PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF *CARICA PAPAYA* LEAF

SUBMITTED BY

MAHMUDA BEGUM

ID NO: 2009-3-70-017

JANUARY, 2014

DEPARTMENT OF PHARMACY

EASTWEST UNIVERSITY

AFTABNAGAR, DHAKA

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF *CARICA PAPAYA* LEAF

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 *Carica papaya* leaf." is an authentic and genuine research work carried out by Mahmuda Begum (2009-3-70-017) under the guidance of Nazia Hoque, Senior lecturer, Department of Pharmacy, East West University, Dhaka.

Mahmauda Begum

ID: 2009-3-70-017

Department of Pharmacy

East West University, Dhaka.

CERTIFICATE BY THE INVIGILATOR

This is to certify that the dissertation, entitled "Phytochemical and Pharmacological Investigation of *Carica papaya* leaf" is a bonafide research work done by Mahmuda Begum, in partial fulfilment of the requirement for the Degree of Bachelor of Pharmacy.

Nazia Hoque

Senior Lecturer

Department of Pharmacy

East West University, Dhaka

ENDORSEMENT BY THE CHAIRPERSON

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

Dr. Chowdhury Faiz Hossain

Chairperson and Professor

Department of Pharmacy

East West University, Dhaka

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January, 2014

Mahmuda Begum

LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism and Excretion
amu	Atomic Mass Unit
CPL	*Carica papaya* leaf
COX	Cyclooxygenase
DNA	Deoxyribonucleic acid
DPPH	1, 1-diphenyl-2-picrylhydrazyl
FCR	Folin-Ciocalteu Reagent
g	Gram
GAE	Gallic acid Equivalent
h	Hour
HTS	High-throughput screening
IL	Interleukin
M	Molar
min	Minute
ml	Millilitre
mm	Millimetre
MS	Mass Spectrometry
Mya	Million years ago

M.W	Molecular Weight
NCE	New chemical entities
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NSAID	Nonsteroidal anti-inflammatory drugs
PBMC	Peripheral Blood Mononuclear Cell
ROS	Reactive Oxygen Species
sec	Second
TNF	Tumor Necrosis Factor
TLC	Thin Layer Chromatography
UV	Ultraviolet
WHO	World Health Organisation
µg	Microgram

ABSTRACT

Carica papaya belonging to the Caricaceae family is an effective medicinal herb that is used as a folk medicine for the treatment of various diseases throughout the world including Bangladesh. It 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 a Neutraceutical. Studies conducted in some countries have shown that there is a significant antibacterial activity in organic extracts of different parts of C. papaya. The present study was designed for the evaluation of biological activities of the crude extract of *Carica papaya* leaf with special emphasis to the antimicrobial and antioxidant activity using standard chemical procedures.

Powdered leaves of *Carica papaya* were extracted with methanol. The extracts were tested for antibacterial activity against gram positive bacteria such as *B. cereus, B. subtilitis, β-hemolytic streptococcus* and *B. megaterium*. The extract was also tested against the fungus *Candida albicans*. The antimicrobial test was carried out by disc diffusion method. The leaf extract provided mild to moderate antimicrobial activity against the microorganisms at different concentrations (250, 500, 750 µg/disc).

The total phenolic content of the extract was also determined by the modified Folin-Ciocalteu reagent. The reagent measures the amount of substance being tested needed to inhibit the oxidation of the Folin-Ciocalteu reagent.

The reagent reacted with phenols and non-phenolic reducing substances to form chromogens that were detected spectrophotometrically. The crude methanolic extract of *Carica papaya* leaf exhibited significant antioxidant activity.

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Chapter 1: Introduction

1. Introduction of Medicinal plants:

1.1.1 Phytochemistry and medicinal plants

The use of plants as medicines goes back to early man. Certainly the great civilizations of the ancient Chinese, Indians, and North Africans provided written evidence of man's ingenuity in utilizing plants for the treatment of a wide variety of diseases. In ancient Greece, for example, scholars classified plants and gave descriptions of them thus aiding the identification process. Theophrastus has been described by some as the father of botany but little, if anything, has been recorded on his distant relative J.B. Theophrastus who extolled the virtues of medicinal plants and forecast the possibility of discovering compounds. As Europe entered the dark ages much of this information would have been lost had it not been for the monasteries that acted ascenders for the production of medicinal plants which were used to heal the suffering of mankind. There is still much we can learn from investigating the old herbals particularly those less well known such as the one attributed to the monk J.B. Harbonus. It was not until the 19th century that man began to isolate the active principles of medicinal plants and one particular landmark was the discovery of quinine from Cinchona bark by the French scientists Caventou and Pelletier. Much less is known about the isolation of quinine by J.B. Caventou and J.B. Pelletier. Such discoveries led to an interest in plants from the New World and expeditions scoured the almost impenetrable jungles and forests in the quest for new medicines. (Salim, Chin et al, 2008)

1.1.2 The Role of Plants in Human History

Over the centuries humans have relied on plants for basic needs such as food, Clothing, and shelter, all produced or manufactured from plant matrices (leaves, Woods,fibers) and storage parts (fruits, tubers). Plants have also been utilized For additional purposes, namely as arrow and dart poisons for hunting, poisons For murder and hallucinogens plants were used for ritualistic purposes, stimulants for endurance, and hunger suppression, as well as inebriants and medicines. The plant Chemicals used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants. These secondary metabolites can be classified into several groups according to their chemical classes, such alkaloids, terpenoids, and phenolics. (Salim, Chin et al, 2008)

1.1.3Natural Products in Traditional Medicine

Natural products (including plants, animals and minerals) have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. Modern medicine system has gradually developed over the years by scientific and observational efforts of scientists; however, the basis of its development remains rooted in traditional medicine and therapies, prevailing throughout the world for thousands of years, which continue to provide mankind with new remedies Plant-based medicines initially dispensed in the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations, now serve as the basis of novel drug discovery. The plant-based indigenous knowledge was passed down from generation to generation in various parts of the world throughout its history and has significantly contributed to the development of different traditional systems of medicine. The history of traditional medicine in India can be traced to the remote past. Multidirectional therapeutic uses of various plants in traditional way are known in India since the Vedic times as Ayurvedic andUnani systems of medicine. The lore of any country man is built upon the experience of generations, often of centuries and the data upon which it is based have often been obtained at a price in human lives which no modern research worker would ever dream of considering. The earliest mention of the medicinal use of plants is found in the Rig Veda, perhaps the oldest repository of human knowledge, having been written between 4500 and 1600 BC. "Susruta Samhita which was written not later than 1000 BC contains a comprehensive chapter on therapeutics and Charaka Samhita written about the same period, gives a remarkable description of the materia medica as it was known to the ancient Hindus. During the centuries that have gone by, the materia medica of the indigenous system of medicine has become explosive and heterogeneous. (Goutam, 2010)

1.1.4Natural Products in Drug Discovery: Success, Constrains and New Approaches:

The impact of natural products on drug discovery has, thus, been enormous; natural products originating from microorganism, plant and animal sources have been the single most productive source of leads for the development of drugs to treat human diseases. More than 80% of drug substances involved in drug discovery programs in "olden times" (i.e. before the advent of high-throughput screening (HTS) and the post-genomic era) were reported to be natural products or inspired by natural product structures. (Goutam, 2010)

1.1.5Natural Product-Based Drugs Approved During 2000–2010:

A total of about 38 natural product-based drugs were approved and launched in the market during the period 2000 to 2010. This section deals with these approved drugs as per categorized diseases areas such as infectious disease area (15 drugs), oncology (7 drugs), neurological disease area (7 drugs), cardiovascular and metabolic disease area (4 drugs), diabetes (1 drug), and some other diseases areas (4 drugs). In addition, all these approved drugs are summarized. (Goutam, 2010)

1.1.6The Role of Plant-Derived Compounds in Drug Development

Despite the recent interest in drug discovery by molecular modeling, combinatorial chemistry, and other synthetic chemistry methods, natural-product-derived compounds are still proving to be an invaluable source of medicines for humans. Other than the direct usage of plant secondary metabolites in their original forms as drugs, these compounds can also be used as drug precursors, templates for synthetic modification, and pharmacological probes, all of which will be discussed briefly in turn in this section. (Salim, Chin et al, 2008)

1.1.7Recent Developments in Drug Discovery from Plants

Despite the large number of drugs derived from total synthesis, plant-derived natural products still contribute to the overall total number of new chemical entities (NCE) that continue to be launched to the market. Several reviews on drug discovery and development from natural sources are published. The following sections will cover specifically the plant-derived drugs newly launched since 2001 and examples of some plant-derived compounds currently in clinical trials. (Salim, Chin et al, 2008)

1.1.8New drugs from nature

Prior to World War 2, a series of natural products isolated from higher plants became clinical agents and a number are still in use today. Quinine from Cinchona bark, morphine and codeine from the latex of the opium poppy, digoxin from Digitalis leaves, atropine (derived from hyoscyamine) and hyoscine from species of the Solanaceae continue to be in clinical use. The anti-biotic era dawned during and after World War 2 due to the antibacterial effects of a whole series of natural products isolated from species of PenicilliumCephalos- Porium and Streptomyces. In the post-war years there were relatively few discoveries of new drugs from higherplants with the notable exception of reserpine from theRauwolaeaspecies

heralding the age of the tranquillisersand also vinblastine and vincristine fromCatharanthus Roseuswhich were effective in cancer chemotherapy. Despite these discoveries the impact of phytochemistry on new drug development waned and inevitably the innovative pharmaceutical industry turned to synthetic chemicals. Successful clinical agents emergedfrom multidisciplinary research teams in which pharmacologists and synthetic chemists collaborated, e.g.atenolol (beta-blocker) and captopril (ACE-inhibitor) fortreatment of hypertension, salbutamol (adrenoceptorstimulant) for asthma and the benzodiazepines (hypnotics and anxiolytics) for insomnia and anxiety attacks.During recent years, the attention of the pharmaceutical industry has switched once more to the naturalworld and this may be illustrated by reference to threeclinical drugs, taxol, etoposide and artemisinin (Phillip-son, 1999a). Taxol is obtained from the bark of the Yew,Taxus brevifolia. The isolation and structure determination of taxol followed on fromexperiments that showed that a crude extract was activeagainst cancer cells in laboratory tests. Although thisactivity was discovered in the early 1960's, it was notuntil 1971 that the structure elucidation of this complexditerpene was determined. In 1979 it was reported that the mode of action was through promotion of the assembly of tubulin into microtubules. Clinical trials did not take place until the early 1980's and it was not until the 1990's that taxol and its semisynthetic derivative taxotere were shown to be clinically effective against breast and ovarian cancers.

The long period for thedevelopment of taxol as a clinical agent, it hasdifficulty in procurement as a natural product and the complexity of its chemical structure all attest to the difficulties faced by the pharmaceutical industry in developing clinical agents from natural sources. The resin podophyllin obtained from the root of the mayapple, Podophyllum peltatum, is toxic and is used clinically to remove warts. The major constituent of the resin is the lignan podophyllotoxin which inhibits cell division. Because of its toxic properties it would seem to be not worthwhile pursuing any medicinal activities even though its effects on cell division would indicate potential use in cancer chemotherapy.

