Pharmacognostic, Physicochemical and Phytochemical Studies on *Carica papaya* Linn. Leaves

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**Abstract**

Papaya (*Carica papaya* Linn.) is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine. During the last few decades considerable progress has been achieved regarding the biological activity and medicinal application of papaya and now it is considered as valuable nutraceutical fruit plant. The present study deals with the microscopic evaluation of leaves of *Carica papaya* Linn., to establish the salient diagnostic features for the leaf. The leaf shows abundant sphaeraphides and rhomboidal calcium oxalate crystals. The leaf shows no trichomes. Micromorphological studies conducted on the leaf gave value of stomatal index to be 31.56 ± 3.41, vein termination number 3-4, and palisade ratio to be 12.65 ± 1.57. The leaf shows a continuous network of veins. Histochemical tests performed indicate the presence of alkaloids and starch. Powder study shows the presence of sphaeraphides, starch grains and rhomboidal calcium oxalate crystals, apart from regular characters such as stomata and spiral xylem. Physicochemical parameters such as extractive values, ash values and moisture content have also been studied for the leaf. The results of this study could be useful in setting some diagnostic indices for identification, authentication and preparation of the monograph of the plant.

**Key words:** *Carica papaya*, anatomy, micromorphology, sphaeraphides

**Introduction**

*Carica papaya* Linn. (Caricaceae) is a fast-growing, semi-woody tropical herb reaching 3-10 m in height. The fleshy stem is single, straight and hollow and contains prominent leaf scars. Papaya exhibits strong apical dominance rarely branching unless the apical meristem is removed, or damaged. *Carica papaya* contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Papain also is used to treat arthritis. The level of the compounds varies in the fruit, latex, leaves, and roots. Since, each part of papaya tree possesses economic value; it is grown on commercial scale.[1]

The present investigation of *Carica papaya* Linn leaves was taken up to establish pharmacognostic profile which will help in crude drug identification as well as standardization of the quality and purity of the drug in crude form. The present study comprises the macroscopical, microscopical and phytochemical studies of the leaves of *Carica papaya* Linn., since no proper report is available on the pharmacognosy and anatomy of the leaf of the plant.

The ash content of a crude drug is the inorganic residue remaining after incineration. It includes not only the inorganic salts, e.g. Calcium oxalate, occurring naturally in the drug; but also inorganic matter from external sources.[2] Four types of ash values used in routine pharmaceutical analysis are Total ash content, Acid insoluble ash, Water soluble ash and Sulphated ash. **Total ash content** - A figure for total ash content is useful when the contamination with calcium oxalate is very little. If more quantity of calcium oxalate is present, then the value for the acid insoluble ash is a better criterion of purity. **Acid insoluble ash** - Crude drugs containing larger quantity of calcium oxalate, can give variable results depending upon the conditions of ignition. Treatment of the ash with Hydrochloric acid leaves virtually only silica. Hence acid insoluble ash forms a better test to detect and limit excess of soil present as an impurity.
in the drug, than does the total ash. **Water soluble ash** - It is a measure of detection of water soluble impurities in drug or raw material. **Sulphated ash** - The determination of sulphated ash is widely used to control the extent of contamination by non-volatile inorganic impurities in organic substances. For sulphated ash, the substance is ignited using small quantities of sulphuric acid, which decomposes and oxidises the organic matter, leaving a residue of inorganic sulphates. Reproducible results are readily obtained in this determination than in total ash determination, due to the higher stability of metal sulphates.

Plants and their parts such as roots, stems, barks, leaves, flowers, fruits, seeds and exudates constitute major portion of drugs used in traditional herbal systems of medicine. The therapeutic efficiency of the drugs used in these systems greatly depends on the use of proper and genuine raw materials. Due to this reason, the assurance of safety, quality and subsequent efficacy of the medicinal plants and herbal products has now become a major and key issue. Both the general public as well as health care professionals require updated authoritative information on the safety and efficacy of medicinal plants.[3] Pharmacognosy includes mainly micromorphological, anatomical and powder studies, though TLC fingerprinting also is an essential feature.

