PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY OF THE LEAVES OF PLANT *Carica papaya* (FAMILY: CARICACEAE)

A Thesis

Submitted In Partial Fulfillment of The Requirements for the Degree of

MASTER OF PHARMACY

Submitted by

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EXAM ROLL NO: M4PHP24007

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2024

CERTIFICATE OF APPROVAL

This is to certify that the research work embodied in this thesis entitled **"Pharmacognostical, Phytochemical and Pharmacological study of the leaves of plant *Carica papaya* (family: caricaceae)"** was carried out by **Sougata Dey**, **(Exam Roll No: M4PHP24007, Registration No: 163686 of 2022-2023** for the partial fulfillment of the requirements for the degree of **Master of Pharmacy**, Jadavpur University, is absolutely based upon her own research project work under my supervision, in the Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032.

She has incorporated her findings into this thesis. This thesis has not been submitted before for any degree/diploma or other academic award elsewhere. I am satisfied that she has carried out this thesis work with proper care and confidence to my satisfaction.

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DECLARATION

I hereby declare that the thesis contains literature survey and the original research work entitled "**Pharmacognostical, Phytochemical and Pharmacological study of the leaves of plant *Carica papaya* (family: caricaceae)**" has been carried out by me under the guidance of Prof. (Dr.) Subhash C. Mandal, Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032. The work is original and has not been submitted in part or in any form to any other university for the award of Degree or Diploma or Fellowship. The result reported here are the findings of work carried out by me.

I also declare that, as required by the rules and conduct, I have fully cited and referenced all the materials and results that are not original to this work.

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Sougata Dey

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CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

Abbreviation	Full form
%	Percentage
%	Percentage
ml	Millilitre
CPLE	*Carica papaya* leaves extract
g	Gram
Fig.	Figure
Sl.No.	Serial number
cm	Centimeter(s)
DPPH	2,2-diphenyl-1-picryl hydrazyl
GAE	Gallic acid equivalent
kg	Kilogram
mg	Milligram(s)
mM	Millimolar
QE	Quercetine equivalent
pH	Potential of hydrogen
v/v	Volume by volume
w/v	Weight by volume
µl	Micro liter
TLC	Thin Layer Chromatography
Hb	Haemoglobin
STD	Standard
Rf	Retention factor
ABTS	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
FRAP	Ferric reducing ability of plasma
TPC	Total Phenolic Content
TFC	Total Flavonoid Content

$scCO_2$	Supercritical Carbon di-oxide
DNS	Dinitro salicylic acid
RSC	Radical scavenging capacity

1. INTRODUCTION

Pharmacological, phytochemical, and pharmacognostic analyses are crucial to the study of natural products, particularly medicinal plants. Understanding the botanical traits, chemical composition, and biological functions natural compounds, these evaluations are important. To provide accurate identification and quality control, pharmacognostic assessment entails the identification and characterisation of medicinal plants based on their morphological, anatomical, and histological properties.

The goal of phytochemical assessment is to analyse plant extracts in order to identify and measure bioactive components that contribute to the medicinal qualities of the plants, such as alkaloids, glycosides, flavonoids, terpenoids, tannins, and phenolic acids [2]. The pharmacological properties of these substances, which include antibacterial, anti-inflammatory, antioxidant, and anticancer actions which are often studied [3]. In vivo and *in vitro* investigations are done in pharmacological evaluations to investigate the mechanism of actions and biological activities of plant extracts or isolated chemicals [4]. These investigations seek to identify novel phytochemical compounds and confirm the conventional use of medicinal plants. Conjointly, these evaluations provide a thorough understanding and knowledge of the therapeutic possibilities of natural products, in relation with traditional use with cutting edge scientific methods for drug discovery and development.

The tropical fruit tree Carica papaya, or papaya, is indigenous to the Americas. Scientific research has focused on its leaves, roots stem, flower, seeds, stalks, fruits for different possible therapeutic benefits. Rich in bioactive constituents, including phenolic compounds, alkaloids, flavonoids and enzymes like chymopapain and, papain are found in papaya leaves [5, 6] It is believed that these components have ampoules of pharmacological and therapeutic activity. Research has indicated that papaya leaf extracts show noteworthy anti-inflammatory and antioxidant [6, 7], anti-diabetic, anti-thrombotic properties. These characteristics have a critical role in mitigating inflammation, oxidative stress, Diabetic, which are nowadays becoming most common diseases.

1.1 Inflammation and Anti-inflammatory agent

Inflammation is a protective response, we feel pain in respond to injury or infection characterised by redness, burn, swelling, heat. An anti-inflammatory agent is a substance that reduces inflammation or swelling in the body, those work by inhibiting the processes or mediators that contribute to inflammation.

Anti-inflammatory agents:

According to the National Center for Biotechnology Information 2021, An anti-inflammatory agent is a class of medication or substance that acts by decreasing inflammation, which is a phenomenon marked by pain, heat, swelling and redness. These medicines alleviate the symptoms of inflammatory diseases by preventing the body from producing or absorbing pro-inflammatory chemicals.

Table 1. Plants having anti-inflammatory property are following:

Plant Name	Scientific Name	Active Compound
Turmeric	*Curcuma longa*	Curcumin [8]
Ginger	*Zingiber officinale*	Gingerols [9]
Green Tea	*Camellia sinensis*	Epigallocatechin gallate (EGCG) [10]
Boswellia	*Boswellia serrata*	Boswellic acids [11]
White Willow Bark	*Salix alba*	Salicin [12]
Aloe Vera	*Aloe barbadensis miller*	Aloin, emodin [13]
Garlic	*Allium sativum*	Allicin [14]

Rosemary	*Rosmarinus officinalis*	Carnosic acid, carnosol [15]
Devil's Claw	*Harpagophytum procumbens*	Harpagoside [16]
Bromelain	*Ananas comosus*	Bromelain enzyme [17]
Frankincense	*Boswellia species*	Boswellic acids [18]
Cayenne	*Capsicum annuum*	Capsaicin [19]

1.2. Free radicals and anti-oxidants

An imbalance between synthesis of antioxidants in our body and reactive oxygen species (ROS) is referred to as oxidative stress. This imbalance can lead to cellular damage, which promote the aging process and causes a number of diseases.

When the body's antioxidant defences are overpowered by high quantities of ROS, a state known as oxidative stress results in damage to cells and tissues. Numerous chronic illnesses, like cancer, neurological problems, and cardiovascular disease are associated with it [20].

Anti-oxidants:

Reactive oxygen species (ROS) are chemicals that antioxidants neutralize and help shield cells from oxidative damage. They function by providing electrons to reactive oxygen species (ROS), which lessens their reactivity and prevents them from damaging lipids, proteins, and DNA in cells.

Table 2. Plants having anti-oxidant property and their scientific name

Plant Name	Scientific Name	Key Antioxidants
Blueberries [21]	*Vaccinium spp*	Anthocyanins, Vitamin C
Green Tea [22]	*Camellia sinensis*	Catechins, EGCG

Turmeric [23]	*Curcuma longa*	Curcumin
Goji Berries [24]	*Lycium barbarum*	Vitamin C, Zeaxanthin
Acai [25]	*Euterpe oleracea*	Anthocyanins, Flavonoids
Pomegranate [26]	*Punica granatum*	Punicalagins, Ellagic acid
Kale [27]	*Brassica oleracea var. sabellica*	Vitamin C, Beta-carotene

1.3. Diabetes and Anti-diabetics

Diabetes mellitus is a chronic disease characterized by elevated blood glucose levels due to defects in insulin secretion, action, or both. It can lead to serious complications if not managed properly. World Health Organization (WHO).

Diabetes mellitus, commonly referred to as diabetes, is a group of chronic metabolic disorders characterized by high blood glucose levels (hyperglycaemia) resulting from defects in insulin secretion, insulin action, or both. The condition is generally classified into two main types:

Type 1 Diabetes:

An autoimmune condition in which the body's immune system attacks and destroys the insulin-producing β-cells in the pancreas, leading to little or no insulin production. It often manifests in childhood or adolescence.

Type 2 Diabetes:

A condition typically resulting from a combination of insulin resistance (where the body's cells do not respond properly to insulin) and relative insulin deficiency. It is more common in adults but increasingly observed in children and adolescents. Lifestyle factors and genetic predisposition play significant roles.

Other Forms of Diabetes:

Gestational Diabetes: Diabetes that develops during pregnancy and usually disappears after delivery, but it increases the risk of developing type 2 diabetes later in life.

Table 3. Plants having anti-diabetic property

Plant Name	Scientific Name	Key Active Compounds
Bitter Melon	*Momordica charantia*	Charantin, vicine, polypeptide-p
Fenugreek	*Trigonella foenum-graecum*	4-hydroxyisoleucine, galactomannan
Gymnema	*Gymnema sylvestre*	Gymnemic acids
Cinnamon	*Cinnamomum verum*	Cinnamaldehyde, cinnamic acid
Aloe	*Aloe vera*	Acemannan, phytosterols
Ginger	*Zingiber officinale*	Gingerols, shogaols
Turmeric	*Curcuma longa*	Curcumin

2. PURPOSE OF RESEARCH

Purpose of this research is to find out Pharmacognostic property of the plant leaves, *Carica papaya*, Physico-chemical evaluation, Phytochemical screening, tincture preparation and characterization, *In-vitro* study of anti-inflammatory, anti-oxidant and anti-diabetic activities of the leaves extract in three different solvents like Petroleum ether and Acetone and methanol.

3. PLAN OF WORK

- Collection, drying and grinding of leaves
- Extraction of the plant leaves with three different solvents
- Authentication of the plant specimen
- Pharmacognostic study of the plant leaves *Carica papaya*
- Physico-chemical evaluation of the leaves powder
- Phytochemical screening of the leaves extract
- Chromatographic analysis of CPLE by TLC profiling
- Preparation and characterization of tincture
- Study of *In-vitro* anti-inflammatory property by HRBC membrane stabilization method.
- Study of *In-vitro* antioxidant property of CPLE with three solvents
  - Determination of DPPH radical scavenging activity
  - Determination of Hydrogen peroxide radical scavenging activity
- Study of *In-vitro* anti-diabetic property of CPLE with three solvents

4. PLANT DESCRIPTION

Synonym: *Carica carica*

Vernacular Name

English: Papaya, Melon tree

Hindi: Papita

Bengali: Pepe

Tamil: Pappali

Malayalam: Omakka

4.1. Taxonomical classification

Division: Magnoliophyta

Class: Magnoliopsida

Order: Brassicales

Family: Caricaceae

Genus: Carica

Species: Papaya L.

Botanical name- *Carica papaya*

Figure 1. *Carica papaya* plant

4.2. Morphology

Simple, spiral-arranged leaves on 30 to 70 cm long petioles that form a terminal cluster make up the morphology. The boundaries of the lobes may be well defined, gently sloped, or entirely distinct. The leaves have thick central irradiated veins and are smooth, fairly palm shaped, and extensively visible, measuring 25–75 cm in diameter. The leaves have a spherical shape, measure 60 to 90 cm in diameter, are placed alternately, are bundled at the tip of the stem and branches, and have long petioles. The bundle of leaves is characterized by reticulated vein and embedded off-white nerves that give it a vivid, dark green to yellow-green colour. The underside surface is opaque, pale green-yellow, and shows clear vascular systems; the petioles are spherical, yellow-green, fistulous, and delicate.