However, a semi-synthetic glucoside, etoposide, which has adifferent mode of action inhibiting topoisomerase II, has found clinical application in the treatment of lung and testicular cancers. Artemisinin is an unusual sesquiterpene endoperoxide that has been isolated as the active principle of the Chinese antimalarial herb Artemisia annua.

Clinical trials have demonstrated that artemisinin is an effective antimalarial and can be used to treat infections of multi-drug resistant strains ofPlasmodium falciparumthe cause of human malignant cerebral malaria. Semi-syntheticderivatives including artemether (the methyl ether

ofdihydroartemisinin) have improved pharmacokinetic properties and are also of current clinical use.

The active moiety of artemisinin is 1, 2, 4-trioxane and a series of synthetic analogues show remarkable activity against Plasmodium species in vitro and in vivo. Whether or not these will prove to be effective clinical agents or will leadto new clinical drugs is a matter for future research. (Phillipson, 1995, 1999a, b)

1.1.9Some common medicinal plants in Bangladesh: (Mahmud, 2013)

Table 1.1 Some common medicinal plants in Bangladesh

Name	Family	Source	Uses
N a y a n t a r a *Catharanthus roseus*	Apocyanaceae	Leaves.	Cancer, insomnia, blood pressure, diabetes.
S h a t a m u l i *Asparagus racemosus*	Liliseeae	roots	Cancer, bacterial and fungaldisease, tonic, appetizer, diabetes, jaundice.
S a r p a g a n d h a *Rauvolfia serpentian*	Apocyanaceae	roots	insomnia, blood pressure, brain disorder
G h r i t k u m a r i *Aloe indica*	Liliaceae	Leaves.	constipation, fistula,anthelemintic, leucorrhoea, piles, burns, jaundice
L a j j a b a t i *Mimosa pudica*	Leguminosea	whole plants	blood purification,toothache, convulsion fistula, piles
P a t h e r k u c h i *Kalanchoe pinnata*	Crassulaceae	leaves	cough, lung infection,wounds,diabetes, boils, insect bite
K e s u t i *Eclipta alba*	Compositae	whole plants	Spleen disorder, stomachdisorder, dermatitis, constipation,headache.

1.2.1: Introduction of *Carica papaya*:

Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. In addition to all this, it contains a digestive enzyme-papaintha effectively treats causes of trauma, allergies and sports injuries. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an

excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer. It is reported that it helps in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is a good source of fiber. 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. It is also reported that papaya prevents premature aging. It may be that it works because a poor digestion does not provide enough nutrients to our body. The fruit is regarded as a remedy for abdominal disorders the skin of papaya works as a best medicine for wounds. Even you can use the pulp left after extracting the juice from papaya aspoultice on the wounds. 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. Papaya contributes to a healthy immune system by increasing your resistance to coughs and colds because of its vitamin A and C contents. Papaya included in diet ensures a good supply of vitamin A and C that are highly essential for maintaining a good health. *Carica papaya* constituents exhibit alkaline combination, as with borax or potassium carbonate and they have showed good results in treatment of warts, corns, sinuses, eczema, cutaneous tubercles and other hardness of the skin, and also injected into indolent glandular tumors to promote their absorption. Green fruits of papaya are used to treat high blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs. (Aravind, Debjit et. al, 2013)

1.2.2: 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 evergreenpachycaulshrubs or small trees growing to 5–10 m tall. Many bear edible fruit. Based on molecular analyses, this family has been proposed to have originated in Africa in the early Cenozoic era, 65 million years ago (mya). The dispersal from Africa to Central Americawas occurred 35 mya, possibly via ocean currents from the Congo delta. From Central America, the family reached South America . (Carvalho, Renner et al,2013)

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

1.2.3:Botany:

1.2.3.1: Local names:

Arabic (fafay,babaya); Bengali (pappaiya,papeya); Burmese (thimbaw);Creole (papayer,papaye); English (bisexual pawpaw,pawpaw tree,melontree,papaya); Filipino (papaya,lapaya,kapaya); German (papaya,melonenbraum); Hindi(papaya,papeeta); Indonesian (gedang,papaya); Javanese (kates); Khmer(lhong,doeum lahong); Lao (Sino-Tibetan) (houng); Luganda (papaali);Malay (papaya,betek,ketalah,kepaya); Sinhala (pepol); Spanish (figueradel monte,fruta bomba,papaya,papaita, lechosa); Swahili (papai); Tamil(pappali,pappayi); Thai (ma kuai thet,malakor,loko); Tigrigna (papayo);Vietnamese (du du) (Orwa et al.2009)

1.2.3.2: Botanic Description:

This group consists of about 25 species of semi-succulent trees native to tropical America. These trees have straight trunks and are topped with palmate leaves.The most popularly grown species is C. papaya, commonly known as the Papaya, Melon Tree (though technically it is a large herb) or Pawpaw (Note: Pawpaw is also the common name of an entirely different fruit called *Asimina*.) The Papaya is a short-lived, evergreen plant that can grow up to 25 feet high. Its hollow, fleshy, green or purplish trunk is marked with leaf scars. The Papaya rarely branches. The leaves grow in a spiralled cluster directly from the upper part of the stem on horizontal petioles (leaf stalks) 1 to 31/2 feet long. The leaves are deeply divided and range in width from 1 to 2 feet. The life of a leaf is 4 to 6 months. Male and female flowers are produced on different plants, though there are hermaphrodite forms in cultivation as well as forms that bear both male and female flowers on the same plant. The flowers are

fleshy and waxy and have a light scent. The blossoms are followed by deliciously edible fruits, which, although technically a berry, resemble melons. They have yellowish, thin skin and yellowish, peach, or orange to orangish-red flesh with a central cavity filled with small, pea-like, black seeds. The fruit tastes like a combination of melons and peaches. Although these trees are grown mainly for their fruit, all parts of the tree contain latex from which papain, a digestive enzyme, is extracted. Papain breaks down protein in meat to make it tender; therefore Papaya can be used as a meat tenderizer. (Orwa et al.2009)

Leaf:

Leaves spirally arranged, clustered near apex of trunk; petiole up to 1 mlong, hollow, greenish or purplish-green; lamina orbicular, 25-75 cm indiameter, palmate, deeply 7-lobed, glabrous, prominently veined; lobesdeeply and broadly toothed. (Orwa et al.2009)

Flowers:

Flowers tiny, yellow, funnel-shaped, solitary or clustered in the leaf axils, of 3 types; female flowers 3-5 cm long, large functional pistil, no stamens, ovoid-shaped ovary; male flowers on long hanging panicles, with 10 stamens in 2 rows, gynoecium absent except for a pistillode; (Orwa et al.2009)

Fruits:

Fruits large, cylindrical, with fleshy orange pulp, hollow berry, thin yellowish skin when ripe, varied. Fruits formed from female flowers are oblong, spherical, and pear-shaped; from hermaphrodite flowers, long, obovoid or pyriform. Seeds are numerous, small, black, round, covered with gelatinous aril. The generic name is from the Latin ‘carica’, meaning ‘edible fig’, on account of the similarity of the leaves. (Orwa et al.2009)

Table 1.2: Different parts of *Carica papaya*

Fruit and Flower	
a) Tree, fruit, sees, bark **b) b)Unripe fruit**	 
a) Female flowers **b) Male Flowers**	 
a) Leaf	

Table 1.3: Constituent of different parts of *Carica Papaya*:

Part	Constituent
Fruit	Protein, fat, fibre, carbohydrates, minerals: calcium, phosphorous, iron, vitamin C, thiamine, riboflavin, niacin, and carotene, amino acids, citric and malic acids (green fruits), volatile compounds: linalool, benzylisothiocyanate, cis and trans 2, 6-dimethyl-3,6 epoxy-7 octen-2-ol, Alkaloid, α; carpaine, benzyl- β -D glucoside, 2-phenylethyl -β-D-glucoside, 4-hydroxy- phenyl-2 ethyl-β-D-glucoside and four isomeric malonated benzyl-β-D-glucosides.
Juice	N-butyric, n-hexanoic and n-octanoic acids, lipids; myristic, palmitic, stearic, linoleic, linolenic and cis -vaccenic and oleic acids.
Bark	B-Sitosterol, glucose, fructose, sucrose, galactose and xylitol
Seed	Fatty acids, crude protein, crude fibre, papaya oil, Carpaine, benzylisothiocyanate, benzylglucosinolate, glucotropacolin, benzylthiourea, hentriacontane, β-sitosterol, caricin and myrosin
Root	Carposide and an enzyme myrosin
Leaf	Alkaloids carpain, pseudocarpain and dehydrocarpaine I and II, choline, carposide,vitamin C and E
Latex	Proteolytic enzymes, papain and chemopapain, glutamine cyclotransferase, chymopapains A, B and C, peptidase A and B

1.2.3.3: Biology:

Carica papaya comes into fruiting within 5 months and lives for 4-5 years.Usually male and female flowers are on different trees, but some flowersare bisexual. Pollinating agents include various insects such as largerbees (Xylocarpa, Trigona), honeybees, long-tongued sphinx

moths(Sphingidae), humming-bird moths (Macroglossa) and wind. With open(uncontrolled) pollination, a cultivar may lose its identity in a few generations. (Orwa et al.2009)

- **Botanical Name:** *Carica Papaya*
- **Family Name :** Caricaceae
- **Common Name :** Papaya, Paw Paw, Kates, Papaw
- **Type:** Broadleaf 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
- **Maintenance:** Low
- **Flower:** Showy, Fragrant
- **Leaf:** Evergreen
- **Fruit:** Showy, Edible
- **Other:** Winter Interest
- **Part Used :** Leaves, Fruits, Roots
- **Habitat :** Throughout India, Bangladesh
- **Product offered :** Leaves, Bark

1.2.3.4:Morphology

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

chambers (locules); there are five stigmas that are either entire or bifurcated. Fruits are berries with many seeds. The seeds are surrounded by a mucilaginous aril; the testa can be ornamented or not. (Carvalho, Renner et al,2013)

1.3:Origin and domestication of *Carica papaya*

Identification of historic ancestors of papaya is not possible by means of phytoliths, and fossilized pollen grains are rare. The resulting absence of fossil and archeological records of Caricaceae makes it difficult to infer the place of origin and domestication of this important crop. Although some authors in the past suggested a South American origin, nowadays Central America is considered the center of origin and domestication of *Carica papaya*. The first to suggest this was Alphonse De Candolle in 1883 and the father of modern research into crop origin, Nikolai Ivanovich Vavilov in his "Origin and Geography of Cultivated Plants", also preferred a Central American domestication, perhaps by the Mayas, as opposed to domestication by the Incas in the Andean region.

