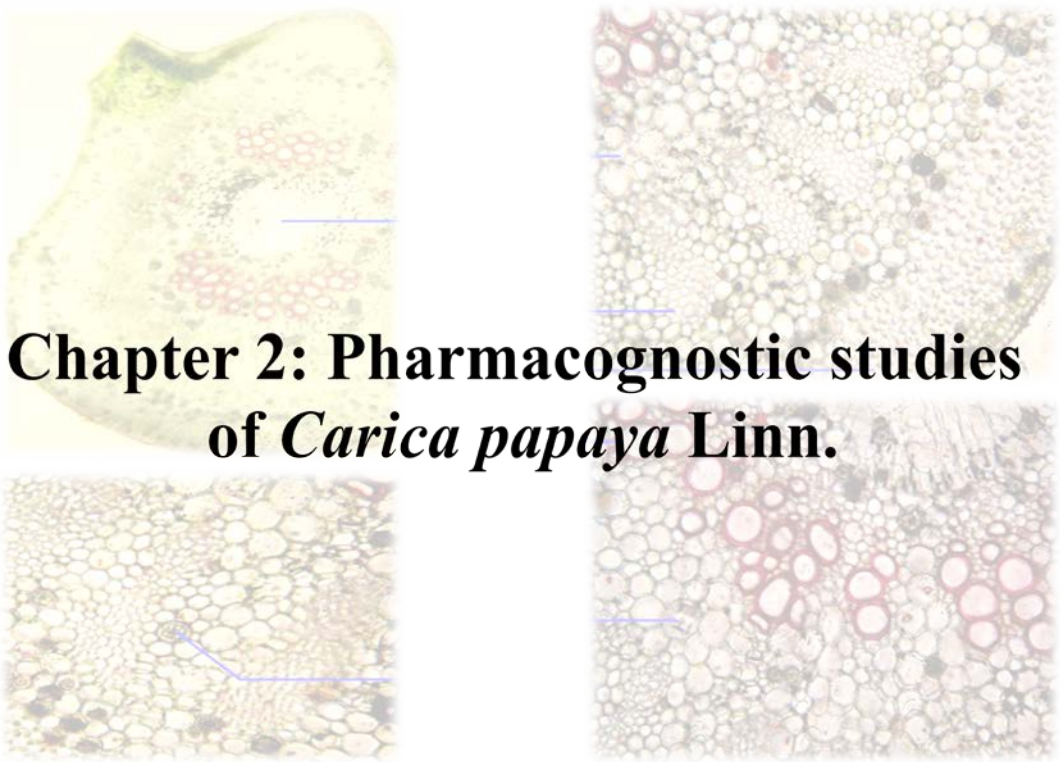

Chapter 2: Pharmacognostic studies of *Carica papaya* Linn.

2. Pharmacognostic studies of *Carica papaya* Linn.

2.1 Introduction

"Pharmacognosy" is derived from Greek word "Pharmakon means drug and gnosis means knowledge [1], [2]. Pharmacognosy is the branch of sciences which involves study of biological, chemical, biochemical and physical properties of the natural sources like plants, microbe and animals [1]–[5]. The terms 'pharmacognosy' and 'pharmacodynamics' were probably first coined by Johann Adam Schmidt (1759-1809) in his hand-written manuscript Lehrbuch der Materia Medica, which was posthumously published in Vienna in 1811. Shortly after the above publication, 'pharmacognosy' appeared again in 1815 in a small work by Chr. Aenotheus Seydler entitled 'Analecta Pharmacognostica'[3]. The term '*material medica*' was used to describe pharmacognosy in the early works of Galen (Greek physician, surgeon and philosopher of 130-200 AD) and Dioscorides (Greek physician, pharmacologist and botanist in 14-90 AD) [6].

Pharmacognosy has its origin in the health-related activities of the most primitive human race of the remote past. The early human sought to alleviate his sufferings of illness and injuries using plants. The knowledge of medicinal properties of plants was acquired by observation of sharp animal behavior, trial and error methods or at times accidently [7]. In a due course of time, a group of people emerged in each community who acquired expertise in collecting, testing and using medicinal plants for treating diseases. These people later became `Medicine Men'. They monopolized the knowledge of drugs and transferred this only to their trusted followers of the successive generations, verbally, or by signs and symbols. As the time progressed, transfer and recording of the knowledge were done in writing [8].

Till the end of 19th century, the subject pharmacognosy was developed in the herbal area mainly, which was gradually expanded to microbes (bacteria, fungi, etc.), and recently it includes various marine organisms as well [9], [10].

With increasing demands for herbal products as an alternative medicinal resource, regulatory requirements based on pharmacognostic studies [3], [11] are introduced in many countries.

Pharmacognosy as mentioned earlier is related to drug source and constituents. Hence it requires good collaborative work among various branches of sciences mainly between botany and chemistry. The scheme of pharmacognostic study comprises of two parts [12]–[14], include macroscropic studies, microscopic and chemical studies. Macroscopic studies consist of identification and authentication of plant materials, biological source and their family, its geographical source, morphological traits. Microscopic and chemical studies include anatomical characters and histochemical tests, physicochemical analysis, phytochemical analysis and pharmacology.