4.3. Distribution

The highly adaptable *carica papaya* developed in southern Mexico and northern Central America, yet it is now found in most tropical and subtropical areas of the world. Sub-Saharan Africa, Oceania, Australia, Pacific islands, South and Southeast Asia, Caribbean islands, South America, and Central America are among the major rising regions. In regions like Florida and Hawaii, papaya is cultivated both commercially and in the backyard.

Warm, tropical regions with temperatures between 20°C and 30°C (68°F and 86°F), high humidity, and well-drained, sandy loam soils are ideal for papaya cultivation. Brazil, Indonesia, and India are important producers; Brazil is well-known for both exports and internal consumption, while Indonesia is another significant producer. India is the greatest producer.

As papayas are easy to grow and bear fruit in a variety of climates, early explorers and traders are primarily responsible for the fruit's broad acceptance through commerce. The plant's widespread acceptance and adaptability have been major factors in its historical expansion.

4.4. Traditional Uses

Carica papaya, occasionally referred to as papaya or pawpaw, is a plant native to tropical and subtropical climates that has been extensively used in traditional Chinese medicine and other civilizations. The fruit, leaves, seeds, and latex of the plant have all been utilized in a range of traditional and alternative medical practices. Below is an overview of its conventional applications:

The fruit is high in antioxidants, papain-like enzymes, and vitamins, especially C. It has historically been consumed for its nutritive value and digestive advantages. Fresh consumption or incorporation into salads, smoothies, and desserts. The fruit is utilized to encourage general health and vigour in a variety of cultures [37]. Papaya leaves have been utilized in traditional medicine, particularly in tropical climates, to cure fever and malaria. Frequently, an extract or tea is made from the leaves. Conventional Use: Made as a tea or juice, then ingested to treat malaria and lower fever [38]. In the past, papaya leaves were also utilized to increase platelet count in diseases like dengue fever. To raise platelet counts, the leaves are prepared into a juice or decoction [39]. The seeds of papaya are traditionally used as a natural remedy for digestive issues. Eating ground seeds can help treat illnesses or parasites that cause stomach problems [40]. Due to its proteolytic enzyme activity, latex derived from papaya fruits is utilized in traditional medicine. Topically applied to the skin to cure acne, warts, and other skin disorders. Because of its proteolytic qualities, papaya latex is also traditionally used to promote wound healing. Applied to wounds to lessen inflammation and promote healing [41]. Papaya extracts have been traditionally used for their antibacterial qualities, as a home cure for skin diseases and infections [42].

4.5. Phytochemistry

Papaya is a fruit rich in vitamins C, A, and flavonoids, including beta-carotene, lycopene, polyphenols, flavonoids, phenolic acids, enzymes like papain, leaves, seeds, and seeds. It contains flavonoids, glycosides, and tannins, with flavonoids like quercetin and kaempferol having antioxidant and anti-inflammatory properties. Seeds contain essential oils like carica seed oil, fatty acids, and alkaloids like carpaine, which have been linked to cardiovascular health. Latex contains proteolytic enzymes like papain, glycosides, and

antidiabetic effects. Papaya roots also contain alkaloids, such as carpaine, which has potential antidiabetic effects.

4.6. Pharmacological activity

Carica papaya, rich in phytochemicals, has numerous pharmacological activities. Its antioxidant properties neutralize free radicals, reduce inflammation, and have antimicrobial properties. Papaya's leaves and seeds can help manage diabetes by lowering blood glucose levels and improving insulin sensitivity. Additionally, papain enzyme aids in protein digestion, alleviating digestive issues.

5. LITERATURE REVIEW

Zuhrotun *et al* Found the antioxidant activity of *Carica papaya* leaves, focusing on two varieties with high antioxidant potential and total flavonoid content. The leaves were extracted using methanol and tested with young and mature leaves. The antioxidant activity was measured using DPPH and FRAP. The golden and Grendel varieties had a higher proportion of radical scavenging properties, with 78.37% and 77.40%, respectively. Mature leaves had higher total flavonoid properties than immature ones. Grendel outperformed purple in terms of total flavonoid content and antioxidant activity. After water extraction, Grendel papaya leaves showed increased antioxidant activity. However, the flavonoid content was lower than methanol and 70% ethanol.

Zhang *et al*. studied by comparing papaya organ tissues, leaves, and flowers from nine cultivars was conducted. Three methods were used to determine antioxidant activities, including DPPH, ABTS, and FRAP assays. The total phenolic and flavonoid content was also examined. Results showed that the leaves and roots of papaya have higher antioxidant properties than all tested organs, with the Daqing cultivar showing the strongest antioxidant ability. This suggests papaya has potential as a natural antioxidant resource.

Banik *et al* Investigated that chloroform leaf extracts of C. papaya showed maximum antibacterial activity against Enterobacter aerogenes, while water and methanolic extracts were less effective. The extract showed high DPPH radical scavenging and H_2O_2 scavenging activity, making it a potential health-promoting constituent for therapeutic purposes.

Adedayo *et al*. Studied that hydromethanolic extract had the highest AChE inhibitory effect, while aqueous extract had the highest BChE inhibitory potential. The hydromethanolic extract was found to be the most potent due to its rich flavonoid content. The biological properties of the extract could be the underlying neuromodulatory mechanism of Pawpaw leaf's actions against neurological disorders.

Palanisamy *et al*. Analysed phytochemical screening revealed the presence of bioactive compounds such as alkaloids, carbohydrates, and amino acids, and TPC and TFC varied among the different solvent extracts, in which methanolic extracts showed the

highest number of phytochemicals and TPC and TFC and antioxidants compared to other solvents.

Yap *et al* Examined to extract and quantify carpaine from papaya leaves, revealing that young leaves had significantly higher amounts of carpaine than older leaves and stalks. Blending treatment significantly affected the amount of carpaine extracted from both leaves, with blended young leaves showing significantly more carpaine than unblended samples. The total polyphenol content (TPC) of young leaves was also higher than that of old leaves and stalks. The results showed a relationship between TPC and the 2, 2-diphenyl-1 picrylhydrazyl (DPPH) assay scavenging activities, with both young and old leaves showing significantly higher DPPH scavenging activities. The study recommends young papaya leaves as a potential source for carpaine extraction for future dengue treatment.

Khor *et al* Assayed C. papaya leaf $scCO_2$ extract with 5% ethanol (CPSCE) and C. papaya leaf $scCO_2$ extract (CPSC) showed stronger DPPH radical scavenging activity than conventional extracts. Hydrophilic extracts (CPEE and CPFD) showed stronger reducing power compared to lipophilic extracts. Cell-based assays showed that CPFD significantly protected skin fibroblasts from H_2O_2-induced oxidative stress. CPSCE had the highest α-tocopherol and squalene contents, while both hydrophilic extracts had higher total phenolic content and rutin content. Overall, CPEs extracted using green and conventional extraction methods showed potential antioxidative property.

Iordănescu *et al.* Aimed to assess the antioxidant activity of unripe and ripe papaya fruits using DPPH kinetics. The peel, pulp, seed, and seed pulp were extracted with ethanol and monitored at 517 nm in the presence of DPPH. The radical scavenging capacity (RSC) and DPPH reaction rates were determined. The highest RSC values were obtained for papaya pulp extracts, consistently higher for ripe samples (86.4% and 41.3%). The DPPH kinetic approach can be useful for a fast and simple evaluation of the overall antioxidant properties of fruit extracts.

Vuong *et al*. The study aimed to optimize extraction conditions and determine the impact of water extraction on polyphenol yield from papaya leaves. The optimal extraction conditions were 70°C for 20 minutes and a water-to-leaf ratio of 100:7.5 mL/g. Water extraction yielded higher polyphenols than organic solvents, while ethanol extract

provided the highest saponin level. The crude powder contained 6.3% polyphenols and had lower scavenging and total antioxidant activity.

Fadare *et al.* Evaluated the primary components of the male *Carica papaya* leaves and flowers were investigated using Fourier transform infrared spectroscopy (FTIR), which operates in the mid-infrared range of 4000–650 cm–1. The results showed that the FTIR spectrum is capable of differentiating and identifying different functional groups that are present in the pawpaw portions. Four water-soluble vitamins were analysed using HPLC: folic acid, thiamine, riboflavin, and niacin. High amount of folic acid was detected in flowers, but lower concentrations of niacin and folic acid were discovered in leaves. Elements including Cu, Ca, Cd, K, Na, Mg, Fe, Zn, and Ni were found using AAS analysis.

Khila *et al.* Found *carica papaya* contains therapeutic qualities that are used to cure a variety of illnesses. According to Ayurvedic literature, papaya leaf extract can boost platelet count and has haemostatic qualities. 21 elements, including Phyto chemicals, alkaloids, phenolics, flavonoids, and amino acids, were found in papaya leaf extract according to a study that used liquid chromatography-mass spectroscopy (LCMS). To find out active bio components for platelet augmentation, anticancer activity, acne activity, menstrual pain reduction activity, and nausea treatment, more research is needed.

Kadiri *et al*. Examined the antioxidant properties and phenolic components of *Carica papaya* Linn processed seed flour samples. The results showed a higher radical scavenging capacity and phenolic compounds with ferulic acid concentrations, suggesting potential use in the food and pharmaceutical industries for nutraceuticals.

6. MATERIALS AND METHOD

6.1. Collection and authentication of plant

In November 2023, leaves from the *Carica papaya* plant were collected from Jadavpur, West Bengal. For identification and verification, a specimen of the plant had been deposited in the Central National Herbarium at the Botanical Survey of India, Shibpur, Howrah. After being collected, the leaves were properly cleaned in water and allowed to dry for few weeks in shaded area. The dried leaves were mechanically processed into powder and stored for later use in an airtight container.

6.2. Morphological Character of Papaya leaves

The fresh leaves of the *carica papaya* were collected and subjected for evaluation of morphological characteristics like colour, size, shape, and other macroscopic characteristics. Other organoleptic characteristics including taste, odor and texture were also evaluated.

6.3. Microscopic Characteristics

Thin transverse sections of the fresh leaf lamina were cut in order to characterize the leaves microscopic characteristics. Glycerine was applied to mount the pieces on a glass slide, and cover slips used to protect them. 10X magnification, compound microscope was used to examine the internal anatomical features of the leaves. Leaves were ground with the help of mortar and pestle, the powder were separated from the larger fragments by passing it through a sieve. Next, a small amount of the powder was applied on a glass slide and glycerine was used to mount it. The glass slide was covered with a cover slip, and the powder characteristics were examined under a compound microscope at a 10X magnification.

6.4. Physicochemical Assessment

6.4.1. Loss on Drying (LOD)

2g of the powdered leaves were placed in a shallow, dried weighing bottle. The sample bed's height was kept 10 mm or less. The sample was then dried in a hot air oven at 105^{0} C for 30 minutes. Then, the shallow weighing bottle was taken out and cooled in a desiccator, and weighed at room temperature. The procedure was repeated until the

difference between two successive weights was less than 0.5 mg. The LOD was determined as a percentage of w/w.

6.4.2 Determination of ash values

Ash value is an essential parameter of a drug for the extent of adulteration and also establishes the quality and purity of the drug.

6.4.3. Determination of total ash values

After the ignition of medicinal plant yield, total ash constituting in which both physiological and non-physiological ashes were present. The drug was incinerated in a silica crucible at temperature which not more than 450°C, then was cooled and weighed to get the total ash content

6.4.4 Determination of acid-insoluble ash values

Sand and siliceous earth both forming acid insoluble ash represent. Ash is boiled with dil. HCl (6 N) for 5 min. After that, the insoluble matter collected on an ashless filter paper, rinsed with hot water, and ignited at a temperature which not more than 450°C to a constant weight.