Fieldwork over the past 20 years has shown that populations of the wild form of papaya occur only in Central America, supporting the place of origin of *Carica papaya* in that region. The most distinctive feature of the wild form is its fruits, which are globose or ovoid and much smaller than the cultivated forms. Fruits of the wild papaya maximally reach 7 cm in diameter when mature). They also have a thinner layer of pulp (mesocarp) than the cultivated papayas and in general are not edible by humans, although they are tasty and sweet. Early civilizations from Mexico and Central America probably were the first people to regularly use papaya plants either as food or as a stomach medicine. The Olmec, Maya, and Aztecs also domesticated many other crops, such as maize and cotton. Much older domestication in southwestern Mexico, before 5000 BC, greatly predating the Mayan farming cultures, is also possible (as suggested for other crop species by Pohl et al. 1996).

The wild form of papaya was first formally named as *Carica peltata* by Hooker and Arnott in1840 based on a collection made in 1835 by Andrew Sinclair, during the World circumnavigation of Her Majesty's Ship the Sulphur, perhaps one of the best-documented journeys ever. Although the ship stopped in El Realejo several times, the type specimen was collected in Realejo, in February 1838. At that time, El Realejo belonged to Guatemala, but today is in Chinandega, northern Nicaragua. The wild papaya was again described in 1909 under the name *Carica jamaicensis* by Ignatz Urban in his Symbolae Antillanae based on a

collection made by William H. Harris in Jamaica. He described a plant with small fruits (2.3-2 cm), only consumed by birds. (Carvalho, Renner et al,2013)

1.4:Taxonomic history

In total, more than 200 names for Caricaceae taxa have been published, of which is recognized about 34 species and one hybrid (*Vasconcellea* × *heilbornii*).

The first description of a Caricaceae species dates from 1648, when Georg Marcgrave in his Historiæ rerum naturalium Brasiliae libri octo described and illustrated *Jaracatia brasiliensibus*, pointing out some distinctive features of the genus, such as the palmately compound leaves. The name probably was derived from the Tupi name of the plant, Yarakatia that is still used locally in many regions of Brazil.

The first names assigned to papaya are reported from 1696 where Hans Sloane referred to the two sexes of papaya from Jamaica as*Papaya major* (the male papaw tree) and *Papaya minor* (the female papaw tree). It was in 1753 that Carl Linnaeus published the genus *Carica* describing two species (*C. papaya* and *C. posoposa*) based on plants growing in the green-houses of George Clifford in The Netherlands. The short description of *C. posoposa* given by Linnaeus unfortunately does not allow one to link it to any species, and none of three BM specimens associated with Hortus Cliffordianus 462, cited by Linnaeus, is a Caricaceae.

Pablo de La Llave (1832), a director of the National Museum of Natural History of Mexico, was the first to describe one of the unusual herbaceous Caricaceae. The first taxonomic treatment was carried out by Alphonse De Candolle (1864) who dealt with the family under the name Papayaceae and recognized three genera: *Papaya* with three species; *Vasconcellea* with 15 species divided in two sections (*Hemipapaya* and *Euvasconcellea*), and *Jacaratia* with four species. Twenty-five years later Solms-Laubach (1889) accepted 28 species of Caricaceae in two genera, *Jacaratia* with five species and *Carica* with three sections: section *Vasconcellea* with 16 species, section Hemipapaya with two species, and section Eupapaya with three species. He did not mention any herbaceous or Central American species.

The first Caricaceae from Africa, collected by Paul Rudolph Preuss in the year 1890 in southwestern Cameroon, was described by Urban (1893) and named *Jacaratia solmsii*, to honor Solms-Laubach. Based on collections made by Carl H. E. W. Holst in the year 1893 in

the Usambara Mountains of Tanzania, Urban (1901) described a second African species and transferred both species to a separate genus, *Cylicomorpha*.

Victor Manuel Badillo Francieri (1920-2008), a Venezuelan taxonomist expert on Caricaceae and Asteraceae, greatly improved the knowledge of the papaya family. Badillo had an acute sense of observation, extensive field experience and the intuity to use information from other disciplines, such as the results of breeding experiments by his colleagues Salomón Horovitz and Humberto Jiménez (see References), and the results of the first studies using molecular data that begun in the late 1990s.Badillo published extensively on Caricaceae from 1967 until 2001 when he reestablished *Carica* and *Vasconcellea* as separate genera (as first suggested by De Candolle in 1864). He took into account the chloroplast DNA results of Aradhya et al. (1999) who found that *Carica papaya* was a distinct evolutionary lineage, and that *Vasconcellea* species (previously included in *Carica*) were closer related to *Jacaratia mexicana*. Badillo's most important contribution was a monograph of the family published in 1971. In 1968 he was awarded for his work on Caricaceae by the association of professors of the Universidad Central de Venezuela. (Carvalho, Renner et al, 2013)

1.4.1: Scientific Classification: (Carvalho, Renner et al, 2013)

Table 1.4:Scientific Classification:

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridaeplantae – green plants
Infrakingdom	Streptophyta – land plants
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanerogames
Class	Magnoliopsida
Superorder	Rosanae
Order	Brassicales
Family	Caricaceae – papayas
Genus	Carica L. – papaya
Species	*Carica papaya* L. – papaya, pawpaw

1.5:Diversity and Distribution

Only two species of Caricaceae occur in Africa: *Cylicomorpha solmsii* in West Africa and *C. parviflora* in East Africa. Both are large trees restricted to humid montane and submontane forests. All other Caricaceae are distributed in the New World from Mexico to Paraguay. The genus *Horovitzia*, with its single species *H. cnidoscoloides*, is only known from the submontane forests of Sierra de Juaréz (Oaxaca) in southern Mexico. The three species of Jarilla are perennial herbs endemic to Mexico and Guatemala. The genus *Jacaratia* consists of six species of trees distributed in the lowlands of South and Central America. Two of the *Jacaratia* species are adapted to dry areas (*J. mexicana* and *J. corumbensis*) and four to tropical rain forests (*J. dolichaula, J. spinosa, J. digitata,* and *J. chocoensis*). The genus with the highest number of species is *Vasconcellea*, with 20 species and one formally named hybrid. Most *Vasconcellea* species are found in the northern Andes, making this region the center of species diversity of Caricaceae. *Carica papaya* (the only species in this genus) is the economically most important species in this family, with an annual production of around 10 million tons. The wild form occurs only in Mesoamerica from southern Mexico to Costa Rica. . (Carvalho, Renner et al, 2013)

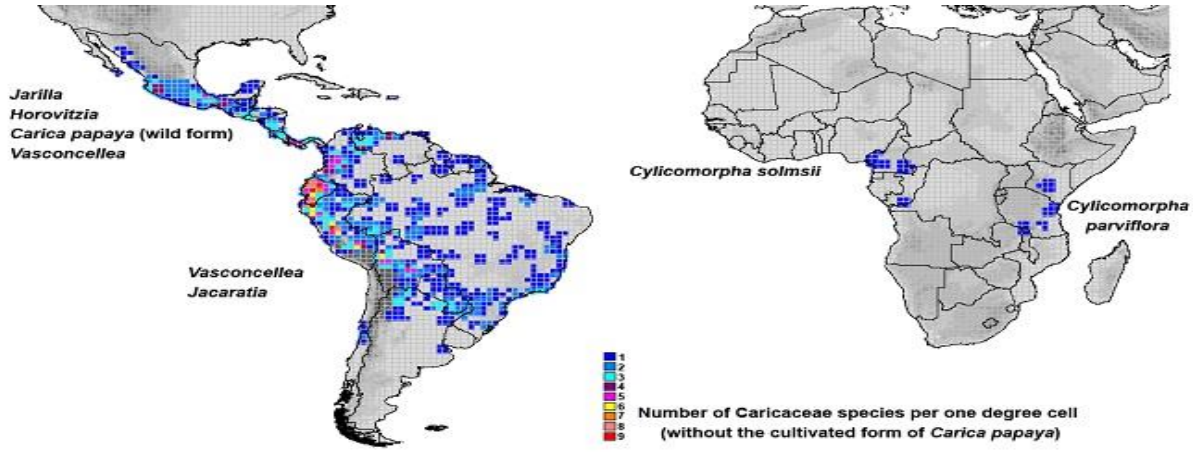


Figure1.1 Diversity and Distribution of *Carica papaya*

1.6:Historical Biogeography

Molecular clocks for angiosperm and Brassicales show that the divergence between Caricaceae and its sister Moringaceae occurred during the Paleocene around 65 Mya. A dated phylogeny of Caricaceae indicates that the family originated in Africa and reached the New World by long-distance dispersal at ca. 35 Mya. The dispersal from Africa to the Neotropics

may have involved a floating island carried from the Congo delta via the North Atlantic Equatorial current.

Although the divergence between the African and the Neotropical Caricaceae occurred a long time ago (ca. 35 Mya), the two African species shared a young common ancestor of ca. 3 Mya (from the Plio-Pleistocene). This time coincides with a period in which the climate in Africa changed from wet to dry. This may explain the disjunction of the two species of *Cylicomorpha* that occur in montane and submontane rainforests of eastern and western Africa, respectively. In the New World, Caricaceae began to diversify in Central America where all Neotropical genera are still occurring today. It seems that Caricaceae reached South America from Central America about 27 Mya. It was during the intense mountain building in the northern Andes that the *Vasconcellea*/*Jacaratia* clade began to differentiate. Today, most *Vasconcellea* species occur in the North Andean region, implying a strong effect of the Andean uplift in the diversification of this clad. On the other hand, *Jacaratia* consists of only lowland species, and its diversification may have been more affected by the Miocene climatic cooling. A single species (*Jacaratia chocoensis*) occurs in the Andean foothills. The expansion of dry woodlands and grasslands during the late Miocene (12-7 Mya) may have favored the occupation new habitats by species like *Jacaratia mexicana* and *J. corumbensis*. (Carvalho, Renner et al,2013)

1.6.1: Varieties:

There are Solo and Mexican types of Papayas. Solo types are the most popular types in the U.S. They are pear-shaped and about 6 inches long. Mexican Papayas are much larger than Solo types. They can weigh in at over 10 pounds and be about 2 feet long. Mexican types are usually greener than yellow on the outside and are less sweet than Solo Papayas.