The quality of herbal products is the major concern in pharmacognostic study. The accuracy of plant material identification, authentication and quality assurance of taxonomy must be scientifically validated. The quality of herbs get reduced due misidentification of collected herbs, contamination with extraneous things, and adulteration with other species [15]. Monograph preparation of the recognized plant must be identified and authenticated by experts on the subject and certified by research institution affiliated to botanical survey of India. Authentication tools include preparation of herbaria of the respective plant material, constant evaluation of active phytochemicals in the collected plant material using biomarkers, morphological, microscopical, histochemical, phytochemical and genetic approaches [16].

The initial step involves proper identification, collection and documentation of the plant material which includes proper bionomical nomenclature of the plant, person's name who collected, place and location from where the material was collected, organoleptic characteristics (e.g. smell, taste, color) and plant material processing steps used (drying method, time, and temperature) as well as a visual representative image (digital picture or sketch) of the plant [16], [17].

Pharmacognostic morphological identification includes the external character of plants [18]–[26]. Morphological studies of collected plant material involve close examination with naked eyes as well as under low magnification [27].

Anatomical characters

Distinct anatomy of any organism is useful to identify the respective organism. Permanent and temporary histological sectioning is done to study different arrangements, size and shape of different cells and tissues of the plant material for its identification. Close examination under microscope provide valuable information which is difficult to differentiate in macroscopic studies. A microscopic approach is developed to evaluate different anatomical characters of the plant such as the types of stomata, trichomes, types of vascular bundles, oil glands, canals, particular cell types, seed or pollen morphology, and vascular traces [28]–[36].

Physicochemical analysis

Herbal medicine powder reflects the amount of mineral content in the plant obtained from a particular geographical location. Microscopic analysis of powder study is used to differentiate between authentic material and the adulterant. This is done by analyzing it under microscope and comparing different components presents in the powder section of collected plant material with the certified one or which are documented earlier. Powder of herbs may contain foreign matter or different cellular organs like stomata, vascular bundles, spheroids, trichomes etc. Apart from powder studies, ash analysis, moisture content, extractive value in water ethanol and ether are also important in crude drug standardization [37]–[39].

Phytochemical analysis

Different parts of plants are known to have diverse medicinal properties due to the presence of different phytochemical group/s. The main bioactivity is due to secondary metabolites present in the plant tissues like alkaloids, phenolics, terpenoids,

flavonoids etc. Phytochemical analysis involves preliminary and confirmatory tests for identification and confirmation of a particular phytochemicals [40]–[42].

Pharmacology

Every secondary metabolite imparts different therapeutic action. The phytochemicals independently or synergistically accounts for the pharmacological function to cure or prevent diseases [43], [44]. Some examples of such activities are given in Table 2.1.

Table 2.1: Few plants with their phytochemicals and pharmacological actions

Plants	Secondary metabolite/s	Pharmacological action
Ephedra sinica	Ephedrine	Antiasthamatic
Vinca rosea	Vincristine	Anticancer
Curcuma longa	Curcumin	Anti-inflammatory
Papaver somniferum	Morphine	Analgesic
Strychnos nux-vomica	Strychnia and Brucia	Antiallergic
Datura mentel	scopolamine, hyoscyamine, and atropine	Antispasmodic, antidotes

2.2 Pharmacognostic studies of *Carica papaya* Linn.

The pharmacognostic characters related to the macroscopic studies are described in the chapter 1 section 1.1.1.

As *Carica papaya* leaves showed anti-thrombocytopenic activity, only leaves were subjected to pharmacognostic studies and the details of the study are described here:

2.2.1 Materials and methods

2.2.1.1 Collection and authentication of plant material

Carica papaya Linn. Collected in August 2010 from Vadodara, Gujarat, India, was identified and authenticated by Prof. M. Daniel at the Department of Botany, M. S.

University of Baroda, Gujarat, India. The voucher specimen of this plant (No. BARO/2010/51) was deposited at the Herbarium, BARO, Department of Botany, M S University of Baroda, Gujarat, India. The plant material was washed, shade dried for a day and then dried completely in an oven at 38 °C. The plants were coarsely powdered using a grinder and stored in air tight plastic containers, and used for phytochemical analysis. Fresh leaves were used for micromorphological and anatomical studies.

2.2.1.2 Chemicals

All chemicals and solvents used were of analytical reagent grade (AR). Methanol, petroleum ether, sulphuric acid, hydrochloric acid, glacial acetic acid, liquor ammonia, ethyl acetate, n-hexane, acetone, ethanol, chloroform, perchloric acid were purchased from the Merck (India). Karl fisher grade methanol and karl fisher reagent (pyridine free) was purchased from Sigma Aldrich India. Celite, Whatman paper 41, Whatman paper 1 was purchased from Merck India. Safranine, ninhydrin, glycerine, potassium hydroxide, sodium hydroxide, mercuric chloride, potassium iodide, iodine, bismuth carbonate, sodium citrate, sodium carbonate, sodium acetate, α-napthol, copper sulphate, copper acetate, sodium potassium tartarate, lead acetate, ferric chloride were purchased from Sigma Aldrich India. Photographs were taken using Leica DM 2000 microscope connected to a digital Canon camera.