6.4.5. Determination of water-soluble ash values

The ash was in dissolved distilled water after that the insoluble part of ash collected on an ashless filter paper which ignited at 450°C to a constant weight. The weight of soluble part of ash is noted by subtracting the weight of insoluble part from the ash.

6.4.6. Extractive Value

Extractive values can help to evaluate crude drug and chemical constituents present in them. It also helps to determine if a drug is adulterated or not.

6.4.6.1. Water-Soluble Extractive Value

Five grams of coarsely ground leaves were macerated with one hundred millilitres of water in a closed conical flask for a full day. Before being allowed to stand for 18 hours, the flask was repeatedly shaken for the first 6 hours. The entire mixture was filtered after a day, and 25 millilitres of the filtrate were measured and evaporated in a little porcelain plate. The measurement and recording of the residue on the porcelain plate was done. The

percentage (w/w) of water-soluble extractive value was computed with respect to the dried sample.

6.4.6.2. Alcohol (Ethanol) Soluble Extractive Value

In a closed conical flask, 5g of coarsely powdered leaves were macerated with 100 ml of ethanol for 24 hours. Alcohol soluble extractive value was determined in the same way as water soluble extractive value.

6.4.7. Analysis of Crude Fiber

Two grams of the dried leaf powder and 200 millilitres of 1.25% (v/v) sulfuric acid were heated together while being continuously stirred. Following heating, a filter paper was used to filter the mixture, and it was repeatedly cleaned with hot water. The filtrate was then boiled for 30 minutes with 200ml of a solution containing 1.25% (w/v) sodium hydroxide added. After the mixture was rinsed with hot water, it was filtered, and the filtrate was ignited at 110°C until it reached a consistent weight. The percentage of crude fiber was calculated using the dried sample.

6.4.8. Swelling Index

The mixture of 1g of dried powder and 25ml of water was placed within a 100ml measuring cylinder. The cylinder was shaken every ten minutes for an hour. After that, the measuring cylinder was stored for three hours. The mixture volume change was noted in order to determine the leaves' swelling index.

6.4.9. Foaming Index

500 millilitres of boiling water were placed in a conical flask, and 1 gram of the dried sample was added. After cooling, it was filtered into a volumetric flask. Water was added till 100 millilitres of content was there. acquired 10 test tubes with stoppers and labelled them. The drug was put into separate tubes in increments of 1 ml, 2 ml, and up to 10 ml. The leftover volume was then adjusted with water up to 10 ml. The stoppers were used to seal the tubes, and after shaking them for 15 seconds and letting them stand for another 15 seconds, the height of the foam in each tube was measured. The foaming index is less than 100 if the foam in each tub is less than 1cm high. The dried sample solution decoction volume in this tube is used to determine the index if the first through tenth tubes have a foam height of 1 cm each. If this is the first or second tube in a

series, perform an intermediate dilution to obtain a more accurate result. If the foaming index reaches 1000, the foam height in the first through tenth tubes is larger than 1 cm.

$$\textbf{Foaming index} = \frac{\mathbf{1000}}{\boldsymbol{a}}$$

Where, a is the amount of decoction in milliliters that was used to prepare the dilution in the tube where foaming to a height of one centimeter is seen.

6.5. Preparation of Extracts

The powdered leaves were extracted using a Soxhlet apparatus and the following solvents, in increasing polarity order: petroleum ether (boiling point 60–80°C) acetone (boiling point-56°) and methanol (boiling point 64°C). Powdered plant material (50g) was first extracted with petroleum ether. A sample was taken out of the syphon tube of the Soxhlet extractor to confirm that all of the contents had been extracted. The extraction process was continued until this was done. After that, it was assessed by TLC utilizing the suitable solvent from the extraction process. The completion of the extraction procedure was indicated by the lack of a spot on the TLC plate in the UV chamber. As seen in the entire Soxhlet extractor assembly.

Figure 2. Soxhlet apparatus

6.6. Phytochemical Screening

To identify the presence of various Phyto compounds, different chemical analyses were run on the three drug extracts, petroleum ether, acetone and methanolic extract.

6.6.1 Test for Carbohydrates

Molisch Test(general)

A few drops of the Molisch reagent were applied to 2–3 ml of the aqueous extract. Sulphuric acid in the amount of two ml was introduced from the test tube's side. Carbohydrates are present when a purple ring forms at the intersection of two liquids.

Test for Reducing Sugars

Fehling's Test

Boiling for one minute after combining 1 ml each of Fehling's solutions A and B. equal volume of the test solution was added. heated for five to ten minutes in a bath of boiling water. When reducing sugars are present, first yellow, then brick red ppt are seen.

Benedict's Test

In a test tube, combine benedict's reagent and test solution in an equal volume. 5 minutes of heating in a pot of boiling water. The colour of the solution depends on how much reducing sugar is present in the test solution; it might be green, yellow or red.

Test for Monosaccharide

Barfoed's Test

Combine Barfoed's reagent and test solution in an equal volume. heated in a bath of boiling water for one to two minutes. Red ppt is formed if monosaccharides are present.

For Non-Reducing Sugar

Test solution does not show positive results for Fehling's and Benedict's test.

Test for Proteins and Amino Acids

Biuret Test (general test)

1 ml of biuret reagent were added to 2 ml of test solution (extract). Protein is present when the colour is violet.

Ninhydrin Test (general test)

2 ml of test solution and 2 drops of 5% Ninhydrin solution were heated for 10 minutes in a boiling water bath. The test solution turns purple or bluish when amino acids are present.

Millon's Test

Combine 5 ml of Millon's reagent with 3 ml of test solution. White ppt is formed which turns brick red after warming if protein is present.

6.6.2 Test for Steroids

Salkowski's Test

Add 2 ml of chloroform and 2 ml of conc. Sulphuric acid to 2 ml of extract and thoroughly shaken. The appearance of a reddish-brown tint in the bottom layer is a sign of steroids.

6.6.3. Test for terpenoids

Noller's Test

Noller's reagent, which contains 0.1% stannic chloride in thionyl chloride, was applied to the extract solution. Terpenoids are present when red hue appears, indicating their existence.

6.6.4. Test for Glycosides

For Cardiac Glycosides

(For cardenolides) Legal's test

1 ml pyridine and 1 ml sodium nitroprusside were added to the extract. Glycosides are indicated by the colour pink.

Keller-Killiani test (for deoxy-sugars)

Glacial acetic acid, one drop of 5% FeCl3, and sulfuric acid were added to the 2 ml of extract. If glycosides are present, a reddish-brown colour occurs at the junction of the two liquid layers, while the upper layer appears bluish green.

Test for anthraquinone glycosides

Borntrager's test

Diluted sulfuric acid was added to 3 ml of extract. filtered and boiled. Chloroform was added in an equal amount to the cold filtrate. firmly shaken. Make the organic solvent separate. Added ammonia. Anthraquinone glycosides cause the ammoniacal layer to turn pink or crimson.

Modified Borntrager's test

5% FeCl3 and 5 ml of diluted HCl were added to 5 ml of extract. heated in a bath of boiling water for five minutes. Chloroform was added after cooling. firmly shaken. poured an equal proportion of diluted ammonia to the separate organic layer. The colouration of the ammoniacal layer is pinkish red.

6.6.5. Test for Saponins

Foam Test

Shake the water and powdered extract vigorously. Saponin is detected when there is persistent froth.

6.6.6 Test for Flavonoids

Shinoda test

5 ml 95% ethanol and a few drops of conc HCl were added to the dried extract. 0.5 gm of magnesium turnings was added to it. Flavonoid's give off an orange, pink, red, or purple tint when they are present.

Ferric Chloride Test

Added a few drops of 10% Fecl3 to the extract. It emits a green tint to show flavonoids are present.

Sodium hydroxide Test

Solution of sodium hydroxide was added to the extract. The presence of flavonoids is shown by the appearance of yellow hue, which is destroyed by the addition of acid.

6.6.7. Test for Alkaloids

Mayer's test

Mayer's reagent in a few drops with 2- 3 ml of filtrate results in a cream-coloured ppt.

Dragendorff 's Test

Add a few drops of Dragendorff's reagent to 2 to 3 ml of filtrate. Orange and brown ppt. is created.

Hager's test

With Hager's reagent, 2- 3 ml of filtrate produces yellow ppt.

Wagner's test

With Wagner's reagent, 2–3 ml of filtrate yields reddish brown ppt.

6.6.8. Test for Tannins and Phenolics

$Fecl_3$ test

Dark green or deep blue colouring after treatment with 5% Fecl3 solution suggests the presence of tannin and phenolics.

Gelatin Test

10% sodium chloride and 1% gelatin solution were added to the test solution. Precipitate formation suggests the presence of tannins.

10%NaOH test

When 4 ml of 10% NaOH solution is added to the extract solution and it is thoroughly agitated, an emulsion forms that shows tannins and phenolics are present.

6.7. Chromatographic analysis of the prepared extracts

Thin layer chromatographic (TLC) analysis was performed on three extracts prepared from the powdered leaves of the plant drug.

Preparation of a sample:

Dried petroleum ether, acetone and methanol extract, each weighing 1gm, were diluted in adequate quantity of the extraction solvents i.e., petroleum ether, acetone and methanol, and utilized as samples for TLC analysis.

Stationary phase

Pre-coated Silica gel G was used as stationary phase for performing TLC analyses of the extracts.

Mobile phase

For methanol extract

Observation

Retention factor (R_f) value for each of the spots was determined after the spots were observed in an UV chamber at wavelengths of 254 nm and 366 nm.

6.8. Preparation and characterization of tincture

A total of 21 amber-coloured bottles with 10 ml capacity were used. To these, 10 ml of alcohol with varied strengths—40, 50, 60, 70, 80, and 90 percent (V/V) and absolute alcohol were added. Each of the twenty-one bottles contained 1g of *Carica papaya* leaves powder. Each batch was allowed to macerate for a specific number of days: 7, 14, and 21. The materials were filtered through a sintered glass crucible (G4) while under reduced pressure after the required amount of maceration time. Physical characteristics were investigated, including colour, pH and total solid content [58, 59].

Figure 3. Tinctures of different alcohol concentration after maceration for different time period

6.9. In-*vitro* anti- inflmmatory studies

HRBC (Human red blood cell) membrane stabilization

Blood was collected from healthy volunteers, mixed with equal volume of Alsever's solution and the HRBC suspension was made. To 200µl of suspension equal volume of extract (0- 200 µg/ml) was added, incubated and centrifuged. Diclofenac sodium (Positive standard) and the control (without extract) was also treated similarly and simultaneously. The haemoglobin content was estimated at 560nm using a microplate reader [56].

The percentage protection was calculated as $\frac{(\textbf{Abs of blank} - \textbf{Abs of extract})}{\textbf{Abs of control}} \times \mathbf{100}$.