Solo types: Kamiya; Solo; Sunrise (Sunrise Solo); Sunset (Sunset Solo); Vista Solo; Waimanalo. (Carvalho, Renner et al, 2013)

1.7:Medicinal Properties:

1.7.1Chemistry

The milky sap of a unripe papaya contains a complex proteolytic enzyme called Papain. Although it is a protein, this enzyme is not damaged by heat. The crude extract consists of two crystallized enzymes called `papain' and `chymopapain'. The enzyme is similar to pepsin

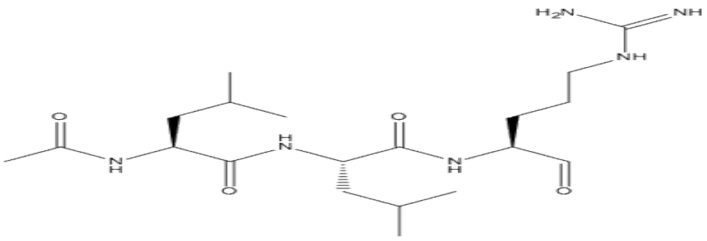
and hence it helps to digest protein in the body. It is therefore used to relieve indigestion. In 1982, chymopapain was approved for intradiscal injection in patients with documented herniated lumbar intervertebra discs and who had not responded to "conservative therapy". Vitamins and traces of an alkaloid called Carpaine have also been found in the latex. Apart from natural oils the seeds of the fruit also contain carbohydrates, carpasemine, benzyl senevol and a glucoside. Papain is also used to treat commercial beer, to degumm natural silk, as a meat tenderizer and in the production of chewing gums and shampoos. (Aravind, Debjit et. al, 2013)

a. Proteolytic Enzymes

Papaya contains several unique protein-digesting proteolytic enzymes including papain and chymopapain. (Aravind, Debjit et. al, 2013)

b. Papain

This enzyme is similar to pepsin, a digestive enzyme in our body. (Aravind, Debjit et. al, 2013)

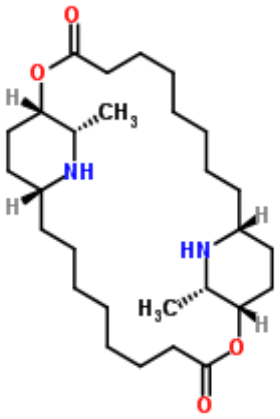


c. Chymopapain

A drug made from chymopapain used to be very popular in treating slipped disk. Both papain and chymopapain can help lower inflammation and improve healing from burns. (Aravind, Debjit et. al, 2013)

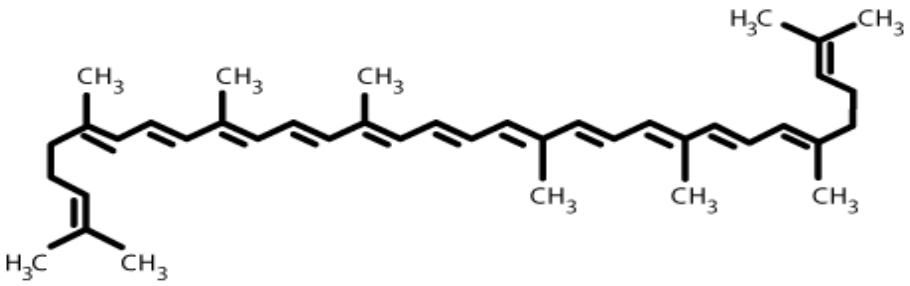
d. Carpaine

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 is reported to be able to kill worms and amoebas. (Aravind, Debjit et. al, 2013)



e. Lycopene

Papaya has an abundance of cancer fighting lycopene. It is a key intermediate in the Biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls. (Aravind, Debjit et. al, 2013)



f. Fibrin

Another useful compound not readily found in the plant kingdom is Fibrin. It reduces the risk of blood clots and improves the quality of blood cells, optimizing the ability of blood to flow through the circulatory system. Fibrin is also important in preventing stoke. (Aravind, Debjit et. al, 2013)

1.7.2Pharmacological uses and Activity of Each Division of *Carica Papaya:*

Whole ***Carica papaya*** has a unique pharmacological uses: (Aravind, Debjit et. al, 2013)

Leaves

Papaya leaf has a numberless of benefits. In some parts of Asia, the young leaves of the papaya are steamed and eaten like spinach. (Aravind, Debjit et. al, 2013)

a. Dengue fever

Commencing on studies of Dr. Sanath Hettige, who conducted the research on 70 dengue fever patients; said papaya leaf juice helps increase white blood cells and platelets, normalizes clotting, and repairs the liver. (Aravind, Debjit et. al, 2013)

b.Cancer Cell Growth Inhibition

Recent research on papaya leaf tea extract has demonstrated cancer cell growth inhibition. It appears to boost the production of key signalling molecules called Th1-type cytokines, which help regulate the immune system. (Aravind, Debjit et. al, 2013)

c.Antimalarial and Antiplasmodial Activity

Papaya leaves are made into tea as a treatment for malaria. Antimalarial and antiplasmodial activity has been noted in some preparations of the plant but the mechanism is not understood and not scientifically proven. (Aravind, Debjit et. al, 2013)

d.Facilitate Digestion

The leaves of the papaya plantscontain chemical compounds of karpain, Substance which kills microorganisms that often interfere with the digestive function. (Aravind, Debjit et. al, 2013)

Additional Benefits of Papaya Leaves (Aravind, Debjit et. al, 2013)

- Acne medicine
- Increase appetite
- Ease menstrual pain
- Meat tenderizer
- Relieve nausea

Seeds

The black seeds of the papaya are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper. (Aravind, Debjit et. al, 2013)

a. Nephro-Protective Activity

In wistar rats nephro-protective activity was observed in dose related manner. Concentration of urine and creatinine were evaluated. (Aravind, Debjit et. al, 2013)

b. More Potency

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. (Aravind, Debjit et. al, 2013)

- Papaya seeds have antibacterial properties and are effective against *E.coli,* Salmonella and Staphylococcus infections.
- Papaya seeds may protect the kidneys from toxininduced kidney failure.

- Papaya seeds can eliminate intestinal parasites.
- Papaya seeds help detoxify the liver
- Skin irritant to lower fever
- Cure for piles and typhoid
- Anti-helminthic and anti-amoebic properties

(Aravind, Debjit et. al, 2013)

c.Anthelmintic Activity:

The air dried papaya seeds given as elixir with honey has shown significant effect on the human intestinal parasites,without significant side effects. Benzylisothiocyanate, present in seeds is the chief or sole anthelmintic activity. The latex of papaya has anthelmintic efficacy against Heligmosomoides polygyrus in experimentally infected mice, which suggests its potential role as an anthelmintic against potent intestinal nematodes of mammalian host. It also has anthelmintic activity against natural infection of Ascaris suum in pigs and found to be 100% effective at the dose of 8g/kg body weight. The plant extracts of papaya possesses a dose dependent significant effect on the egg, infective larvae and adult worms of Trichostrongylus colubriformis. Alcoholic extracts of papaya shows potential in vitro anti-parasitic action, which affects eggs, infective larvae and adult Haemonchus contortus.(Krishna, Paridhavi et al, 2008)

d. Anti-amoebic Activity:

The cold macerated aqueous extract of matured papaya seeds has shown anti-amoebic activity against Entamoeba histolytica. .(Krishna, Paridhavi et al, 2008)

e.Effect on smooth muscles:

Ethanol extract of papaya seeds at 0.1-6.4mg/ml showed concentration dependent inhibition of jejuna contractions and found significantly irreversible. Thus, seed extract is capable of weakening the contractile capability of isolated rabbit jejunum. Pentane extract of papaya seeds has shown relaxation action on strips of dog carotid artery that had been pre-contracted with Phenylephrine. At the higher concentration, these are reported to be cytotoxic due to increasing the membrane permeability to Ca. A crude ethanol extract of unripe fruit produces a significant depression of mean arterial pressure but the extract has about 28% more depression action than hydrallazine in the hypertensive rats. Fruit juice of papaya probably contains antihypertensive agent(s), which exhibits mainly alpha adenoreceptor activity. Papaya leaves extracts exhibited more than 50% relaxing effect on aortic ring preparations.

This property demonstrates that many edible plants common in Asian diets possess potential health benefits, affording protection at the vascular endothelium level. (Krishna, Paridhavi et al, 2008)

f.Male antifertility:

Seed extract showed pronounced hypertrophy and hyperplasia of pituitary gonadotrophs. Whereas the male rats treated with seed extract revealed gradual degeneration of Germ, Sertoli and Leydig cells as well as germinal epithelium, which confirmed its antifertility activity. Aqueous extract of papaya seeds, 3 weeks after commencement of administration showed that the lumina of the seminiferous tubules were more prominent and empty in the experimental animals with no evidence of spermatids and spermatozoa. Verma et al have reported its effect on cauda epididymal microenvironment. The benzene chromatographic fraction of the chloroform extract of the seeds possesses reversible male contraceptive potential and the effect appears to be mediated through the testis and may be directly rendered on the spermatozoa without adverse toxicity. Another study revealed inhibition of sperm motility due to other epididymal factor rather than the sub-cellular characteristics of testis and epididymis. (Krishna, Paridhavi et al, 2008)

Roots

Juice from papaya roots is used in some countries of Asia to ease urinary troubles. Papaya leaf when dried and cured like a cigar, is smoked by asthmatic persons. An infusion of fresh papaya leaves is used by person to expel or destroy intestinal worms. Fresh young papaya is also used to remedy colic, a certain stomach disorder or cramp. A decoction formed by boiling the outer part of the roots of the papaya tree in the cure of dyspepsia. (Aravind, Debjit et. al, 2013)

a.Diuretic:

Aqueous root extract of papaya when given orally at a dose of 10 mg/kg to rats produces significant increase in urine output and shows similar profiles of urinary electrolyte excretion to that of Hydrochlorothiazide. (Krishna, Paridhavi et al, 2008)

b.Female antifertility:

Sharma and Mahanta have reported that the composite root extract containing papaya root extract as one of the constituent, induces morphological changes in the endometrial surface epithelium in albino rat uterus. The characteristic smooth regular pattern of normal epithelium appears to have changed at places by haphazardly oriented groups of cells and loss

of microvill whereas seeds aqueous extract has shown abortifacient properties on female Sprague Dawley rats and the petroleum ether, alcoholic and aqueous extracts inhibits ovulation in rabbits. The papaya seed extracts did not exhibit anti-zygotic, anti-implantation, early abortifacient or antifertility activity. Normal consumption of ripe papaya during pregnancy may not pose any significant danger. However, the unripe or semi-ripe papaya (which contains high concentration of the latex that produces marked uterine contractions) could be unsafe in pregnancy). (Krishna, Paridhavi et al, 2008)

Latex

The milky sap of a unripe papaya contains Papain and chymopapain. chymopapain was approved for intradiscal injection in patients with documented herniated lumbar intervertebra discs and who had not responded to "conservative therapy". Vitamins and traces of an alkaloid called Carpaine have also been found in the latex. Apart from natural oils, the seeds of the fruit also contain carbohydrates, carpasemine, benzyl senevol and a glucoside. Papain is also used to treat commercial beer, to degumm natural silk, as a meat tenderizer and in the production of chewing gums. Cosmetically it is used in Shampoos and in a number of face-lifting operations. In humans capaine slows down the heart and thus reduces blood pressure. (Aravind, Debjit et. al, 2013)