2.2.2 Physicochemical parameters

All physicochemical parameters were performed as per the WHO standard procedures [45].

2.2.2.1 Total moisture content

The moisture content of powdered sample was carried out on Karl Fischer instrument (Lab India). The leaves were ground and sieved through a 0.84 mm sieve (20 mesh) and placed in a sealed jar. Initially 30 mL of Karl Fisher grade methanol were run into the vessel and titrated to dryness with Karl Fisher reagent (pyridine free). After 10 mins leave powder sample was titrated in the methanol against stabilized Karl Fisher

reagent. The determination of titration end point was done by polarized double-pin electrode and the moisture value (%) was displayed on the Karl Fisher titrator screen [46].

2.2.2.2 Extractive values

2.2.2.2.1 Water soluble extractive

5 g of air-dried powdered plant material (leaves) was added to 50 mL of water at 80 °C in a glass stoppered flask. It was shaken well and allowed to stand for 10 mins. It was cooled to 15 °C and filtered through celite filter. The filtrate was transferred to a tarred evaporating basin. The solvent was evaporated on a water bath for 30 mins and then dried in a steam oven. Water soluble extractive was calculated with reference to the air-dried powdered plant material.

2.2.2.2.2 Alcohol soluble extractive

5 g of air-dried powdered plant material (leaves) was macerated with 100 mL of alcohol in a closed flask for 24 h, shaken frequently for 6 h and allowed to stand for 18 h. The filtrate was evaporated to dryness in an evaporating dish and dried at 105 °C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried powered plant material.

2.2.2.2.3 Ether soluble extractive

5 g of air dried powdered plant material (leaves) was macerated with 100 mL of petroleum ether in a closed flask for 24 h, shaken frequently for 6 h and allowed to stand for 18 h. The filtrate was evaporated to dryness in an evaporating dish and dried at 105 °C and weighed. The percentage of ether soluble extractive was calculated with reference to the air-dried powered plant material.

2.2.2.3 Ash Analysis

2.2.2.3.1 Total ash value

5 g of air dried powdered plant material (leaves) was ignited in an electric furnace at 500–550 °C in a previously weighed silica crucible until the sample reached a constant weight. The total ash value was calculated with reference to the air-dried powdered plant material.

2.2.2.3.2 Water soluble ash value

0.50 g of the total ash obtained after ignition of the air dried plant material (leaves) was transferred to 100 mL beaker and treated with distilled water. The water insoluble matter was collected on an ashless filter paper (Whatman paper 41), washed with water repeatedly, dried and ignited in an electric furnace at 450 °C in a previously weighed silica crucible until reached a constant value. The weight of insoluble matter was subtracted from the weight of the total ash to indicate the weight of water soluble ash.

2.2.2.3.3 Acid Insoluble Ash Value

0.50 g of total ash obtained was treated with 25 mL of 1:1 dilute HCl for 10 mins. It was filtered in an ashless filter paper (Whatman No. 41) the residue was washed with distilled water till free from acid, dried and ignited in the furnace to get a constant weight. The acid insoluble ash value was calculated with reference to the total ash.

2.2.2.3.4 Sulphated ash value

0.50 g of total ash powder obtained after ignition of the air dried powdered plant material (leaves) was moistened with 1 mL of H_2SO_4 and ignited to 800 ± 25 °C in a previously weighed silica crucible until reached a constant weight. The sulphated ash value was calculated with reference to the total ash.

2.2.3 Qualitative phytochemical screening

The shade dried and coarsely powdered leaves were extracted successively with different solvents by using Soxhlet apparatus and analysed using simple chemical tests for preliminary screening of various groups of phytoconstituents such as alkaloids, flavonoids, phenolic acids, sterols, cardiac glycosides, tannins, and so on [47].

The following tests were performed on extracts to detect various phytochemical groups present in them.

2.2.3.1 Detection of Alkaloids

0.50 g of plant material was refluxed with 2 mL of dilute hydrochloric acid for 10 min on water bath, cooled and filtered. The filtrate was tested carefully with various alkaloidal regents as follows.

A. Mayer's Test

Mayer's Reagent was prepared by dissolving mercuric chloride (0.14 g) and potassium iodide (0.50 g) in 10 mL of distilled water.

Mayer's Test was performed by adding two drops of Mayer's reagent by the side of the test tube containing to 2 mL of filtrate. A white or creamy precipitate indicated the test to be positive.

B. Wagner's test

Wagner's reagent was prepared by dissolving iodine (0.13 g) and potassium iodide (0.20 g) in 10mL of water.

Wagner's test was performed by adding few drops of Wagner's reagent to a 2 mL of filtrate, by the side of the test tube. A reddish–brown precipitate confirmed the test as positive.