6.10. Determination of Total Phenolic content

The total phenolic content of the sample was determined using the Folin-Ciocalteu in 96-well plate with slight modifications [60]. About 25µl of the extract solution was mixed with 100µl of Folin-Ciocalteau reagent (FCR). After 3 min, 75µl of sodium carbonate solution was added and the mixture was allowed to stand for 1 h at room temperature followed by absorbance was measured at 765 nm using a microplate reader. From the calibration curve of gallic acid prepared in different concentrations (200,400,600,800 and 1000µg/ml). The amount of phenolic compounds was determined and expressed as mg gallic acid equivalent (GAE) of dried or fresh extract exudates. The total phenolic content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the following formula:

$$\mathbf{T=C\ X\ V\ /\ M}$$

Where, T- Total phenolic contents in mg/g plant extract, in Gallic Acid Equivalent(GAE) C-Concentration (mg/ml) of Gallic acid obtained from the calibration curve, V=Volume of extract (ml),

M= Weight (mg) of plant extract.

6.11. Determination of total Flavonoid content

Total soluble flavonoid content of the fractions was determined with aluminium chloride using quercetin as a standard in 96 well plate. Free radicals

concentration(200,400,600,800,1000µg/ml) of quercetin, 150µl of methanol was added. Then it was mixed with 10µl of aluminium chloride and 10µl Extract solution of different concentration was also prepared using the same method. All the samples were incubated for 30 minutes at room temperature and absorbance was measured at 415 nm using a microplate reader against control. The total flavonoid content in the fractions was determined as µg quercetin equivalent by using the standard quercetin graph and using the following formula:

T= C X V/ M

Where, T-Total flavonoid content in mg/g plant extract, in Quercetin Equivalent (QE), C=Concentration (mg/ml) of Quercetin obtained from calibration curve,

V= Volume of extract (ml),

M= Weight (mg) of plant extract**.**

6.12. *In-vitro* antioxidant studies

The word antioxidant simply means " against oxidation". An antioxidant is any substance that retards or prevents the deterioration, damage, or destruction of cells by oxidation [62]. It is a classification of a number of organic compounds, including selenium, the carotenoids chemical family, vitamin A (which is made from beta carotene), and the vitamins C and E.

6.12.1 Determination of DPPH radical scavenging activity

Minor adjustments were made to an earlier approach [63] for measuring DPPH activity while using Ascorbic acid as the Standard for comparison in 96 well plate. In order to create a series of sample solutions with various pre-set concentrations(200,400,600,800,1000 µg/ml)the extract was dissolved in methanol. 50 µl of the extract sample solution were combined with 150µl of a 0.3 mM DPPH solution in methanol, and the mixture was then incubated for 30 min at 37°C. Absorbance was measured at 517 nm using a microplate reader against control. The same method was applied to standard solutions with comparable concentrations. The ability of the sample/standard to scavenge the DPPH radical was determined as the free radical inhibition percentage using the following formula:

$$\% \text{ Inhibition of DPPH scavenging activity } = \frac{(A_c - A_t)}{A_c} \times 100$$

Where, A_c is the absorbance of the control reaction and A_t is the absorbance of the test/standard. The extract's antioxidant activity was quantified as IC50. The IC50 values were computed using linear regression of plots, where the ordinate represents the average percent of scavenging capacity and the abscissa indicates the concentration of the studied plant extracts. The IC50 value is defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

6.12.2 Determination of hydrogen peroxide radical scavenging activity

The ability of three T.orientalis extracts to scavenge hydrogen peroxide was determined according to the method of Oktay Munir and others[**64**]. In phosphate buffer with a pH of 7.4, hydrogen peroxide (2 mmol/L) was produced as a solution. Hydrogen peroxide concentration was determined microplate reader from absorption at 230 nm with molar absorbtivity81 mol/L-1 /cm. Extracts samples (100–400µg/ml) in distilled water were added to a hydrogen peroxide solution (30 µl). After 10 minutes, the absorbance of hydrogen peroxide at 230 nm was measured in comparison to a blank solution made up of phosphate buffer and no hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both T.orientalis extract and standard compounds(Ascorbic acid).

$$\% \text{ Inhibition of DPPH scavenging activity } = \frac{A_c - A_t}{A_c} \times 100$$

Where, Ac is the absorbance of the control reaction and At is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC50. The IC50 value is defined as the concentration (µg/ml) of dry extract that inhibits the formation of H_2O_2 radicals by 50%.

6.13. *In vitro* anti-diabetic activity

Inhibition of α -Amylase

70µl of root extract and positive standard (acarbose) of concentration ranging from 100-400µg/ml were taken. To each concentration 70µl of α-amylase solution dissolved in phosphate buffer was added and incubated at 30°C for 10 minutes in 96 well plate. Then 70µl of 1% starch solution was added, incubated for 3 minutes. Finally the reaction was stopped by adding 30µl 0f 3,5-dinitrosalicylic acid (DNSA), kept in boiling water bath for

10 minutes, cooled and the absorbance was measured at 540nm using a microplate reader. [Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar [65].Control was also treated similarly without the extract. a-amylase inhibitory activity was measured using the formula:

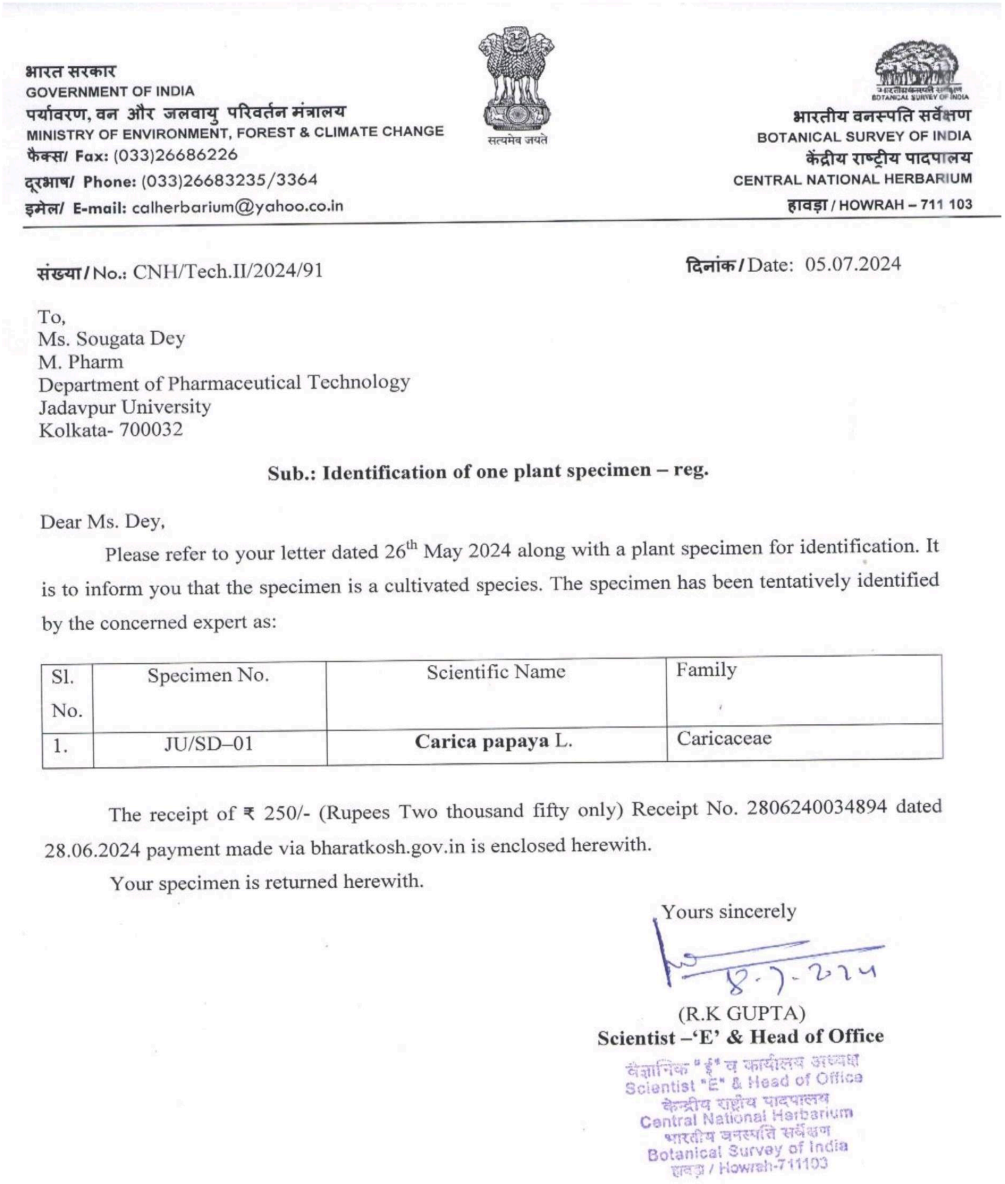
$$\% \text{ Inhibition } = \frac{\text{Abs Control} - \text{ Abs Test}}{\text{Abs Control}} \times 100$$

The IC50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate . All the measurements were done in triplicate and results are expressed in terms of Mean ± standard deviation and IC50 values were calculated using MS-Excel software.

7. RESULTS AND DISCUSSION

7.1. Authentication of the plant material

An illustration of the *Carica papaya* plant's fully grown leaves is included. The Central National Herbarium, Botanical Survey of India, has provided the authenticity certificate, which is shown in figure. The collected species is identified as *Carica papaya*, a member of the caricaceae family, according to the certificate issued by the CNH.



भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
फैक्स/ Fax: (033)26686226
दूरभाष/ Phone: (033)26683235/3364
इमेल/ E-mail: calherbarium@yahoo.co.in

सत्यमेव जयते

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या / No.: CNH/Tech.II/2024/91

दिनांक / Date: 05.07.2024

To,
Ms. Sougata Dey
M. Pharm
Department of Pharmaceutical Technology
Jadavpur University
Kolkata- 700032

Sub.: Identification of one plant specimen – reg.

Dear Ms. Dey,

Please refer to your letter dated 26th May 2024 along with a plant specimen for identification. It is to inform you that the specimen is a cultivated species. The specimen has been tentatively identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	JU/SD–01	**Carica papaya** L.	Caricaceae

The receipt of ₹ 250/- (Rupees Two thousand fifty only) Receipt No. 2806240034894 dated 28.06.2024 payment made via bharatkosh.gov.in is enclosed herewith.

Your specimen is returned herewith.

Yours sincerely

8.7.2024

(R.K GUPTA)
Scientist –'E' & Head of Office

वैज्ञानिक "ई" व कार्यालय अध्यक्ष
Scientist "E" & Head of Office
केन्द्रीय राष्ट्रीय पादपालय
Central National Herbarium
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
हावड़ा / Howrah-711103

Figure 4. Authentication certificate from Botanical Survey of India

7.2 Morphological characteristics of the species

Morphology: Simple, spiral-arranged leaves on 30 to 70 cm long petioles that form a terminal cluster make up the morphology. The boundaries of the lobes may be well defined, gently sloped, or entirely distinct. The leaves have thick central irradiated veins and are smooth, fairly palm shaped, and extensively visible, measuring 25–75 cm in diameter. The leaves have a spherical shape, measure 60 to 90 cm in diameter, are placed alternately, are bundled at the tip of the stem and branches, and have long petioles. The bundle of leaves is characterized by colour. The underside surface is opaque, pale green-yellow, and shows clear vascular systems; the petioles are spherical, yellow-green, fistulous, and delicate

Table 4. Morphological characteristics of CPLE

Sl No.	Characteristics	Leaves part
1.	Colour	deep, rich green
2.	Odour	pungent aroma
3.	Taste	bitter taste
4.	Texture	Smooth leathery
5.	Shape	pungent aroma
6.	Size	50-70 cm in diameter

Figure 5. *Carica papaya* leaves

7.3. Microscopical characteristics of the species

The microscopic characters from the transverse section of the leaves of the plant is represented in Figure. The section shows the presence of various anatomical characters of the leaves including single layered epidermal cells, vascular bundle and diagnostic character like unicellular trichomes. On Peeling of lower epidermis presence of another diagnostic characteristic in the form anomocytic stomata stomata is present as in Figure. Powder characterization of the leaves reveals the presence of anomocytic stomata along with spiral vessel and annular xylem fibers, Spongy parenchyma cells as shown in Figure.