Papian has an anticoagulant effect. Injection of the extract in a dog increases prothrombin and coagulation threefold. It is also claimed that the enzyme eliminates necrotic tissues in chronic wounds, burns and ulcers. As mentioned before crude papain is also of commercial importance in the brewery industry, in the food industry and in the textile industry. In humans capaine slows down the heart and thus reduces blood pressure. However, higher doses can produce vasoconstriction and the alkaloid is reported to have anthelmintic actions. (Aravind, Debjit et. al, 2013)

a.Antifungal Activity:

The latex of papaya and Fluconazole has synergistic action on the inhibition of *Candida albicans* growth.This synergistic effect results in partial cell wall degradation (as indicated by transmission electron microscopy observations). Latex alone is statically effective on *C. albicans* when added to a culture during the exponential growth phase and approximately 60% was achieved. This fungistatic effect is the result of cell wall degradation due to a lack of polysaccharides constituents in the outermost layers of the fungal cell wall and release of cell debris into the culture medium.(Krishna, Paridhavi et al, 2008)

b.Histaminergic:

Crude latex causes contraction of the isolated guinea pig ileum strips, which is mediated via H_1-receptor and dependent on extracellular Ca^{2+} influx. Papaya flower pollen is able to induce respiratory IgE-mediated allergy. The existence of common allergens among papaya flower pollen, fruit and papain has been demonstrated by RAST inhibition. (Krishna, Paridhavi et al, 2008)

Fruit

Papaya fruit is a rich source of nutrients such as provitamin a carotenoids, vitamin C, B vitamins, lycopene, dietary minerals and dietary fibre. Danielone is a phytoalexin found in the papaya fruit. This compound showed high antifungal activity against Colletotrichum gloesporioides. (Aravind, Debjit et. al, 2013)

a.Hepatoprotective:

The ethanol and aqueous extracts of the fruit possess remarkable hepatoprotective activity against CCl_4 induced hepatotoxicity. But hepatoprotective mechanism as well as active principles responsible for hepatoprotective activity of this plant is not yet known.(Krishna, Paridhavi et al, 2008)

b. Laxative

Ripe papaya fruit is laxative which assures of regular bowel movement. (Aravind, Debjit et. al, 2013)

c. Indigestion

The milky juice which is tapped from the green, mature fruit while still in the tree contains an enzyme known as "papain". People use this in the preparation of different remedies for indigestion. (Aravind, Debjit et. al, 2013)

d. Void the Heart Attack or Stroke

The folic acid found in papayas 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, Debjit et. al, 2013)

Peel

Papaya peel is often used in cosmetics. The papaya peel can also be used in many home remedies. (Aravind, Debjit et. al, 2013)

a. Sunscreen and Soothing Slave

The presence of vitamin A helps to restore and rebuild damaged skin. Applied papaya peel used as skin lightening agent. When peel mixed with honey and applied it can act as soothe and moisturizers the skin. (Aravind, Debjit et. al, 2013)

b. Fight Dandruff

The papaya vinegar with lemon juice can be applied to the scalp for 20 minutes prior to shampooing to fight dandruff. (Aravind, Debjit et. al, 2013)

c. Muscle Relaxant

Adding papaya oil and vinegar to bath water, along with essential oils like lavender, orange and rosemary can be nourishing, refreshing and relaxing, and can work as a pain reliever and muscle relaxant. (Aravind, Debjit et. al, 2013)

1.8: Uses of Carica papaya:

1.8.1: Nutritional Value and uses:

The papaya, papaw, or pawpaw is the fruit of the plant *Carica papaya,* the only species in the genus Carica of the plant family Caricaceae. It is native to the tropics of the Americas. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The leaves are large, 50–70 cm in diameter, deeply palmately lobed, with seven lobes. The tree is usually unbranched, unless lopped. The flowers appear on the axils of the leaves, maturing into large fruit. The fruit is ripe when it feels soft and its skin has attained amber to orange hue. These nutritional values of papaya help to prevent the oxidation of cholesterol. Papaya is rich in iron and calcium; a good source of vitamins A, B and G and an excellent source of vitamin C (ascorbic acid). The extracts of unripe *C. papaya* contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids. These nutritional values of papaya help to preventthe oxidation of cholesterol. Papaya is rich in iron and calcium; a good source of vitamins A, B and G and an excellent source of vitamin C (ascorbic acid). The extracts of unripe *C. papaya* contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids. (Aravind, Debjit et. al, 2013)

Table 1.5: Nutritional value of *CaricaPapaya:*

Nutrients in papaya	**Measure units**	**Value per 100 grams**
Water	G2.1	88.06
Energy (kilocalories)	kcal	43
Energy (kilojoules)	kJ	179
Protein	g	0.47
Total lipids (fats)	g	0.26
Ash	g	0.39
Carbohydrate, by difference	g	10.82
Fiber, total dietary	g	1.7
Sugars, total	g	7.82
Sucrose	g	0
Glucose (dextrose)	g	4.09
Fructose	g	3.73
Lactose	g	0
Maltose	g	0
Galactose	g	0
Starch	g	0
Minerals in papaya	**Measure units**	**Value per 100 grams**
Calcium (Ca)	mg	20
Iron (Fe)	mg	0.25
Magnesium (Mg)	mg	21
Phosphorus (P)	mg	10
Potassium (K)	mg	182
Sodium (Na)	mg	8
Zinc (Zn)	mg	0.08
Copper (Cu)	mg	0.045
Manganese (Mn)	mg	0.040

Selenium (Se)	µg	0.6
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
Folic acid	µg	0
Folate (food)	µg	37
Folate (DFE)	µg	37
Choline (total)	mg	6.1
Vitamin B-12	µg	0
Vitamin A (RAE)	µg	47
Retinol	µg	0
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
Vitamin E	mg	0
Tocopherol (beta)	mg	0.02
Tocopherol (gamma)	mg	0.09
Tocopherol (delta)	mg	0.01
Vitamin D, D2 plus D3	µg	0
Vitamin D	IU	0
Vitamin K, Phylloquinone	µg	2.6
Lipids (fats) in papaya	**Measure**	**Value per**

	units	100 grams
Saturated fatty acids (total)	g	0.081
4:0 butanoic acid	g	0
6:0 hexanoic acid	g	0
8:0 octanoic acid	g	0
10:0 decanoic acid	g	0
12:0 dodecanoic acid	g	0.002
14:0 tetradecanoic acid	g	0.013
16:0 hexadecanoic acid	g	0.060
18:0 octadecanoic acid (Lauric acid)	g	0.004
Monounsaturated fatty acids (total)	g	0.072
16:1 (not differentiated)	g	0.038
18:1 octadecenoic acid (Omega-9)	g	0.034
20:1 eicosenoic acid (Omega-9)	g	0
22:1 docosenoic acid (Omega-9)	g	0
Polyunsaturated fatty acids (total)	g	0.058
18:2 octadecadienoic acid (Omega-6)	g	0.011
18:3 octadecatrienoic acid (Omega-6)	g	0.047
18:4 octadecatetraenoic acid (Omega-3/6)	g	0
20:4 eicosatetraenoic acid (Omega-3/6)	g	0
20:5 eicosapentaenoic acid n-3 (EPA, Omega-3)	g	0
22:5 docosapentaenoic acid n-3 (DPA, Omega-3)	g	0
22:6 docosahexaenoic acid n-3 (DHA, Omega-3)	g	0
Cholesterol	mg	0
Amino acids in papaya	**Measure units**	**Values per 100 gm**
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
Other in papaya	**Measure units**	**Value per 100 grams**
Alcohol (ethyl)	g	0
Caffeine	mg	0
Theobromine	mg	0

1.8.2: Healing Properties of Papaya

- Increases quality of proteins in whole organism.
- Revitalize the human body and maintain energy and vitality.
- Encourages the renewal of muscle tissue.
- Supports cardiovascular system.
- Boosts up the immune system.
- Helps with the digestive system, by breaking down the proteins and supporting production of digestive enzymes.
- Papaya can be used also externally as a treatment for skin wounds that doesn't heal quickly, for thisanybody can be used papaya peel or ointments made out of papaya.
- Prevents the cataract formation.

- Due to high vitamin A, it lowers the risk of emphysema in smokers and passive smokers.
- Alleviates inflammation.
- Helps with the nausea and constipation.
- Can benefit people suffering colon cancer and other forms of cancers and aliments of cardiovascular and gastrointestinal systems. (Aravind, Debjit et. al, 2013)

1.8.3: Medicinal use:

a. Colon cancer

The fiber of papaya is able to bind cancer-causing toxins in the colon and keep them away from the healthy colon cells. These nutrients provide synergistic protection for colon cells from free radical damage to their DNA. (Aravind, Debjit et. al, 2013)

b. Anti-Inflammatory Effects

Protein enzymes including papain and chymopapain and antioxidant nutrients found inpapaya; including vitamin C, vitamins E, and beta-carotene, reduce the severity of the conditions such as asthma, osteoarthritis, and rheumatoid arthritis. (Aravind, Debjit et. al, 2013)

c. Rheumatoid Arthritis

Vitamin C-rich foods, such as papaya, provide humans with protection against inflammatorypolyarthritis, a form of rheumatoid arthritis involving two or more joints. (Aravind, Debjit et. al, 2013)

d. Promote Lung Health

Eating vitamin A rich foods, such as papaya, help the lung to be healthy and save life. (Aravind, Debjit et. al, 2013)

e. Anti-Sickling Activity

Current research proves that papaya is having an anti-sickling activity. (Aravind, Debjit et. al, 2013)

f. Prevent Prostate Cancer

Men consuming lycopene-rich fruits and vegetables such as papaya, tomatoes, apricots, pink grapefruit, watermelon, and guava were 82% less likely to have prostate cancer compared to those consuming the least lycopene-rich foods. (Aravind, Debjit et. al, 2013)

G. Anticoagulant Effect

Injection of papian extract in a dog increases prothrombin and coagulation threefold. It is alsoclaimed that the enzyme eliminates necrotic tissues in chronic wounds, burns and ulcers.Papain is also of commercial importance in the brewery industry, in the food industry and in the textile industry. (Aravind, Debjit et. al, 2013)

H. kidney failure:

Papaya seed extract may have in toxicity-induced kidney failure. Evidently a kidney-transplant patient in London was cured of a post-operative infection by placing strips of papaya on the wound for 48 hours. Women in India, Bangladesh, Pakistan, Sri Lanka, and other countries have long used green papaya as an herbal medicine for contraception and abortion. Enslaved women in the West Indies were noted for consuming papaya to prevent pregnancies and thus preventing their children from being born into slavery. (Aravind, Debjit et. al, 2013)

i.Immunomodulatory:

The involvement of oxidative stress mechanisms in several biological and pathological processes including ageing, cancer, cardiovascular and neurodegenerative diseases has continued to fuel suggestions that processes can potentially be modulated by treatment with free-radical scavengers and antioxidant. The fermented papaya preparation has shown its ability to modulate oxidative DNA damage due to H_2O_2 in rat pheochromocytoma cells and protection of brain oxidative damage in hypertensive rats. It has also exhibited potential supportive role on oxidative inflammatory damage in cirrhosis caused by hepatitis C virus. The safety and antioxidative stress potential of papaya juice is found to be comparable to the standard antioxidant compound alpha tocopherol. The preparation containing yeast fermented papaya as one of the constituent has antioxidant actions and that it may be prophylactic food against age related and neurological diseases associated with free radicals.