C. Dragendorff's test

For the preparation of Dragendorff's reagent, Solution A: 0.17 g of bismuth subnitrate in 2 mL glacial acetic acid diluted upto 10 mL with distilled water. Solution B: 4 g of potassium iodide was taken in 10 mL of glacial acetic acid and diluted upto 20 mL with water. Solution A and B were mixed together and diluted upto 100mL.

Dragendorff's test was performed by adding 2 mL of Dragendorff's reagent to 2 mL of filtrate. A prominent yellow precipitate indicated the test to be positive.

2.2.3.2 Detection of Carbohydrates

Decoction of 2 g of leaves material were prepared by 20 mins refluxing in 10 mL distilled water and filtered through Whatman no. 1 filter paper. The filtrate was subjected to the following tests.

A. Molish's test

Two drops of alcoholic solution of α-naphthol were added to 2 mL of filtrate, the mixture was shaken well and 1 mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

B. Fehling's test

Fehling's solution A was prepared by dissolving 0.35 g of Copper sulphate in 5.0 mL of distilled water. Fehling's solution B was prepared by dissolving potassium sodium tartarate (0.17 g) and sodium hydroxide (0.50 g) in 5.0 mL of water.

Fehling's test was performed by boiling 1 mL of filtrate with 1 mL each of Fehling solutions A and B on a water bath. A red precipitate indicated the presence of sugar.

C. Barfoed's test

Barfoed's reagent was prepared by dissolving 3.5 g copper acetate in 10 mL of glacial acetic acid.

Barfoed's test was performed by adding 1 mL of Barfoed's reagent to 1 mL of filtrate, followed by heating on a water bath for 2 mins. Red precipitate indicated presence of sugar.

D. Benedict's test

Benedict's reagent was prepared by dissolving sodium citrate (1.73 g) and sodium carbonate (1 g) in 8 mL of distilled water and mixing the clear solution with 1 mL aqueous solution of copper sulphate (0.17 g).

Benedict's test was performed by adding 0.5 mL of Benedict's reagent to 0.5 mL of filtrate and heating the mixture on a boiling water bath for 2 mins. A characteristic colored precipitate indicated the presence of sugar.

2.2.3.3 Detection of Proteins and Amino Acids

Decoction of 2 g of leaves material were prepared by 20 mins refluxing in 10 mL distilled water and filtered through Whatman No.1 filter paper. The filtrate was subjected to tests for proteins and amino acids.

A. Biuret test

An aliquot of 2 mL of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, pink color in the ethanolic layer indicated the presence of proteins.

B. Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of the filtrate. A characteristic purple color indicated the presence of amino acid.

2.2.3.4 Detection of Glycosides

For detection of glycosides, 5 g of water extract of *Carica papaya* leaves was hydrolyzed with 2 N concentrated hydrochloric acid for 2 h on water both, filtered through Whatman no. 1 filter paper and the hydrolysate was subjected to the following tests.

Borntrager's test

3 mL chloroform was added in 2 mL filtered hydrolysate, well shaken. The chloroform layer was separated and 10% ammonia solution was added to it, a pink color indicated the presence of glycosides.

2.2.3.5 Detection of Saponins by Foam Test

2g decoction of papaya leaves material was shaken in a graduated cylinder for 15 mins. 2 cm layer of foam indicated the presence of saponins.

2.2.3.6 Detection of Steroidal and Cardiac glycosides

A. Libermann – Burchard's test

The decoction of papaya leaves extract (2 mL) was dissolved in 2 mL acetic anhydride. To this, one or two drops of concentrated sulphuric acid were added slowly along the side of the test tube. An array of color changes showed the presence of Steroidal and Cardiac glycosides.

B. Salkowski's test

Salkowski's reagent was prepared by dissolving 2% of $FeCl_3$ in 35% of $HClO_4$ solution.

For Salkowski's test, 2 mL decoction leaves extract was treated with 2 mL of Salkowski's reagent. The appearance of yellowish color with green fluorescence indicated the presence of Steroidal and Cardiac glycosides.

2.2.3.7 Detection of Fixed Oils and Fats by Spot test

A small quantity of dried leaves was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

2.2.3.8 Detection of phenolic compounds and Tannins

A. Ferric chloride test

Five drops of neutral 5% ferric chloride solution were added in the decoction of papaya leaves (5 mL). A dark green color indicated presence of phenolic compounds.

B. Lead acetate test

The decoction of papaya leaves (3 mL) was diluted with distilled water and 3 mL of 10% lead acetate solution was added. A bulky white precipitate indicated presence of phenolic compounds.

2.2.3.9 Detection of Gum and Mucilage

The decoction of papaya leaves (2 mL) was dilute with 10 mL distilled water and 25 mL of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage.

2.2.4 Anatomy

Sections of fresh leaf were subjected to Safranine (1% in water) staining. The slides were then mounted and sealed using DPX. The slides were then observed under the microscope and the sizes of various cells observed in the tissues were measured using an ocular micrometer. The least count of the micrometer was calculated for this purpose. The sections were photographed under a Leica DM 2000 microscope connected to a digital Canon camera.