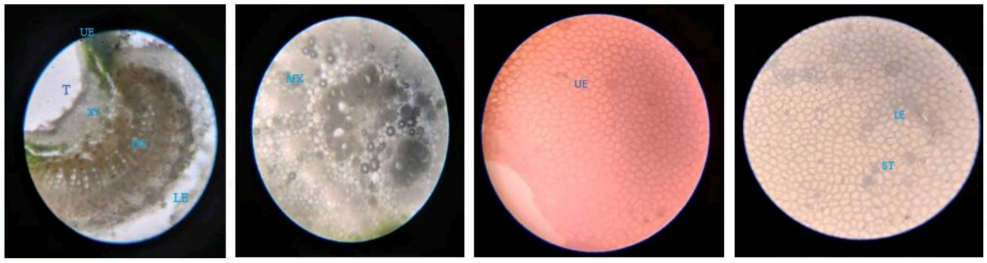


Figure 6. Transverse section of leaf (UE- upper epidermis; T –trichome; XY- xylem; CC –cortical cells; LE – lower epidermis; MX- meta xylem; ST- stomata)

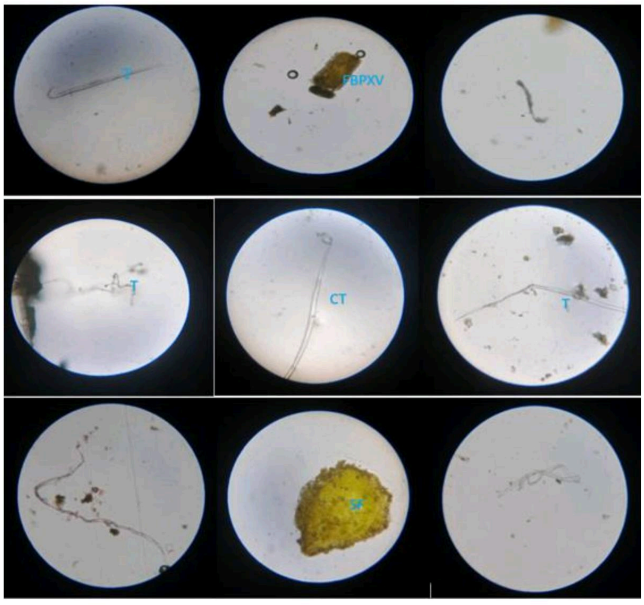


Figure 7. Powder characteristic of Carica papaya leaves under 10 × 45 magnifications

(T-trichome; CT– conical trichome; FBPXV – fragment of border pitted xylem vessel; SF – simple fiber)

7.4. Physicochemical Characteristics of Leaves Powder

Table 5. Result of different physicochemical properties of leaves powder

Sl No.	Physico-Chemical Parameters	Result % w/w
1	Loss On Drying	13
2	Total Ash	13.4
3	Acid Insoluble Ash	10
4	Water Soluble Ash	4.2
5	Water Soluble Extractive	15.5
6	Ethanol soluble extractive	7
7	Moisture content	4
8	Crude fiber Content	12
9	Swelling Index	<100
10	Foaming Index	<100

7.5. Phytochemical screening of the extracts

The results of phytochemical screening of petroleum ether, acetone and methanolic extracts of leaves are presented in the table. This phytochemical study shows that the petroleum ether extract contains terpenoids, steroids, cardiac glycosides. Methanolic extract on the other hand has carbohydrates, reducing sugar, steroids, terpenoids, glycosides, and glycosides, alkaloids, tannins. Acetone extract contain alkaloid only.

Table 6. Phytochemical screening of three extracts

Chemical Constituents	Chemical Test	Petroleum ether extract	Acetone extract	Methanol extract
Carbohydrates	Molisch Test	-	-	+
Reducing Sugars	Fehling's Test	-	-	+
	Benedict's Test	-	-	+
Monosaccharides	Barfoed's Test	-	-	+
Proteins and Amino Acids	Biuret Test	-	-	-
	Ninhydrin Test	-	-	-
	Millon's Test	-	-	-
Steroids	Salkowski reaction	+	-	+
	Liebermann-Burchard Test	+	-	+
Terpenoids	Noller's Test	+	-	-
Glycosides (Cardiac Glycosides)	Legal's test	+	-	+
	Keller–killiani test	+	-	+
Anthraquinone glycosides	Borntrager's test	-	-	+
	Modified Borntrager's test	-	-	+
Saponins	Foam Test	-	-	
Flavonoids	Shinoda Test	-	-	+
	Ferric Chloride Test	-	-	+
	Sodium hydroxide Test	-	-	+
Alkaloid	Mayer's test	-	-	+
	Dragendorff'stest	-	+	+
	Hager's test	-	+	+
	Wagner's test	-	+	+
Tannins and Phenolics	Ferric Chloride Test	-	-	+
	Gelatin Test	-	-	+
	10% NaOH test	-	-	+

.

7.6. TLC Profiling

For Petroleum ether extract mobile phase used as methanol and hexane in the ratio of 1:2; chloroform and hexane in the ratio of 1:1 and 3:1. For acetone extract mobile phase is methanol and chloroform in the ratio 1:2 and 1:1. For methanol extract mobile phase used as Chloroform and Hexane in the ratio of 1:1.

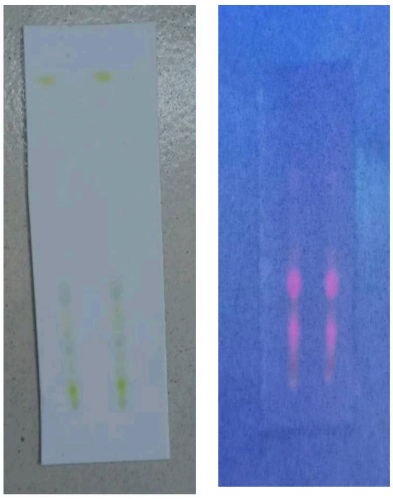


Fig. 8. Methanol: Hexane = 1:2

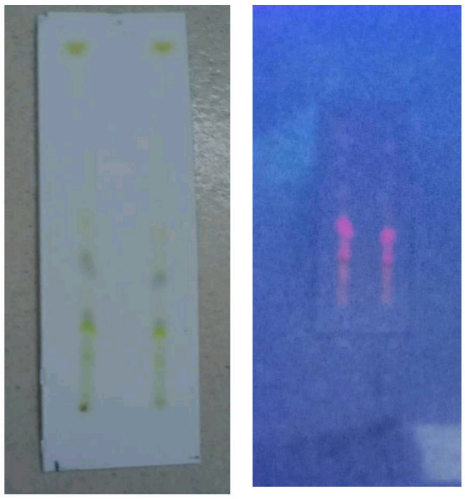
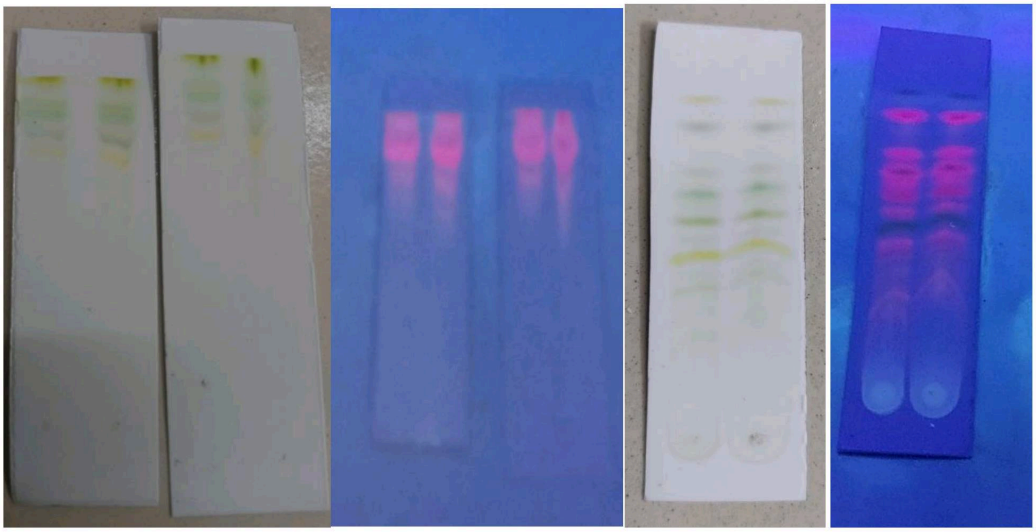


Fig. 9. Chloroform: Hexane = 3:1



(a) (b) (a) (b)

Fig.10. Methanol: Chloroform = 1:2 (a) and 1:1(b) **Fig.11** Chloroform : Hexane =1:1

The TLC characteristics in the form of retention factor (R_f) of petroleum ether, acetone and methanolic extracts of *carica papaya* leaves are shown in the table.

Table 7. TLC Profiling of extracts

Sl. No.	R_f Value		
	Petroleum Ether Extract	**Acetone extract**	**Methanol extract**
1	0.209	0.738	0.65
2	0.208	0.877	-
3	0.075	-	-

7.7. Characterization of tincture

The table displays the different features of the developed tincture. The pH of the produced tincture at various alcohol concentrations is shown in the table:8. The outcomes of the study demonstrate that tinctures with higher alcohol concentrations have a little higher acidity, while tinctures stored for longer periods of time have a slightly higher basicity. As can be observed from the chart, a higher alcohol percentage results in a lower solid content.