Ayurvedic Pharmacopoeia Committee constituted by the Department of AYUSH recently has introduced certain quality parameters for the plants used in Indian System of Medicines. It is now important for the manufacturers of these drugs, to confirm that their raw materials are identified to be genuine. It is a mistaken notion that the classical procedures are obsolete and only chemo-taxonomical profile can provide a true identity of the drug and thus distinguish them from substitutes and adulterants. It is observed that the classical procedure of powder microscopy is still a useful tool for species identification and can play a pivotal role in the authentication of the drug material.[4]

**MATERIALS AND METHODS**

**Collection and authentication of plant material**

*Carica papaya* Linn. Collected in August 2010 from Vadodara, Gujarat, India, was identified and authenticated 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. 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 rotary grinder and stored in airtight plastic containers, and then used for phytochemical analysis. Fresh leaves were used for micromorphological and anatomical studies.

**Physicochemical parameters**

Physicochemical parameters such as extractive values, ash values and moisture content, were performed as per the official standard procedures.[5,6]

**Anatomy**

Sections of fresh leaf were subjected to staining using Safranine (1% in water). 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.

**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 10-12 μm 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 minutes to remove chlorophyll, then washed 2-3 times with water, then boiled again with 10% KOH solution for 2-3 minutes and washed 4-5 times with water. The epidermal layer was peeled off using the help of 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 then calculated using standard procedures.[7,8]

**Powder studies**

Completely dried plant material was finely powdered and sieved through BSS mesh No. 85. The fine powder obtained was stained using 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.

**Histochemical tests**

Specific reagents for identification of important classes of compounds were prepared according to procedures prescribed in the WHO guidelines. Sections of midrib as well as cleared sections of the lamina were treated with these reagents and mounted on slides for observation under a microscope.[6]

**Preliminary 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, as per WHO guidelines.\[6,9\]

RESULTS AND DISCUSSION

Physicochemical parameters
Physicochemical parameters like percentage of moisture content, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and water soluble extractive were determined and depicted in Table 1.

Anatomy
The transverse section of *Carica papaya* Linn. leaf (Plate 1) shows that the leaf was dorsiventral. 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 consist of barrel shaped cells (8 µm × 4 µm) below which there were layer of collenchyma. Vascular bundles was of collateral closed arrangement. Vascular bundles were arranged in a ring with two vascular bundles positioned one on the upper side and other on the lower side. The other bundles were small and represented by 3-4 tracheids. Xylem consist 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 canals 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 × 2 µm). The spongy tissue consisted of 5-6 layers of closely packed mesophyll cells (6 µm × 4 µm). The section also showed the presence of prismatic calcium oxalate crystal.

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 (Plate 2).

Powder study
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 (Plate 3).
standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physicochemical parameters. The present work is undertaken to produce some pharmacognostical standards for *Carica papaya*. The above studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be useful for pharmacognostical study and standardization for the plant.

**REFERENCES**


**Table 3: Results of phytochemical tests performed on powder of *Carica papaya***

<table>
<thead>
<tr>
<th>Group of phytoconstituents</th>
<th>Observations</th>
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<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Gums and mucilages</td>
<td>–</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
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<tr>
<td>Amino acids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>–</td>
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<tr>
<td>Cardiac glycosides</td>
<td>+</td>
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<tr>
<td>Anthraquinone glycosides</td>
<td>–</td>
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<tr>
<td>Saponin glycosides</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
<td>+</td>
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<tr>
<td>Tannins</td>
<td>+</td>
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<td>Phenolics</td>
<td>+</td>
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<tr>
<td>Iridoids</td>
<td>+</td>
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<td>Anthocyanins</td>
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**Histochemical tests**

The results of histochemical detection are furnished in Table 2.

**Preliminary phytochemical screening**

Preliminary phytochemical screening revealed the presence of carbohydrates, amino acids, saponin glycosides, iridoids, flavonoids, phenolics, and alkaloids (Table 3).

**CONCLUSIONS**

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopeia, these...