2.2.5 Micromorphology

Fresh leaves were washed and small fragments of leaves were taken from the middle region of the lamina of mature leaves. For anatomical studies, sections of 10–12μm thick were prepared and stained with Safranine (0.5%) in water and then mounted in 50% glycerine. Clearing of leaf was done to study the venation pattern. Washed leaf fragments were first boiled in 90% alcohol for about 3-5 mins to remove chlorophyll, washed 2-3 times with water, boiled again with 10% KOH solution for 2-3 mins and washed 4-5 times with water. The epidermal layer was peeled off using a pointed needle and forceps and was washed in water, stained with Safranine (0.5%). The margins of the cover slips were sealed with DPX, and the slides were observed under the microscope. Stomatal index, palisade ratio, vein termination number and vein islet number were calculated using standard procedures.

2.2.6 Histochemical tests

Specific reagents for identification of important classes of compounds were prepared according to procedures prescribed in the WHO guidelines [45]. Sections of midrib as well as cleared sections of the lamina were treated with various reagents and mounted on slides for observation under a microscope.

2.2.7 Powder studies

Completely dried plant material was finely powdered and sieved through BSS mesh No. 85. The fine powder was stained with Safranine in water. The stained powder was mounted on a slide and observed under a microscope to locate and identify the characters present. The characters observed were photographed under a Leica DM 2000 microscope connected to a digital Canon camera.

2.3 Results and discussion

2.3.1 Physicochemical parameters

The results of physicochemical parameters namely percentage of moisture content, total ash, acid insoluble ash, water soluble ash, ethanol, ether and water soluble extractive are given in Table 2.2 and Table 2.3.

Water, ethanol and ether soluble extractive value indicate richness of polar and non polar phytochemical contents.

Table 2.2: Various extractive values of *Carica papaya* leaves

Parameter	**Mean ± SD (%)**
Moisture content	05.70 ± 0.05
Water soluble extractive	17.72 ± 0.06
Ether soluble extractive	03.30 ± 0.05
Ethanol soluble extractive	08.40 ± 0.09

The water present in plant tissues is present as free and bound water. Bound water consists of organic matter, hygroscopic water, imbibed water and adsorbed water. Total moisture content represents the water content of vegetal material. Total ash content represents the amount of minerals present in the plant sample.

Table 2.3: Ash analysis of *Carica papaya* leaves

Parameter	Mean ± SD (%)
Total ash	17.45 ± 0.13
Acid insoluble ash	13.30 ± 0.09
Water soluble ash	03.25 ± 0.14
Sulphated ash	27.05 ± 0.07

2.3.2 Preliminary phytochemical screening

The results of preliminary phytochemical screening are given in Table 2.4.

Table 2.4: Results of phytochemical tests performed on powder of papaya leaf

Group of phytoconstituents	Observations
Carbohydrates	+
Gums and mucilages	-
Proteins	+
Amino acids	+
Steroids	-
Cardiac glycosides	+
Anthraquinone glycoside	-
Saponin glycosides	+
Flavonoids	+
Alkaloids	+
Tannins	+
Phenolics	+
Iridoids	+
Anthocyanins	-

The primary screening of phytochemicals revealed the presence of carbohydrates, proteins, amino acids, saponin glycosides, iridoids, flavonoids, phenolics and alkaloids.

2.3.3 Anatomy

The transverse section of *Carica papaya* Linn. leaf (Figure 2.1) shows that the leaf was dorsoventrally flattened. The mid rib portion was almost spherical with a large portion on the lower side. The leaf was glabrous without any hairs on either side. The lamina segregated into upper epidermis, mesophyll and lower epidermis. Epidermis consists of barrel shaped cells (8 µm x 4 µm) below which there were layers of collenchyma. Vascular bundles were of collateral closed arrangements. The two vascular bundles were arranged in a ring, positioned one on the upper side and other on the lower side. The other bundles were small and represented by 3-4 tracheids. Xylem consists of tracheids (3.96 µm-10.56 µm) only and vessels were absent. The phloem was represented by radially elongated patches of cells (1.98 µm-3.3 µm) separated from one another by parenchyma cells, some of which contain chlorophyll. Between the bundles and epidermis was a broad band of collenchyma. The central portion was represented by a hollow region. Articulated laticiferous canal accompany the vascular bundles of the veins and extend into the surrounding mesophyll. A large number of sphaeraphides (2.64 µm- 6.6 µm) were seen throughout the collenchymatous cortex and parenchymatous ground tissue. In the laminal region mesophyll was differentiated into upper palisade and lower spongy. Palisade was two layered, each cell having dimensions (10 µm x 2 µm). The spongy tissue consisted of 5-6 layers of closely packed mesophyll cells (6 µm x 4 µm). The section also showed the presence of prismatic calcium oxalate crystal.