Alcohol Strength (% v/v)	pH		
	7 days	**14 days**	**21 days**
40%	6.13	6.13	6.05
50%	6.19	6.30	6.10
60%	6.09	6.12	6.18
70%	6.16	6.15	6.20
80%	6.20	6.30	6.19
90%	6.10	6.22	6.13
Absolute (100%)	5.86	6.10	6.04

Table 8. pH characterization of the tincture

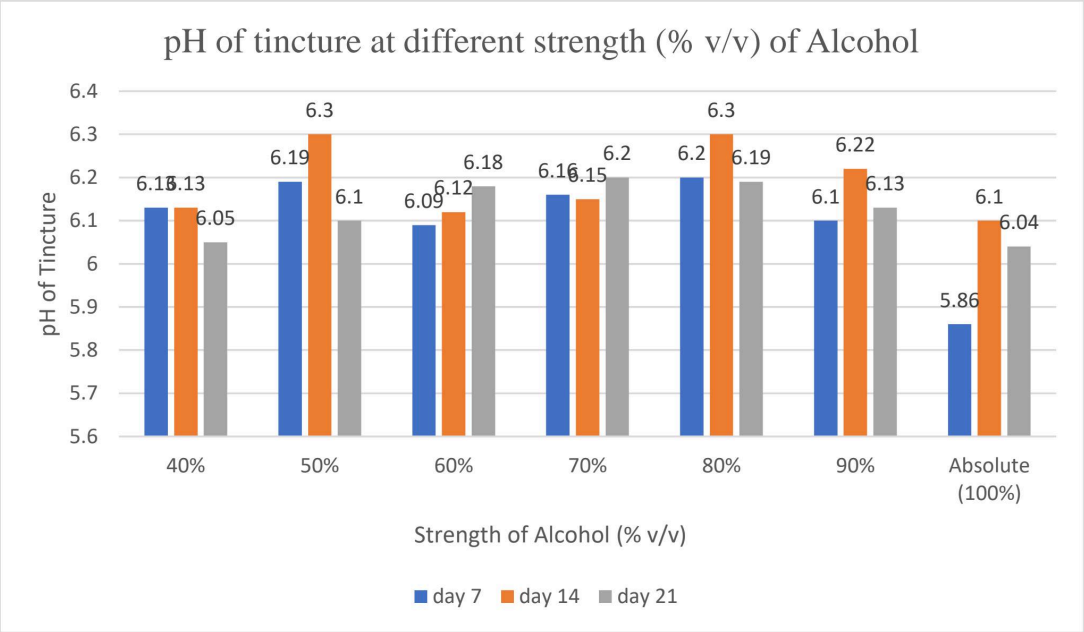


Figure 12. pH of the tincture at different strength (% v/v) of Alcohol

Table 9. Solid content (% w/v) of tincture

	% Solid content		
Alcohol Strength (% v/v)	**7 Days**	**14 Days**	**21 Days**
40%	**0.50**	**0.62**	**0.23**
50%	**0.48**	**0.65**	**0.34**
60%	**0.37**	**0.44**	**0.34**
70%	**0.36**	**0.47**	**0.39**
80%	**0.46**	**0.36**	**0.33**
90%	**0.34**	**0.30**	**0.31**
Absolute (100%)	**0.24**	**0.24**	**0.24**

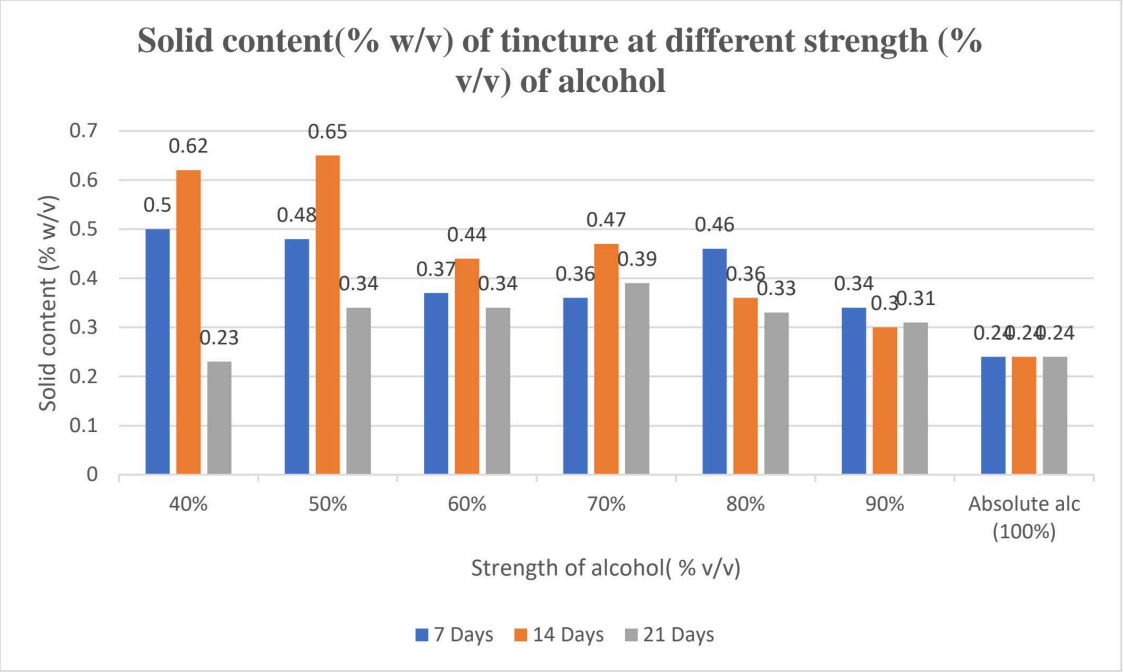


Figure 13. Solid content of tincture at different strength of alcohol

7.8. Anti-inflammatory assay

The *in-vitro* anti-inflammatory activity was investigated using the HRBC membrane stabilization technique. The lysosomal membrane and the erythrocyte membrane are comparable. Lysosomal enzymes are released during inflammation, which leads to the development of several illnesses. These enzymes' extracellular activity and either acute or chronic inflammation are connected. The lysosomal membrane and red blood cell membrane are similar, it may be able to inhibit the release of neutrophil lysosomal material at the site of inflammation. Anti-inflammatory activity is measured by preventing HRBC membrane lysis.

Table 10. HRBC membrane stabilisation by % of hemolysis inhibition

SL No.	Concentration (µg/ml)	% of haemolysis Inhibition			
		Standard drug (Diclofenac Sodium)	Pet Ether Extract (PEE)	Acetone Extract (AE)	Methanol Extract (MEE)
1.	50	75.98	39.151	55.87	75.24
2.	100	71.29	32.67	54.98	69.41
3.	150	67.34	33.16	50.17	65.32

4.	200	60.19	28.07	41.16	60.16

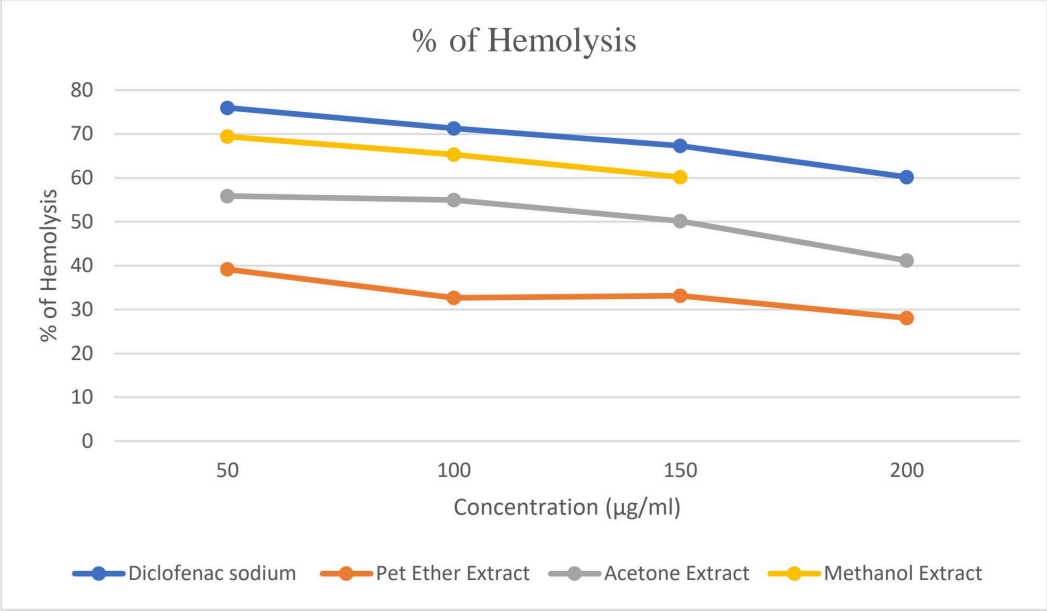


Figure 14. Anti-inflammatory assay % hemolysis

7.9. Total Phenolic Content Assay

The absorbance of the Standard and test solution were represented in Table 11. The total phenolic content in terms of mg Gallic Acid Equivalent (GAE) of Petroleum ether extract was found to be 14.35 mg/g and methanolic extract has found to be 47.96 mg/g and Acetone extract is found to be 22.5. These results suggest that higher the presence of phenolic components is responsible for the levels of antioxidant activity. The Standard curve of Gallic acid has shown in graph:4.

Table 11. Determination of total phenolic content

Sl. No.	Concentration (µg/ml)	Absorbance	Phenolic content of Petroleum ether extract	Phenolic content of Methanolic extract	Phenolic content of Acetone extract
1.	200	0.349	**14.35**	**47.96**	**22.5**
2.	400	0.434			
3.	600	0.505			
4.	800	0.716			
5.	1000	0.869			
6.	Equation of line	Y= 0.0007 X+ 0.178			
7.	Coefficient (R^2)	R^2=0.9594			

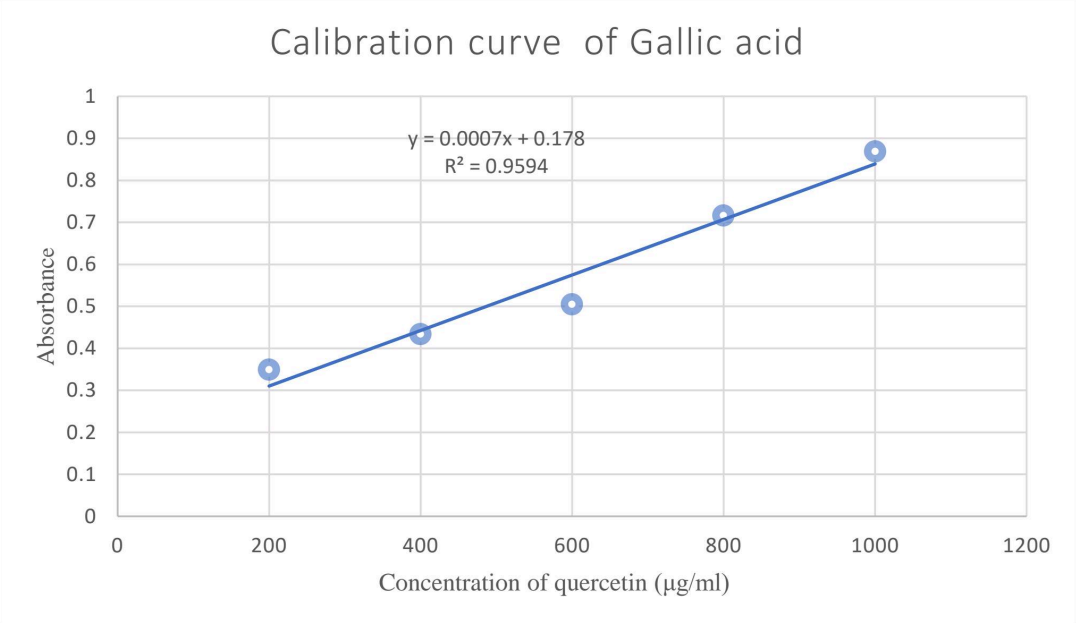


Figure 15. calibration curve of Gallic acid

7.10. Total Flavonoid Content Assay

The content of flavonoid compound in Petroleum ether, acetone and methanolic extract of *Carica papaya* leaves was measured by aluminum chloride reagent in terms of quercetin equivalent. The absorbance of the Standard and test solutions are represented in table no.

12. It is well known that flavonoids have significant antioxidant activity and have a positive impact on human nutrition and health. The methanolic extract of *Carica papaya* leaves contains a substantial quantity of flavonoids which may contribute considerable function to the antioxidant activity of the plant. The Standard curve of Quercetin is shown in graph: 5.