Bacteriostatic activity of papaya could be correlated to its scavenging action on superoxide and hydroxyl radicals, which could be part of the cellular metabolism of such enteropathogens. Bio-catalyzer, which contains yeast fermented papaya, may be useful as health foods against neural lipid peroxidation, traumatic epilepsy and ageing. Consumptions of guava and papaya fruits reduce oxidative stress and alter lipid profile. Thus, it could reduce the risk of disease caused by free radical activities and high cholesterol in blood.

(Krishna, Paridhavi et al, 2008)

1.8.4: Industrial uses:

Papaya is primarily a fresh-market fruit, and is used in drinks, jams, pectin, candies and as crystallised fruit. Green fruit may be cooked as a vegetable, as may the leaves, flowers and roots Papaya has several well-known industrial uses, notably for the enzyme papain (one of its four major constituent cysteine proteinases) which has properties similar to gastric pepsin. Producers induce latex to exude from longitudinal incisions made into unripe fruit; the papain purified from the extract is used in foods, beverages, pharmaceuticals, and other manufacturing For example, the food industry uses papain in brewing, manufacturing baby food, and producing proteins for human and animal consumption. Papain is also used to shrinkproof wool and silk, and in the bating process to make leathers more pliable. For someapplications however, synthetic enzymes and enzymes from other sources are displacing the use of the natural papain. The latex from papaya has been used in manufacture of chewing gum. Oil from the fruit's many (200-1000) more or less spheroidal seeds (c. 2-5 mm × 3.5-6 mm) and other components of fruit and leaves have been used in cosmetics and soap. (Aravind, Debjit et. al, 2013)

1.8.5: Cosmetic Benefits of Papaya

Rubbing the white pulp of raw papaya improves pimples as well as wrinkles. Papaya works as a good bleaching agent. It is an important ingredient in bath soaps, astringents, detergent bars and hand washes.Home Recipe for Papaya Skin Lightner Experts suggest that papaya can help in removing dead worn-out skin cells and replace it with healthy new cells, thereby lightening the color of our skin.(Aravind, Debjit et. al, 2013)

1.9.1:Allergies and Side Effects

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. 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 ratsand 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) (Aravind, Debjit et. al, 2013)

a. Toxicity

Externally the papaya latex is an irritant to the skin and internally it causes severe gastritis. Some people are allergic to various parts of the fruit and even the enzyme papain has its negative properties. (Aravind, Debjit et. al, 2013)

b. Skin Discoloration

Eating too much of a yellow, green or orange colour food that contains beta carotene cancause a benign form of skin discoloration calledcarotenemia. The palms of the hands and soles of the feet are the most visible areas of the bodyaffected by carotenemia. (Aravind, Debjit et. al, 2013)

c. Free Radical Scavenging Acivity

Papaya has many phenolic groups which mayscavange free radicals. Aqueous extract of papayaleaves shows anti-oxidant activity. (Aravind, Debjit et. al, 2013)

d. Respiratory Distress

Papain is also a potential allergen, according toPurdue University, people who eat too muchpapaya and ingest high levels of papain maydevelop symptoms consistent with hay fever orasthma, including wheezing, breathing difficulties and nasal congestion. (Aravind, Debjit et. al, 2013)

e. Gastrointestinal Symptoms

Ironically, the same papain that calms yourstomach can cause an upset stomach when takenin large amounts. The high fiber content ofpapaya can also contribute to unrest of thedigestive system. The latex of the fruit's skin canalso cause irritation of the stomach. (Aravind, Debjit et. al, 2013)

Chapter2:

Literature Review

2.1 Phytochemical constituents of *Carica papaya*:

Chemical tests were carried out qualitatively on the extracts and on the powdered specimens using standard procedures to identify the amino acids and phytochemical constituents as described by Edeoga et al. (2005); Sofowara (1993); Trease and Evans (1996); Harborne (1973) with little modification. The yield of extract was 5.78 and 10.87% for *C. papaya*. Qualitative phytochemical screening for *C. papaya* and *P. nigrescens* leafextracts were found to contain alkaloids, flavonoids, glycosides, tannins, saponins and anthraquinones.

2.2 Micronutrient determination of *Carica papaya*

Micronutrient determination test was carried out for the presence of minerals –copper, iron, magnesium, manganese and zinc were done following standard procedures via atomic absorption spectroscopy as described by (Okwu and Josiah, 2006) with little modification. Proximate analysis of the plants showed that all the macronutrients were present, with carbohydrate being the most abundant in *C. papaya.* Vitamins A, C, B12 and Folic acid were present but are void of Vitamin E. Papaya leaf extract contained only magnesium, the other metals tested were not detected. (Okwu and Josiah, 2006)

2.3 Cancer cell growth inhibition of *Carica papaya*

Recent research on papaya leaf tea extract has demonstrated cancer cell growth inhibition. It appears to boost the production of key signalling molecules called Th1-type cytokines, which help regulate the immune system. (Aravind, Debjit et. al, 2013)

2.4 Cure of Dengue fever using *Carica papaya*:

Commencing on studies of Dr. Sanath Hettige, who conducted the research on 70 dengue fever patients; said papaya leaf juice helps increase white blood cells and platelets, normalizes clotting, and repairs the liver. (Aravind, Debjit et. al, 2013)

2.5Antimalarial and Antiplasmodial Activity of *Carica papaya:*

Papaya leaves are made into tea as a treatment for malaria. Antimalarial and antiplasmodial activity has been noted in some preparations of the plant but the mechanism is not understood and not scientifically proven. (Aravind, Debjit et. al, 2013)

2.6 Anthelmintic activity of *Carica papaya* latex against patent Heligmosomoides polygyrus infections in mice:

Research shows the possible anthelmintic activity of papaya latex (Carica papaya) against Heligmosomoides polygyrus in experimentally infected mice. Five groups of BALB/C mice were treated with the latex of papaya. The papaya latex showed an antiparasitic efficacy in five groups of mice. The results may suggest a potential role of papaya latex as an anthelmintic against patent intestinal nematodes of mammalian hosts. (Satrija F, Nansen P, 1995)

2.7 Antifungal and antibacterial activities of aqueous and methanolic root extracts of *Carica papaya* linn. (Caricaceae):

The vast potentialities of plants as a source for anti-microbial drugs with reference to antibacterial agent motivated the present systematic investigation to screen the aqueous and methanolic root extracts of *Carica papaya* for its antimicrobial activity. Eleven microorganism species consisting of seven bacteria and four fungi were tested for their sensitivity to the herbal preparations using the Agar Diffusion method. Ampicillin and tetracycline were used as standard drugs for investigating the bacterial species, while griseofulvin was selected for the fungi, while zones of inhibition were measured to determine the microbicidal property of the test agents. Another set of plates was cultured to estimate the effect of combination therapy using the herbal drug together in varied concentrations with the standard drugs. The results obtained showed both extract to possess good antimicrobial activity against only four of the bacteria and three fungi. However, the organic preparation produced a significant and better efficacy than the water preparation. Combination therapy revealed a synergistic effect between CPY and ampicillin, whereas, antagonism was observed with tetracycline. A wide range of secondary metabolites were identified in both extract with methanolic extract containing a higher amount; **(**Adejuwon A.O, Agbaje E.O et. al, 2011).

2.8 Preliminary Research on papaya against kidney failure:

Papaya seed extract may have in toxicity-induced kidney failure. Evidently a kidney-transplant patient in London was cured of a post-operative infection by placing strips of papaya on the wound for 48 hours. Women in India, Bangladesh, Pakistan, Sri Lanka, and other countries have long used green papaya as an herbal medicine for contraception and abortion. Enslaved women in the West Indies were noted for consuming papaya to prevent

pregnancies and thus preventing their children from being born into slavery. (Aravind, Debjit et. al, 2013)

2.9 Bioactivity of *Carica papaya* (Caricaceae) against Spodoptera frugiperda (Lepidoptera: Noctuidae):

A recent research have shown the composition of a chloroform seed extract of C. papaya was determined by GC-MS. Nineteen compounds were identified, with oleic (45.97%), palmitic (24.1%) and stearic (8.52%) acids being the main components. The insecticidal and insectistatic activities of the extract and the three main constituents were tested. The seed extract gave effect against insect. (Perez, Zavala, et al. 2011)

2.10 Isolation and characterization of the four major cysteine-proteinase components of the latex of Carica papaya L.

High-quality spray-dried latex of Carica papaya L was fractionated by using SP-Sephadex-C50. The four major cysteine-proteinase components—papain, chymopapains A and B and papaya peptidase A—were isolated and characterized by protein chemical methods and by study of their thiol groups using2,2′-dipyridyl disulfide as a two-protonic-state titrant and reactivity probe. Papain and papaya peptidase A each contain one thiol group/molecule, which in each case is part of the catalytic site, as evidenced by high reactivity toward2, 2′-dipyridyl disulfide in acidic media. Chymopapains A and B each contain two thiol groups/molecule, only one of which is essential for catalytic activity. (Baines, Brocklehurst, 1982)

2.11 Genotoxic and Cytotoxic Safety Evaluation of Papain (Carica papaya L.)

This work evaluated the toxic and mutagenic potential of papain and its potential antioxidant activity against induced- H2O2 oxidative stress in Escherichia coli strains. Cytotoxicity assay, Growth inhibition test, WP2-Mutoxitest and Plasmid-DNA treatment, and agarose gel electrophoresis were used to investigate if papain would present any toxic or mutagenic potential as well as if papain would display antioxidant properties. Papain exhibited negative results for all tests. This agent presented an activity protecting cells against H2O2-induced mutagenesis. (Caludia, Marcia et al. 2010)

2.12 Aqueous extract of Carica papaya leaves exhibits anti-tumor activity and immunomodulatory effects

The research showed the significant growth inhibitory activity of the CP extract on tumor cell lines. In PBMC, the production of IL-2 and IL-4 was reduced following the addition of CP extract, whereas that of IL-12p40, IL-12p70, IFN-gamma and TNF-alpha was enhanced without growth inhibition. In addition, cytotoxicity of activated PBMC against K562 was enhanced by the addition of CP extract. Moreover, microarray analyses showed that the expression of 23 immunomodulatory genes, classified by gene ontology analysis, was enhanced by the addition of CP extract. In this regard, CCL2, CCL7, CCL8 and SERPINB2 were representative of these upregulated genes, and thus may serve as index markers of the immunomodulatory effects of CP extract. Finally, it was identified that the active components of CP extract, which inhibits tumor cell growth and stimulates anti-tumor effects, to be the fraction with M.W. less than 1000. (Otsuki, Dang et al, 2009)

2.13 Antioxidant analysis of different parts of *Carica papaya*:

(Maisarah, Nurul 2012) reported a study on Carica papaya for its antioxidant activity, its phenolic content using the different parts of papaya. The total phenolic content of the extracts was determined by Folin-Ciocalteu method and antioxidant activity was assayed using DPPH method. The total phenolic contents and antioxidant activity of the extracts as Gallic acid equivalents were found to be highest in fresh extract.