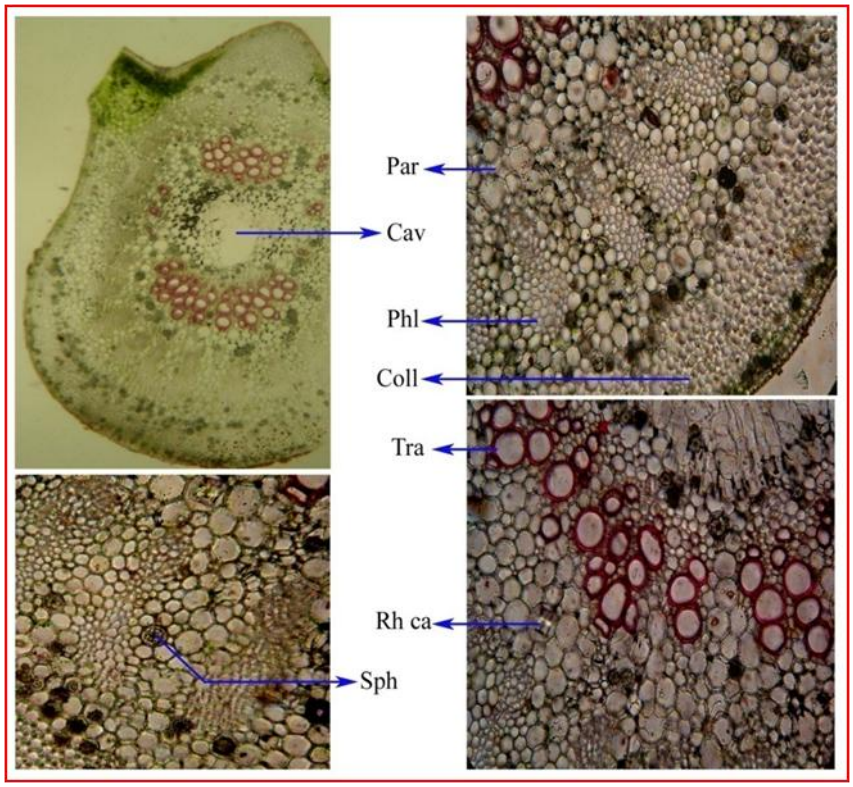

Figure 2.1: Transverse section of leaf of *Carica papaya*, showing the presence of **Par**- parenchyma, **Cav**- Hollow cavity, **Phl**- phloem, **Coll**- collenchyma, **Tra**- tracheids, **Rh ca**- Rhomboidal calcium oxalate crystals, and **Sph**- sphaeraphides.

2.3.4 Micromorphology

Vein termination number in *Carica papaya* leaf showed values close to 3-4, stomatal index was calculated to be 31.56±3.41, while the palisade ratio was calculated as 12.65±1.57. The leaf showed a continuous network of veins hence vein islet number was found to be zero. Anomocytic stomata were found restricted only to the lower epidermis (Figure 2.2).

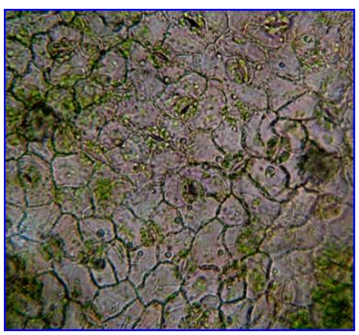

Figure 2.2: Lower epidermis of leaf of *Carica papaya* showing the presence of anomocytic stomata

2.3.5 Histochemical studies

Histochemical test showed [Table 2.5] presence of lignified cell wall, in which cell wall get stained due to presence of lignin (phenolic polymer). On addition with HCl effervescences of carbon dioxide were seen on section slide. Starch, the principal ergastic substance of the protoplast, gives black stain with iodine solution, were observed to be radially arranged. No yellow stain was present in the section slide shows absence of tannin group. Yellow to brown stain with dragendorff's reagent represented the presence of alkaloidal group in the protoplasm of the section. Presence of cellulose in the section was confirmed, as it remained unaffected on application of sulfuric acid, while other parts were decomposed.

Table 2.5: Results of histochemical tests performed of sections of *Carica papaya* leaf

Cells contents	Reagent used	Observations
Lignified cell walls	Phloroglucinol + HCl	+
Calcium oxalate	HCl	+
Starch	Iodine	+
Tannins	$FeCl_3$	-
Cellulose	Iodine + H_2SO_4	+
Alkaloids	Dragendorff	+
Aleurone grains	Iodine	-

2.3.6 Powder studies

Sphaeraphides, starch grains and rhomboidal calcium oxalate crystals form diagnostic characters in the leaf powder of *Carica papaya*. Other regular characters like stomata and spiral xylem were also found in the powder (Figure 2.3).