Table 12. Determination of total Flavonoid content

Sl. No.	Concentration (µg/ml)	**Absorbance**	**Flavonoid content of Petroleum ether extract**	**Flavonoid content of Methanolic extract**	**Flavonoid content of Acetone extract**
1.	200	0.193			
2.	400	0.244			
3.	600	0.29			
4.	800	0.345			
5.	1000	0.378			
6.	Equation of line	y = 0.0002x + 0.1303	**98.83**	**102.7**	**87.96**
7.	Coefficient (R^2)	0.9775			

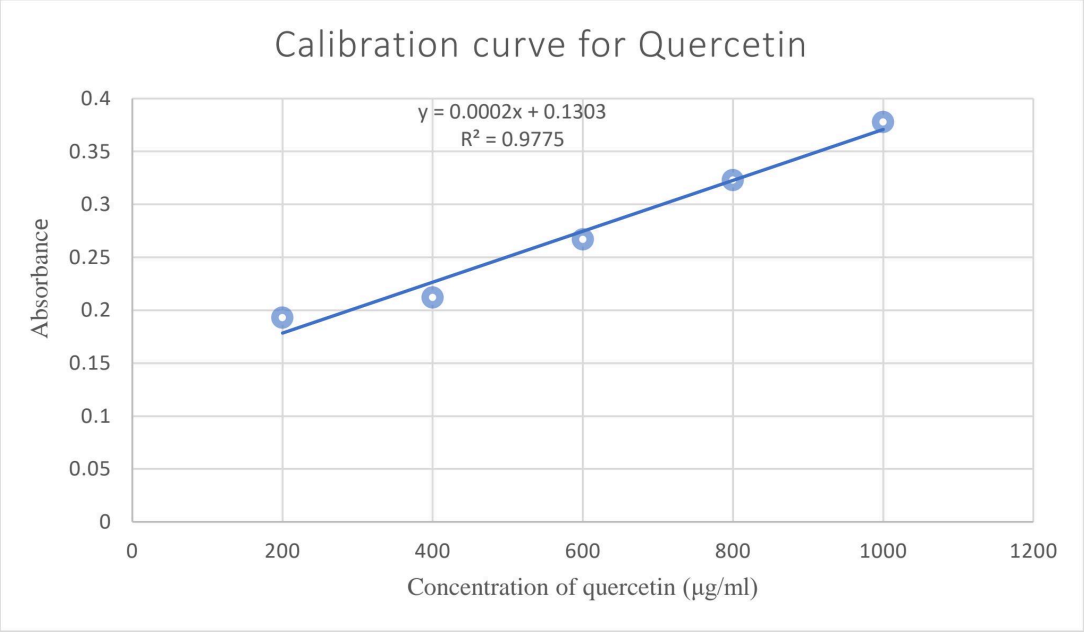


Figure 16. Calibration curve for Quercetin

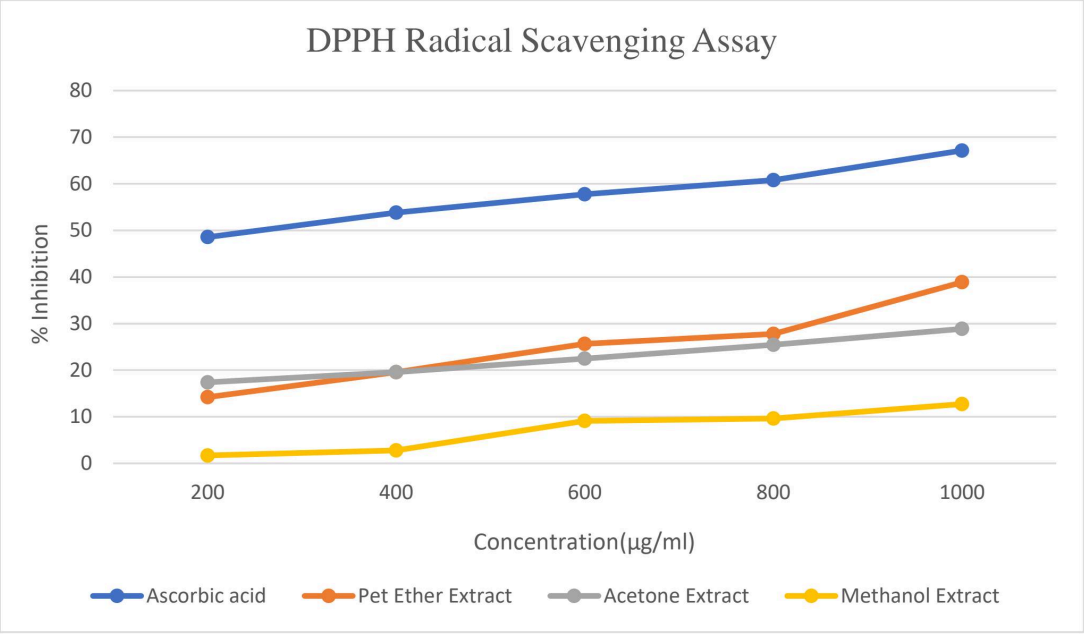
7.11. Antioxidant Assay

7.11.1 DPPH Radical Scavenging Assay

The drop in DPPH's absorbance at 517 nm, which is brought on by antioxidants, was used to assess its capacity for reduction. The extract shows maximum hydrogen donating ability in the presence of DPPH free radicals at high concentrations. *Carica papaya* has significant scavenging effects with increasing concentration when compared with that of Ascorbic acid. The results of the DPPH scavenging activity are shown below in the table:13 represents DPPH radical scavenging assay of standard and test samples. Graph: 6 DPPH Radical Scavenging Assay of Standard sample and Test Sample

Table 13. DPPH Radical Scavenging Assay of Standard sample and Test Samples

SL No.	**Concentration (µg/ml)**	**% Inhibition**			
		(STD) Ascorbic acid	Pet Ether Extract (PEE)	Acetone Extract (AE)	Methanol Extract (MEE)
5.	200	48.5461	14.20455	17.38636	1.704545
6.	400	53.81765	19.567	19.54545	2.768
7.	600	57.76538	25.6363	22.4545	9.090909
8.	800	60.78256	27.7727	25.436	9.647727
9.	1000	67.13634	38.86364	28.86364	12.72727
10.	IC50 Value	1.276	7.205	12.44	16.75



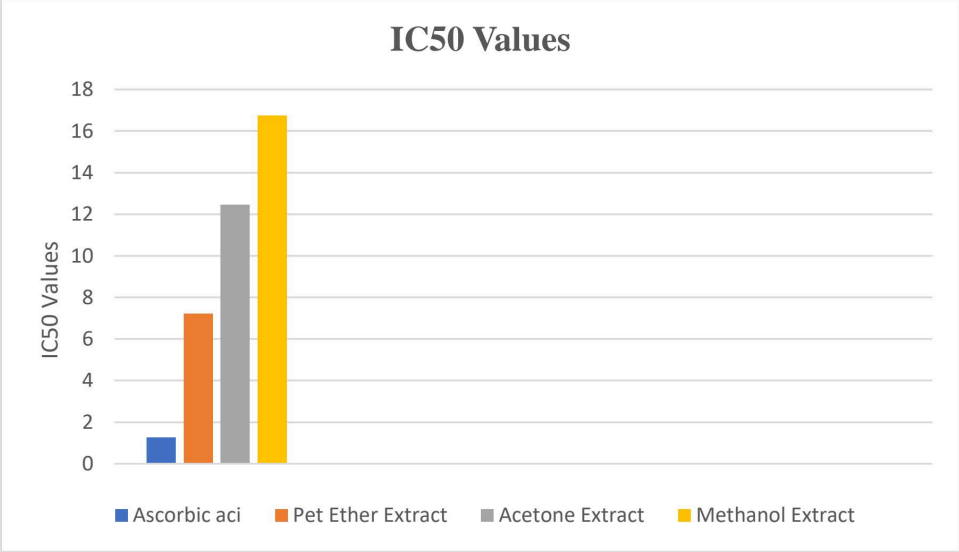


Figure 17. DPPH Radical Scavenging Assay and IC50 Values

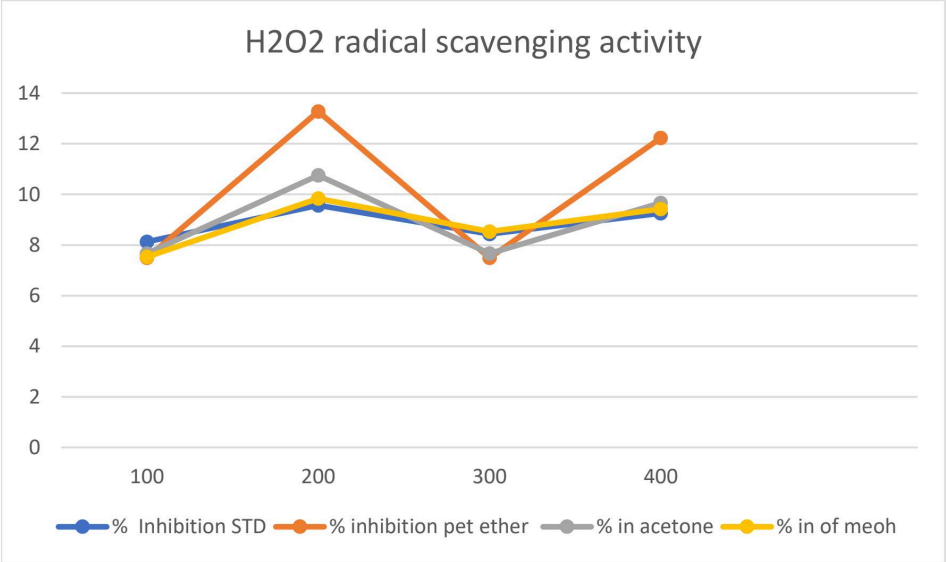
7.11.2. Inhibition of hydrogen peroxide radicals

In addition to being highly reactive, hydrogen peroxide itself can occasionally be hazardous to cells due to the hydroxyl radical it produces inside them. Thus, removing H_2O_2, as well as O_2 is very important for the protection of food systems. Here hydrogen peroxide scavenging activity of extracts are compared with ascorbic acid. The table below displays the outcomes. The IC_{50} value of extracts and ascorbic acid are calculated. The results shows that the test samples are effective in scavenging hydrogen peroxide in a dose-

dependent manner. The outcome is comparable to that of the ascorbic acid reference standard. Table: 14 represents hydrogen peroxide radical scavenging assay of standard and test samples.

