya* leaves can be used in herbal medicine for the treatment of common cold and other diseases like prostate cancer. Other vitamins though in trace amount are essential for body metabolism. The carotenoid in C. papaya fruit and leaves contains antioxidant, anticancer and anti-aging properties. Previous phytochemical screening of *Carica papaya* leaves showed the presence of alkaloids, carbohydrates, saponins, glycosides, proteins and amino acids, phytosterol, phenolic

compounds, flavonoids, Terpenoids and tannins in different extracts. The presence of phytosterol in *Carica papaya* leaf was very prominent in all extracts. The saponins, glycosides, proteins and amino acids, flavonoid, terpenoids showed greater intensity of their presence in methanol.

This study has shown the phytochemicals and antimicrobial activity. Phytochemical results revealed the presence of only Tannin and Saponin. Flavonoid test was supposed to be positive but may be the result was negative due to experimental error. The zone of inhibitions produced by the crude methanolic extract of *C. papaya* leaf ranged from 0-13 mm at a concentration of 50µg/disc; 0-15 mm for 100 µg/disc and 0-10 mm for 150 µg/disc. The Zone of inhibition for standard antibiotic disc (Kanamycin) against the microorganisms is also included in the **Table 4.2**. The crude methanolic extract of *C. papaya* leaf showed mild to moderate antimicrobial activity against the microorganisms. The antimicrobial activity of the plants may be due to the presence of various active principles in their leaves. However, methanolic extract of *C. papaya* leaf was not active against *Streptococcus pyogenes* and *Shigella boydii*. This might be due to the fact that methanol did not contain enough active constituents. Also different solvents have been reported to be able to extract different phytoconstituents depending on their solubility or polarity in the solvent. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

CONCLUSION

The presence of antibacterial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blue print for the development of a drug.

The crude methanolic extract of C. papaya showed significant, antimicrobial activities, some of which supports the traditional use of this plant in various diseases.

The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates. Very few compounds are isolated from the *C. papaya*. Therefore, there is huge potential to find active principles which could be beneficial for mankind for targeting various diseases.

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