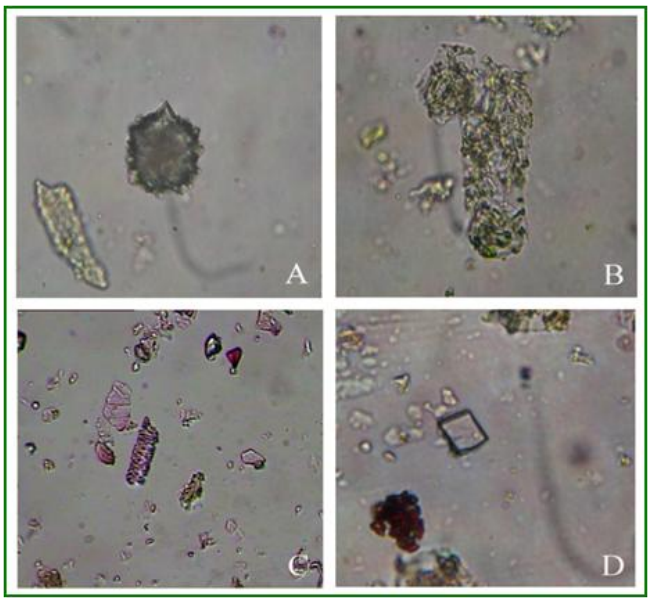

Figure 2.3: Powder of *Carica papaya* showing **A**- Sphaeraphide, **B**- Stomata, **C**- Spiral xylem, starch grains, **D**- Rhomboidal calcium oxalate crystal.

2.4 Conclusion

The present work was undertaken to produce some pharmacognostical standards for the leaves of *Carica papaya*. The study provided following information with respect to identification, chemical constituents and physicochemical characters useful for the standardization of *Carica papaya* leaves.

a) The presence of sphaeraphides, starch grains and rhomboidal calcium oxalate crystals form diagnostic characters in the leaf powder of *Carica papaya*.

b) The *Carica papaya* leaf has a continuous network of veins with zero islet number and 3-4 vein termination number. It has stomatal index of 31.56±3.41and palisade ratio of 12.65±1.57 with anomocytic stomata at the lower epidermis.

c) The *Carica papaya* leaf contains saponin, phenolics and alkaloids.

References

[1] T. Makino, "Introduction of clinical pharmacognosy integration between pharmacognosy and clinical pharmacy," *J. Pharm. Soc. Japan*, vol. 131, no. 3, pp. 375–382, 2011.

[2] N. Dhami, "Trends in Pharmacognosy: A modern science of natural medicines," *Journal of Herbal Medicine*, vol. 3, no. 4. pp. 123–131, 2013.

[3] W. C. Evans, *Trease and Evans Pharmacognosy*. W.B. Saunders, 2002.

[4] J. D. Phillipson, "Phytochemistry and pharmacognosy," *Phytochemistry*, vol. 68, no. 22–24. pp. 2960–2972, 2007.

[5] K. Mangathayaru, *Pharmacognosy: An Indian perspective*. Pearson Education India, 2013.

[6] P. De Vos, "European Materia Medica in Historical Texts: Longevity of a Tradition and Implications for Future Use," *Journal of ethnopharmacology*, vol. 132, no. 1. pp. 28–47, Oct-2010.

[7] B. D. Davis, "The Scientist's World," *Microbiol. Mol. Biol. Rev.*, vol. 64, no. 1, pp. 1–12, Mar. 2000.

[8] M. M. Iwu, *Handbook of African Medicinal Plants, Second Edition*. Taylor & Francis, 2014.

[9] L. Bohlin, U. Göransson, C. Alsmark, C. Wedén, and A. Backlund, "Natural products in modern life science," *Phytochem. Rev.*, vol. 9, no. 2, pp. 279–301, 2010.

[10] I. E. Orhan, "Pharmacognosy: Science of natural products in drug discovery," *BioImpacts*, vol. 4, no. 3, pp. 109–110, 2014.

[11] J. K. H. Gor and H. K. Jain, "Bioactive Natural Products: Isolation Identification and Evaluation," *Asian J. Biochem. Pharm. Res.*, vol. 3, no. 3, pp. 56–69, 2013.

[12] S. B. Gokhale, *Pharmacognosy*. Nirali Prakashan, 2008.

[13] S. H. Afaq and M. M. H. Siddiqui, *Pharmacognosy, Phytochemistry, Pharmacology & Clinical Studies of Unani Medicinal Plants*, no. v. 1. A.K. Tibbiya College, Aligarh Muslim University, 1984.

[14] N. Prakashan, *Pharmacognosy*. Nirali Prakashan, 2009.

[15] O. Folashade, H. Omoregie, and P. Ochogu, "Standardization of herbal medicines -A review," *Int. J. Biodivers. Conserv.*, vol. 4, no. 3, pp. 101–112, 2012.

[16] T. J. Smillie and I. a Khan, "A comprehensive approach to identifying and authenticating botanical products.," *Clin. Pharmacol. Ther.*, vol. 87, no. 2, pp. 175–186, 2010.

[17] C. Howard, E. Socratous, S. Williams, E. Graham, M. R. Fowler, N. W. Scott, P. D. Bremner, and A. Slater, "PlantID – DNA-based identification of multiple medicinal plants in complex mixtures," *Chin. Med.*, vol. 7, p. 18, Jul. 2012.