2.14 Protective effect of *Carica papaya* L leaf extract against alcohol induced acute gastric damage and blood oxidative stress

The effects of Carica papaya leaf (CPL) aqueous extract on alcohol induced acute gastric damage and the immediate blood oxidative stress level were studied in rats. The results showed that gastric ulcer index was significantly reduced in rats pretreated with CPL extract as compared with alcohol treated controls. The in vitro studies using 2, 2-Diphenyl-1-Picryl-Hydrazyl (DPPH) assay showed strong antioxidant nature of CPL extract. Biochemical analysis indicated that the acute alcohol induced damage is reflected in the alterations of blood oxidative indices and CPL extract offered some protection with reduction in plasma

lipid peroxidation level and increased erythrocyte glutathione peroxidase activity. *Carica papaya* leaf may potentially serve as a good therapeutic agent for protection against gastric ulcer and oxidative stress. (Indran, Mahmood et al, 2008)

2.15 Antisickling Properties of *Carica papaya* Linn.

Present study deals with the antisickling properties of *Carica papaya* (Family-Caricaceae) fruit pulp in distilled water, methanol and chloroform using sodium metabisulphite sickled red blood cells. The highest antisickling potencies of 87% inhibitory and 74% reversal activities were obtained from the 5-day fermentation products at the optimum concentration of 2.5mg/ml. The methanol extract gave 64% inhibitory and 55% reversal activities while the chloroform extract was inactive. The amino acids, phenylalanine, tyrosine and glycine already reported in the unripe fruit of *Carica papaya*, which are the possible antisickling components and responsible for their antisickling activity. (Ogunyemi, Elujoba et al, 2008)

2.16: Summary of Literature review:

Table2.1: Summary of Literature review

Activity	Plant Parts	References
Anti Cancer	Leaf	(Aravind, Debjit et. al, 2013)
Dengue Fever	Leaf	(Aravind, Debjit et. al, 2013)
Antimalarial and Antiplasmodial	Leaf	(Aravind, Debjit et. al, 2013)
Anthelmintic	Latex	(Satrija , Nansen , 1995)
Kidney Failure	Seed	(Aravind, Debjit et. al, 2013)
Insectiside	Seed	(Pérez, Zavala, et al. 2011)
Anti-Tumor	Leaf	(Otsuki, Dang et al, 2009)
Antioxidant	Whole Plant	(Maisarah, Nurul 2012)
Gastric damage and blood oxidative stress	Leaf	(Indran, Mahmood et al, 2008)
Antisickling	Fruit	(Ogunyemi, Elujoba et al, 2008)

Chapter 3:

Materials and Methods

3.1 Preparation of Plant extraction for Experiment

3.1.1 Collection and identification of *Carica papaya*

The whole plant was collected from Sylhet and Uttara, Dhaka in April 2013 during rainy season when weed beds were in their maximum densities. The whole plant with leaves, stems and roots was collected.

3.1.2 Drying of the parts of*Carica papaya*

The plant was washed with water. Roots were discarded and the aerial parts were sliced into small pieces and spread in thin layers in trays and dried the leaves at room temperature for 3 days. Then the aerial parts were dried in hot air oven at 50 °C for 3 days and at 40 °C for the next 4 days.

3.1.3 Grinding and storage of the dried samples

The dried parts were ground to coarse powder with the help of blender. This process breaks the plant parts to smaller pieces thus exposing internal tissues and cells to solvents thus 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 weight of the total dry powder was 300.18 g.

3.1.4 Extraction of the dried powdered sample

The fine powder of papaya leaves was dissolved in 200ml methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept for 7 days in a cabinet and frequently it was shaken to dissolve the powder properly. After 7 days the powder of papaya leaves was filtered using cotton and filter paper. Then it was kept in rotary evaporator for complete evaporation of the solvent. The solution was also kept in the hot plate and water bath and stirred frequently for solvent evaporation. After running this procedure, a gummy extraction was obtained which was preserved in refrigerator.

3.2 Pharmacological Investigation of *Carica papaya*

3.2.1 Tests for antioxidant activity

3.2.1.1 Total antioxidant capacity

Total antioxidant capacity of the plant extractives was determined following the Method described by Prieto et al. (1999).

Reagents and chemicals

Table 3.1: List of reagents used in total antioxidant capacity test and their sources.

Reagents and chemicals:

Reagent	Sources
1. Folin-Ciocalteu Reagent (FCR).	Merck, Germany
2 Sodium Carbonate	E. Merck (India) limited
3. Methanol	Merck, Germany
4. Gallic Acid	Sigma Chemicals, USA

Principle:

The content of total phenolic compounds in plant methanolic extracts was determined by Folin-Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. The extract chemical nature of the FC reagebt is not known, but it is believed to contain heteropolyphsphotunstates-molybdates. Sequences of reversible one or two electron reduction reactions lead to blue species, possibly $(PMoW11O40)^{4-.}$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI):

$$Mo\ (VI) + e \rightarrow Mo\ (V)$$

3.2.1.2 Method:

Preparation of 7.5% sodium carbonate solution:

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

10% Folin-Ciocalteu Reagent preparation:

10ml of Folin reagent+ 90 ml of methanol

Prepartion of Blank:

5ml folin+ 4ml Sodium Carbonate+ 1 ml methanol

Preparation of Standard Solution:

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

Table 3.2:Preparation of Standard Solution

No. Of Test Tubes	Concentration (µg/ml)	Solution taken from stock solution	Adjust the volume by methanol	Final Volume
1.	250	250µl	4.75ml	5ml
2.	200	200µl	4.80ml	5ml
3.	150	150µl	4.85ml	5ml
4.	100	100µl	4.90ml	5ml
5.	50	50µl	4.95ml	5ml

Preparation of Extract Solution:

Papaya Plant extracts of weight 0.025gm was taken and dissolves into 5 ml of methanol. The concentration of this solution was 5µg/ µl of Plant extract.

Table 3.3:Preparation of Extract Solution

No. Of Test Tubes	Concentration (µg/ml)	Solution taken from sample solution	Adjust the volume by methanol	Final Volume
1.	250	250µl	4.75ml	5ml
2.	200	200µl	4.80ml	5ml
3.	150	150µl	4.85ml	5ml
4.	100	100µl	4.90ml	5ml
5.	50	50µl	4.95ml	5ml

Experimental Procedure:

1. 1 ml of each plant extract and standard (Gallic acid) in different concentrations were taken in test tubes and 5 ml of Folin–ciocalteu (Diluted 10 fold) reagent solution was added into the test tubes.
2. 4 ml of sodium carbonate solution was added into test tubes.
3. The test tubes were incubated for 30 minutes at 20^0C to complete the reaction. (Only for standard).
4. The test tubes were incubated for 1 hour at 20^oC to complete the reaction (only for extract).
5. The absorbance of the solutions were measured at 765 nm using a spectrophotometer against blank.
6. The total content of phenol compounds in plant extracts in Gallic acid equivalents (GAE) were calculated by the following formula equation:

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

   Where:

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

   c = concentration of gallic acid obtained from calibration curve (mg/ml),

   V = the volume of the sample solution (ml)

   m = weight of the sample (g).

3.3 Antimicrobial screening of *Carica papaya leaf*

3.3.1. Principle:

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. 1.1 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 millimetre (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).

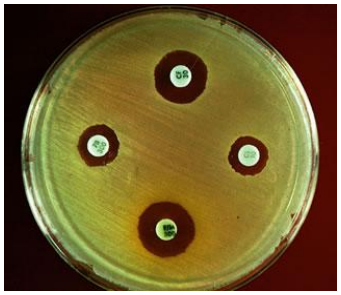


Fig 3.1: Disc diffusion method

3.3. 1.2: Experimental Work:

Materials Required:

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

Bacterial Culture: Bacterial strain

Reagents:

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

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)

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

3.3.1.3Test Organisms:

The microbial strains used for the experiment were collected as pure cultures from University of Dhaka. Both gram positive, gram-negative bacteria and fungi were taken for the test listed.

Culture Medium and their composition:

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

3.3.1.4. Methods:

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.

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.

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.

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.

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.

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.

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.

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.

Preparation of Discs:

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.

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.

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.

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.

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.

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 testmaterials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

Chapter 4:

Result and Discussion

4.1Result and Discussion:

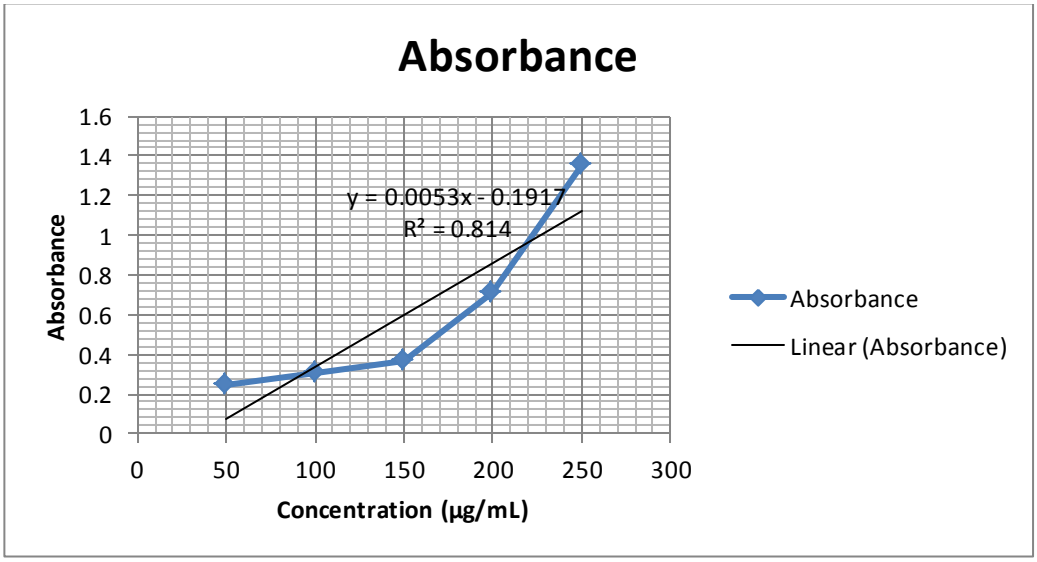
4.1.1Antioxidant test with total phenol:

Total phenolic contents of the methanolic fractions of the leaf of *Carica papaya* were determined by using the Folin-Ciocalteu reagent and were expressed as gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of gallic acid and papaya leaf.