Table 14. H_2O_2 radical scavenging activity of Extracts and Standard

SL No.	Concentration (µg/ml)	% Inhibition			
		(STD) Ascorbic acid	Pet Ether Extract (PEE)	Acetone Extract (AE)	Methanol Extract (MEE)
1.	100	8.127572	7.489712	7.664609	7.530864
2.	200	9.567901	13.27778	10.75129	9.835391
3.	300	8.436214	7.489712	7.664609	8.530864
4.	400	9.259259	12.22634	9.664609	9.427984
5.	IC 50 Value	185.7	49.85	96.4	96.5



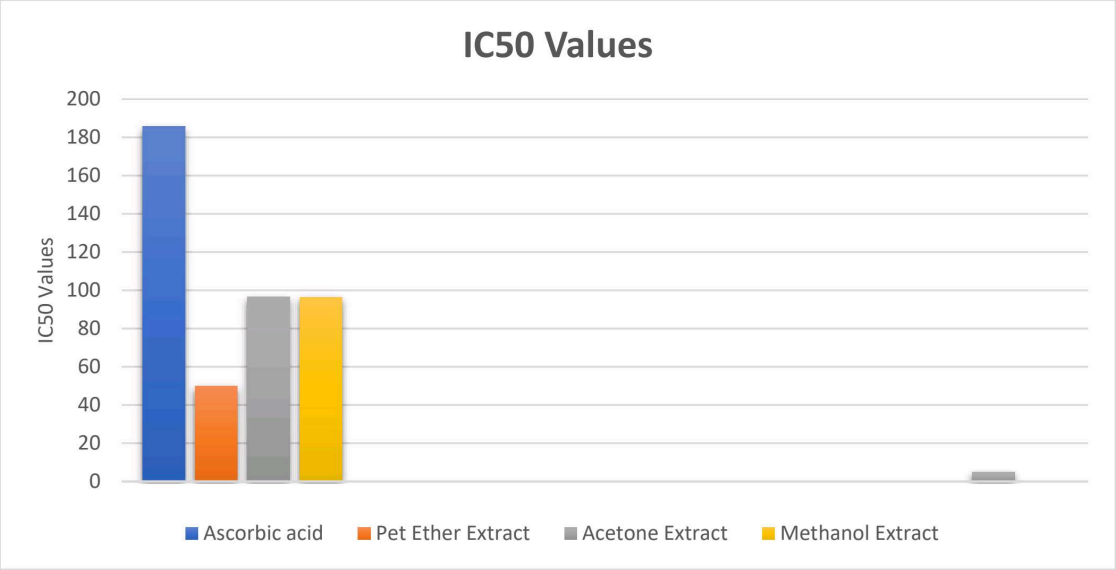


Figure 18. H_2O_2 radical scavenging activity and IC50 Values

7.12. Determination of *in-vitro* Antidiabetic Activity

α-amylase inhibition assay

α-amylase is one of the enzymes that hydrolyses the α-1,4 glycosidic linkages in starch to produce glucose and maltose. α-amylase hydrolyses complex polysaccharides into oligosaccharides and disaccharides and then hydrolyses by α-glucosidase to monosaccharides. The *in vitro* antidiabetic activity has been evaluated by using α amylase inhibitory assay. The pet-ether, acetone and methanolic extracts of *Carica papaya* produces some inhibitory effects on this enzyme. Acarbose has been used as a standard which shows marked inhibitory effect of the enzyme. The IC_{50} of Acarbose and three extracts are calculated. Table: 15 shows the α-amylase inhibitory activity of Standard and test samples. Graph: 8 represents α-amylase inhibition assay of Acarbose and test sample.

Table 15. Alpha- amylase inhibitory activity of the extracts of C. papaya leaves

SL No.	Concentration (µg/ml)	% of amylase Inhibition			
		(STD) Acarbose	Pet Ether Extract (PEE)	Acetone Extract (AE)	Methanol Extract (MEE)
1.	100	23.1	22.36	37.6	64.4
2.	200	48.0	23.44	32.73	60.70
3.	300	70.3	34.5	33.23	66.40
4.	400	95.1	29.63	30.89	66.32

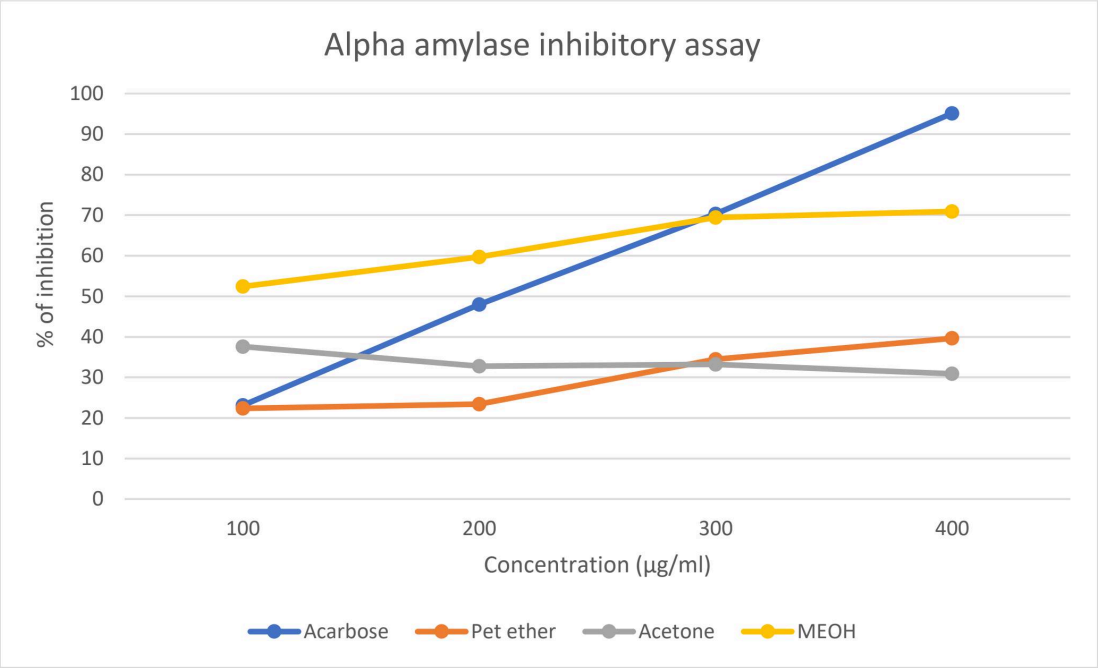


Figure 19. Alpha amylase inhibitory assay

8. DISCUSSION

The Botanical Survey of India's Central National Herbarium verified the authenticity of Carica papaya, an essential step in ensuring accurate identification of the plant material employed. The form, size, and arrangement of the leaves, among other morphological traits, are consistent with the usual features of Carica papaya as recorded in botanical literature. The deep, rich green colour and the straightforward, spiral-arranged leaves with clearly defined lobes are in line with descriptions found in other botanical literature. Papaya leaves from the Carica variety are also known for their strong flavour and scent. This morphological description is consistent with observations made by Morton (1987) and Otsuki (2010), who also mentioned the big, lobed leaves and unique papaya leaf fragrance. These characteristics are crucial for accurate species identification and subsequent studies.

Anomocytic stomata, spiral arteries, spongy parenchyma cells, and unicellular trichomes were among the diagnostic characteristics identified by the microscopical study. These resemble the typical anatomical characteristics of dicotyledonous plants, such as Carica papaya. Sivarajan and Balachandran (1994) found comparable traits in their microscopic analysis of Carica papaya, which supports the existence of these microscopical features. The presence of anomocytic stomata is important since it facilitates the species' taxonomical identification.

To assess the quality and purity of the plant material, the physicochemical characteristics—such as loss on drying, total ash, acid-insoluble ash, and extractive values—are important. The findings indicate the existence of mineral components due to the moderate moisture level (4%) and high total ash content (13.4%). Gowri and Vijayalakshmi (2011) found identical physicochemical properties for Carica papaya leaves, which are similar to these values. The high ash level suggests the existence of inorganic components, which may be related to the plant's potential medicinal benefits.

Phytochemical studies suggested that different extracts contain independent bioactive components. The most diverse mixture of compounds was found in the methanolic extract, which included reducing sugars, alkaloids, flavonoids, terpenoids, glycosides, and steroids. Whereas the petroleum ether extract included terpenoids, steroids, and cardiac glycosides, the acetone extract was primarily rich in alkaloids. The

phytochemical profile of papaya leaves from Carica is consistent with research by Krishna *et al.* (2008), who also found that chemicals are comparable in papaya leaves. The plant's historic usage in treating a range of illnesses is supported by the presence of alkaloids and flavonoids, which have anti-inflammatory and antioxidant effects, among other medicinal benefits. For various extracts, the TLC profiling revealed unique retention factor (Rf) values, which is helpful for the chemical characterization of the plant. A thorough profile of the numerous phytochemicals present was made possible by using multiple solvent systems. The extracts exhibited noteworthy anti-inflammatory properties, especially the methanolic extract that demonstrated hemolysis inhibition at concentrations equivalent to the conventional medication Diclofenac Sodium. Moderate anti-inflammatory effects are shown by the petroleum ether and acetone extracts. The discovered anti-inflammatory qualities are consistent with research by Otsuki *et al.* (2010), which found that papaya leaves from Carica had significant anti-inflammatory effects. These benefits are probably caused by flavonoids and other phenolic chemicals.

The methanolic extract demonstrated the highest potential for antioxidant activity, followed by the acetone and petroleum ether extracts, based on DPPH radical scavenging and hydrogen peroxide scavenging tests. This shows that chemicals that may donate hydrogen atoms to neutralize free radicals are especially abundant in the methanolic extract. These results are similar to earlier studies conducted by Banjarnahor and Artanti (2015), who discovered that the flavonoid and phenolic content of Carica papaya leaves contributes to their high antioxidant content. The plant may have a significant role in preventing illnesses associated with oxidative stress because it has high antioxidant activity.

The α-amylase inhibition assay demonstrated that the methanolic extract had the strongest inhibitory effect on the enzyme, suggesting potential antidiabetic activity. This is significant because α-amylase inhibitors are known to reduce the postprandial rise in blood glucose levels. This antidiabetic potential is supported by a study conducted by Juárez-Rojop *et al.* (2012), which found that Carica papaya leaves exhibit inhibitory effects on carbohydrate-hydrating enzymes, thereby supporting their traditional use in managing diabetes.

9. CONCLUSION

This study provides a comprehensive analysis of the *Carica papaya* leaves, confirming their identity and morphological characteristics through detailed morphological and microscopical evaluations. The authentication was confirmed by the Central National Herbarium, Botanical Survey of India. Physicochemical characterization revealed notable properties such as a loss on drying percentage of 13%, total ash content of 13.4%, and crude fiber content of 12%. The moisture content was found to be 4%, indicating the leaves' stable composition. Phytochemical screening of various extracts demonstrated the presence of significant bioactive compounds. Petroleum ether extract contained terpenoids, steroids, and cardiac glycosides, while methanolic extract exhibited a broader range of compounds, including carbohydrates, reducing sugars, steroids, terpenoids, glycosides, alkaloids, and tannins. The acetone extract was mainly characterized by the presence of alkaloids.

Thin Layer Chromatography (TLC) profiling revealed distinct Rf values for each extract, further confirming the presence of unique phytochemicals in *Carica papaya* leaves. The tincture characterization indicated that higher alcohol concentrations slightly increased acidity over time while reducing solid content, suggesting that tincture preparation should consider these factors for optimal stability. In terms of biological activity, the methanolic extract showed promising anti-inflammatory properties with a significant percentage of haemolysis inhibition, comparable to the standard drug Diclofenac Sodium. The total phenolic and flavonoid content assays underscored the antioxidant potential of *Carica papaya* leaves, with methanolic extract displaying the highest phenolic content.

The antioxidant assays, particularly the DPPH radical scavenging assay and hydrogen peroxide radical scavenging activity, highlighted the significant scavenging effects of the methanolic extract, suggesting its potential use in mitigating oxidative stress-related conditions. Moreover, the *in-vitro* antidiabetic activity, as demonstrated by the α-amylase inhibition assay, showed that the methanolic extract had the highest inhibitory effect, indicating its potential role in managing diabetes.

In conclusion, the study illustrates that *Carica papaya* leaves are a rich source of bioactive compounds with substantial anti-inflammatory, antioxidant, and antidiabetic properties. These findings support the potential use of *Carica papaya* leaves in therapeutic

applications, particularly in managing inflammation, oxidative stress, and diabetes. Further studies and clinical trials are recommended to fully explore and validate these therapeutic potentials.

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