[18] D. Datta, *College Botany*, no. v. 1. New Central Book Agency (P) Limited, 1968.

[19] W. L. Tedders, U. S. Science, and E. Administration, *Important Biological and Morphological Characteristics of the Foliar-feeding Aphids of Pecan*, no. v. 1578-1587. Department of Agriculture, Science and Education Administration, 1978.

[20] R. E. Meyer, S. M. Meola, U. S. A. R. Service, and T. A. E. Station, *Morphological Characteristics of Leaves and Stems of Selected Texas Woody Plants*. Department of Agriculture, Agricultural Research Service, 1978.

[21] B. L. Gartner, *Plant Stems: Physiology and Functional Morphology*. Elsevier Science, 1995.

[22] F. Weberling and R. J. Pankhurst, *Morphology of Flowers and Inflorescences*. Cambridge University Press, 1992.

[23] A. D. Bell and A. Bryan, *Plant Form: An Illustrated Guide to Flowering Plant Morphology*. Timber Press, 2008.

[24] O. W. Caldwell, *Handbook of Plant Morphology: Being the Handbook of Plant Dissection*. H. Holt, 1904.

[25] S. R. Mishra, *Morphology of Plants*. Discovery Publishing House, 2004.

[26] H. H. Rusby and S. E. Jelliffe, *Morphology and Histology of Plants: Designed Especially as a Guide to Plant-analysis and Classification, and as an Introduction to Pharmacognosy and Vegetable Physiology*. The authors, 1899.

[27] E. B. Banning, "Analysing Plant Remains," in *The Archaeologist's Laboratory*, R. S. D. J. Michael A. Jochim, Ed. Springer US, 2002, pp. 213–233.

[28] N. S. Subrahmanyam, *Modern Plant Taxonomy, 1E*. Vikas Publishing House Pvt Limited, 2009.

[29] G. Erdtman, *Pollen Morphology and Plant Taxonomy: Angiosperms*. E.J. Brill, 1986.

[30] C. A. Stace, *Plant Taxonomy and Biosystematics*. Cambridge University Press, 1991.

[31] O. P. Sharma, *Plant taxonomy*. Tata Mcgraw-hill education private ltd, 2011.

[32] C. Jeffrey, *An Introduction to Plant Taxonomy*. Cambridge University Press, 1982.

[33] T. F. Stuessy, *Plant Taxonomy: The Systematic Evaluation of Comparative Data*. Columbia University Press, 2009.

[34] D. R. Walters and D. J. Keil, *Vascular Plant Taxonomy*. Kendall/Hunt Publishing Company, 1996.

[35] R. Gupta and E. R. Institute, *Plant Taxonomy: past, present, and future*. Teri Press, Energy and Resources Institute, 2012.

[36] V. V Sivarajan and N. K. P. Robson, *Introduction to the Principles of Plant Taxonomy*. Cambridge University Press, 1991.

[37] S. B. Gokhale and C. K. Kokate, *Practical Pharmacognosy*. Nirali Prakashan, 2008.

[38] S. Chanda, "Importance of pharmacognostic study of medicinal plants: An overview," *J. Pharcognosy Phytochem.*, vol. 2, no. 5, pp. 69–73, 2014.

[39] V. Sanmugarajah, I. Thabrew, and S. R. Sivapalan, "Phyto , Physicochemical Standardization of Medicinal Plant Enicostemma littorale, Blume," *IOSR J. Pharm.*, vol. 3, no. 2, pp. 52–58, 2013.

[40] P. Tiwari, B. Kumar, M. Kaur, and H. Kaur, "Phytochemical screening and Extraction: A Review," *Int. Pharm. Sci.*, vol. 1, no. 1, pp. 98–106, 2011.

[41] T. Ahmad, S. Singh, and S. Pandey, "Phytochemical Screening and Physicochemical Parameters of Crude Drugs: A Brief Review," *Int. J. Pharma*

Res. Rev., vol. 2, no. 12, pp. 53–60, 2013.

[42] C. Sánchez-Moreno, "Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems," *Food Sci. Technol. Int.*, vol. 8, no. 3, pp. 121–137, 2002.

[43] B. Wang, J. Deng, Y. Gao, L. Zhu, R. He, and Y. Xu, "The screening toolbox of bioactive substances from natural products: A review," *Fitoterapia*, vol. 82, no. 8, pp. 1141–1151, 2011.

[44] K. P. Mishra, L. Ganju, M. Sairam, P. K. Banerjee, and R. C. Sawhney, "A review of high throughput technology for the screening of natural products," *Biomed. Pharmacother.*, vol. 62, no. 2, pp. 94–98, 2008.

[45] W. H. O. (WHO), *Quality control methods for herbal materials*. 2011.

[46] W. Horwitz, G. W. Latimer, and A. International, *Official Methods of Analysis of AOAC International*. AOAC International, 2005.

[47] J. B. Harborne, *Phytochemical Methods: A guide to modern techniques of plant analysis*, Second Edi. London, New York: chapman and hall, 2013.