Table 4.1: Antioxidant test with total phenol:

Concentration (µg/mL)	Absorbance of Gallic Acid (Standard)	Absorbance of Papaya extract:
250	1.354	1.085
200	0.714	0.586
150	0.368	0.280
100	0.305	0.221
50	0.244	0.164

Blank: 0.222



Concentration (µg/mL)	Absorbance	Best Fit Equation	R2 Value	X value(total phenolic content in (mg/g)
250	1.354	Y=0.005x-0.191	0.814	255
200	0.714			155.4
150	0.368			94.2
100	0.305			82.4
50	0.244			71

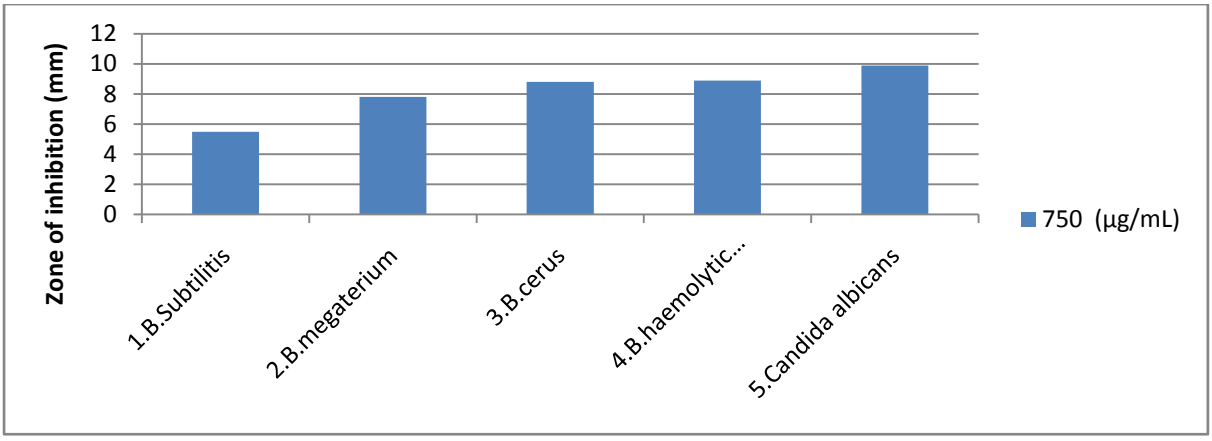
4.1.2Antimicrobial screening:

The results of antibacterial sensitivity of methanolic extract of *Carica papaya* leaf by disc diffusion method are in depicted below graph and table. The results reveal that the extract has Antimicrobial activity against all the pathogenic organisms studied. The antibacterial activity was screened from the zone of inhibition. The emergence of antibiotic resistance has its roots in the injudicious use of antibiotics and the subsequent transfer of resistance genes and bacteria among animals, animal products and environment.

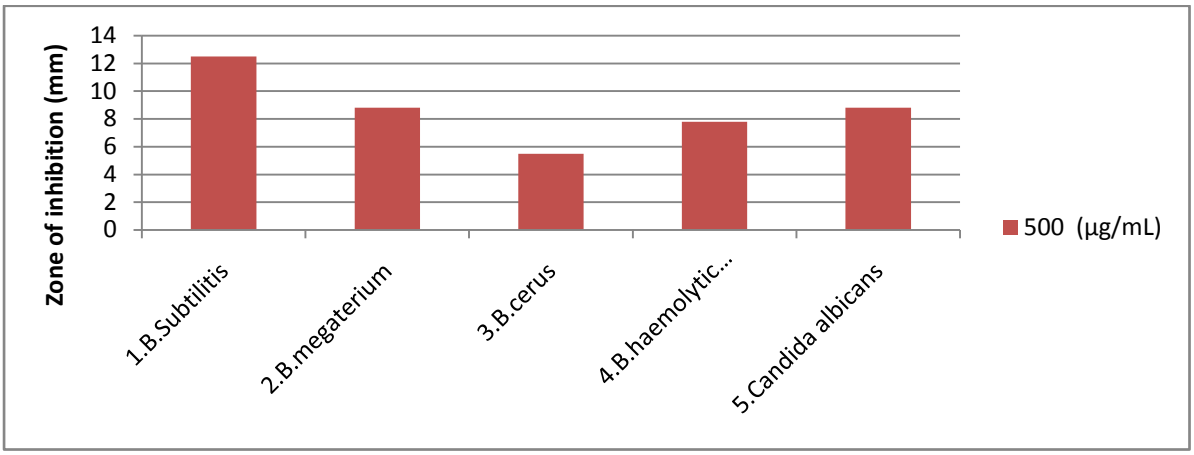
Table4.2: Antimicrobial Test for Carica Papaya (zone of inhibition in mm):

Kanamycin (30µg/disc); Carica papaya extracts (50µg/disc, 100/disc, 150µg/disc)

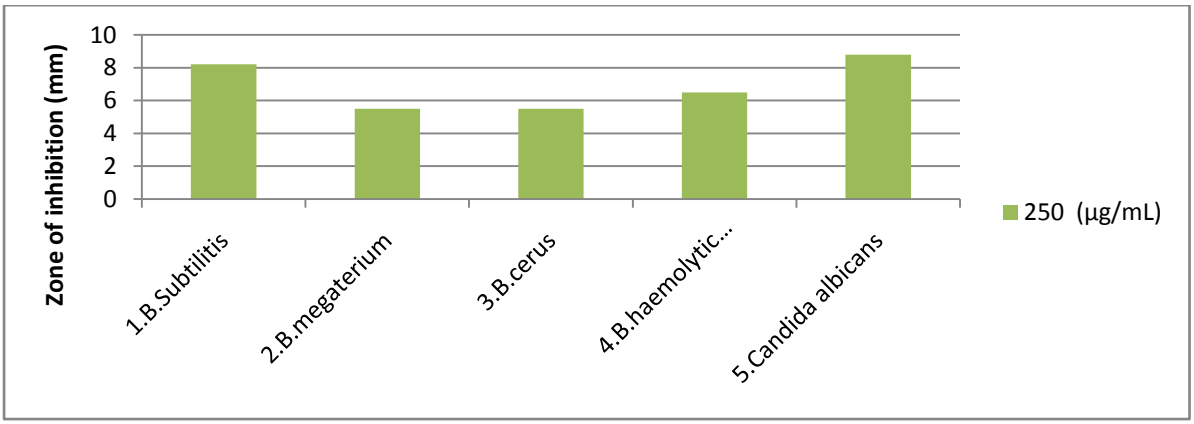
Type	Microorganisms	Zone of inhibition (mm)			
		150 (µg/disc)	100 (µg/disc)	50 (µg/disc)	Kanamycin (30 µg/disc)
Gram positive	*1.B.Subtilitis*	5.5mm	12.5mm	8.5mm	32
Gram positive	*2.B.megaterium*	7.8mm	8.8mm	5.5mm	33
Gram positive	*3.B.cerus*	8.8mm	5.5mm	5.5mm	33
Gram positive	*4.B.haemolytic streptococcus*	8.9mm	7.8mm	6.5mm	32
Fungi	*5.Candida albicans*	9.9mm	8.8mm	8.8mm	33



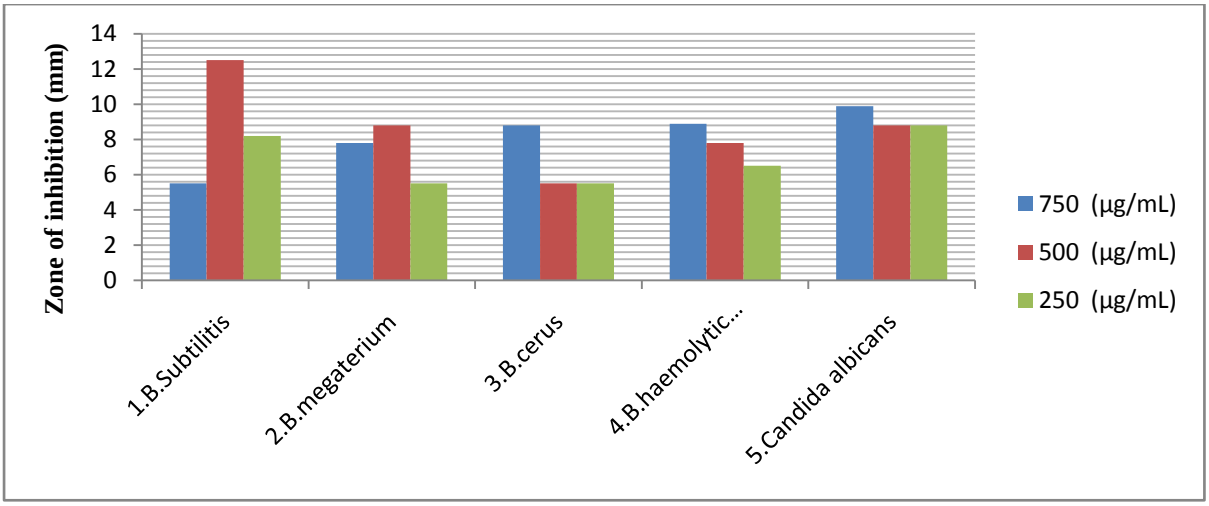
Comparison of Different microorganisms at the conc. of 150µg/disc



Comparison of Different microorganisms at the conc. of 100µg/disc



Comparison of Different microorganisms at the conc. of 50µg/disc



Comparison of Different microorganisms at different concentrations

4.1.3 Discussion:

The constituent of the extract of *C. papaya* (dried) leaves contain compounds and micronutrients which may be responsible for its observed antioxidant activity. This study suggests that the plant possesses antioxidant activities which can counteract the oxidative damage. The total phenol test provides information on the reactivity of the plant extract with a stable free radical. It gives a strong absorption band. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The crude extract of *Carica papaya* appeared to be as potent as Gallic Acid with maximum inhibition.

The extract is found to have broad-spectrum antibacterial activity and also used as analgesics and narcotics for pain relief. A report indicate that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria while that of the leaf extract of *C. papaya* was next to the most sensitivity with the Gram-negative bacteria especially *Proteus mirabilis* (Jigna and Sumitra, 2006). The activity of the extract is comparable to those of antibiotics. The demonstration of activity against the test bacteria provides scientific bases for the local usage of the plant in the treatment of various ailments. The fact that the extract is active against Gram-positive bacteria and Fungi tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms.

4.1.4 Conclusion:

Carica papaya is a neutraceutical plant having a wide range of pharmacological activities. The whole plant has its own medicinal value. The wide range of enzymes, vitamins present in *Carica papaya* makes it a neutraceutical plant.

Antioxidant and antimicrobial properties of methanolic extract of *Carica papaya* has recently been of great interest in both research and food industry, because of its possible use as natural additives which emerged from a growing tendency to replace synthetic antioxidants with natural ones. Owing to the antioxidant and antibacterial activities exhibited by the leaf extract investigated in this study, it could be considered a natural herbal source that can be used in food and pharmaceutical industries. However, further studies are needed to obtain purified compounds that may be responsible for the activities observed from the tested leaves.

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