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An Identity Based Pharmacognostical Profile of *folium Annona squamosa*

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Abstract – The leaves of *Annona squamosa* Linn. (Annonaceae), commonly called as custard apple, are popularly used for the treatment of diabetes by various tribes in India. The leaves are also used for their powerful insecticidal activity, especially for destroying lice. This paper presents a detailed pharmacognostical study of the crude drug *folium Annona squamosa*. The samples were studied using procedures of light, confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *Annona squamosa* leaf and may possibly help to differentiate the drug from its adulterants.

Keywords – *Annona squamosa,* Adulterants, Antidiabetic, Pharmacognosy

Introduction

Annona squamosa Linn. (Annonaceae) (Warrier *et al.*, 1996), commonly known as custard apple, is an important plant in the Indian system of medicine, especially for its haemetinic and tonic nature of the edible fruit pulp (Kirtikar and Basu, 1975). The leaf is reported to be used in diabetes (Atta-Ur-Rahman and Zaman, 1989; Atique *et al.*, 1985; Topno, 1997), hyperthyroidism (Sunanda and Anand, 2003), as an abortifacient (Nadkarni, 2000), insecticidal (Satyavati *et al.*, 1976; Mukerjea and Govind, 1958), and suppurative (Kirtikar and Basu, 1975). Various phyto-constituents namely samoquasine A (Yang *et al.*, 2003; Morita *et al.*, 2000), squamocin-O1and O2 (Araya *et al.*, 2002), cyclosquamosins A-G (Morita *et al.*, 1999), (2,4-cis and trans)-squamolinone (Hopp *et al.*, 1998), squamotacin (Hopp *et al.*, 1996), annonins (Nonfon *et al.*, 1990), squamostatin-A (Fujimoto *et al.*, 1990), bullatacin, bullatacinone and squamone (Li *et al.*, 1990), have been isolated from the plant. The leaf also contains alkaloids (Bhaumik *et al.*, 1979; Bhakuni *et al.*, 1972; Trimurti, 1924), flavonoids, phytosterols, mucilage and terpenes (Shirwaikar *et al.*, 2004).

The leaf is used by different Unani and allopathic physicians for the treatment of diabetes (Atique *et al.*, 1985). Many tribes who live in parts of Northern India also use the leaf of this plant for the same (Topno, 1997). The uses of this plant have been scientifically supported and proved by different workers (Shirwaikar *et al.*, 2004a; Shirwaikar *et al.*, 2004b; Gupta *et al.*, 2005a; Gupta *et al.*, 2005b), which has eventually led to the increase in the use of this drug by physicians of different alternative systems of medicine.

The demand for this drug has led to the increase in the adulteration of *Annona squamosa* with inferior species like *Annona reticulata* and other species resembling the genuine drug. However, there is no pharmacognostical report of the leaf or the plant in whole, particularly to determine the anatomical and other physico-chemical standards required for the quality control of the crude drug. Hence the present study includes morphological and anatomical evaluation, determination of physico-chemical constants and the preliminary phytochemical screening of the different extractives of the leaves of *Annona squamosa.*

Experimental

The leaves of *Annona squamosa* were collected from Painkulam village in Tamil Nadu, (India) during the month of July (30°C ± 2°C, rainy weather, located 100 m above mean sea level). The plant was identified by Dr. N. Stephen Julius, Botanist, Nesamony Memorial College of Arts and Science, Marthandam (India). A reference voucher specimen (PP 519) classified as cultivated *Annona squamosa*, Annonaceae has been deposited in the herbarium of Department of Pharmacognosy, MCOPS, Manipal (India).

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The macroscopy and morphology of the plant were studied according to the method of Brain and Turner, (1975a). For the microscopical studies, cross-sections were prepared and stained as per the procedure of Johansen, (1940). The micropowder analysis was done according to the method of Brain and Turner, (1975b) and Kokate, (1986a). Leaf constants (Evans, 2003), *viz*; vein islet number, vein termination number, palisade ratio and stomatal index were also studied.

Physico-chemical values such as the percentage of ash values and extractive values were performed according to the official methods prescribed (Indian Pharmacopoeia, 1996) and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992). Preliminary phytochemical screening (Chase and Pratt, 1949; Kokoski *et al.*, 1958) was done for different extractives which were prepared by successive exhaustive solvent extraction of the powdered drug with different known solvents in increasing order of polarity *viz*; hexane, petroleum ether, solvent ether, n-butanol, chloroform, ethanol and finally with chloroform water (Kokate, 1986b). All the other standards *viz;* swelling index, bitterness value, foaming index, mucilage content and moisture content were done in compliance with the prescribed quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992).

The different materials used for the study include basic microscopical equipments *viz*; compound microscope, stage micrometer, camera lucida, drawing sheets, glass slides, coverslips, watch glass and other common glass wares. The microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Common solvents *viz*; ethanol (95%), hexane, petroleum ether, solvent ether, n-butanol, chloroform and reagents *viz*; phloroglucinol, glycerin, hydrochloric acid, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals, Mumbai (India).

Results and Discussion

Morphological description of aerial part of the plant (Fig. 1) – The plant grows about 4-6 m high; leaves 3.8-15.2 cm in length and 1.8-5.5 cm in breadth; lanceolate; acute or sub-acute; pellucido-punctate, glabrous above; glaucous and pubescent beneath when young; venation pinnately reticulate; lateral nerves 8-11 pairs; petioles 12 mm in length; flowers solitary, leaf opposite; extra-axillary branchlets; pedicels 12-19 mm in length, bracteate below the middle; sepals minute, triangular and pubescent; petals pubescent on both surfaces; fruit globose, 5-10 cm in diameter usually with a glaucous bloom on the surface when young, yellowish green when ripe, easily broken into large pieces, areoles well marked, granulate or tuberculate, 5-6 gonous; seeds brownish black and smooth. Annona reticulata (Yoganarasimhan, 2000), a common adulterant of Annona squamosa can be differentiated by the leaves which are slightly longer and grows up to 23 cm in length and 5.5 cm in breadth.

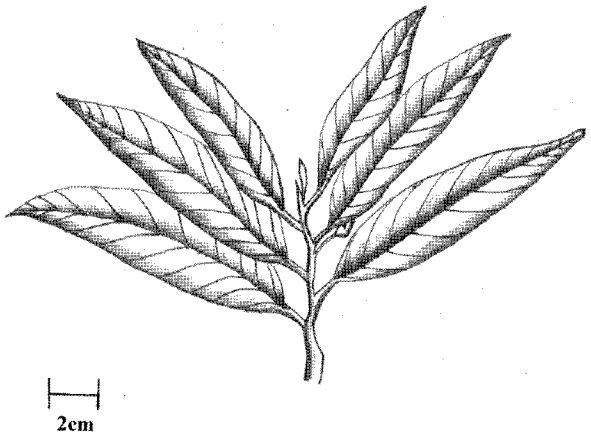

Fig. 1. Macroscopy of the aerial aspect of *Annona squamosa.*

Anatomical characteristics of the leaf (Fig. 2) – The transverse section of *folia Annona squamosa* shows a dorsiventral nature. The section is broadly divided into the lamina and the midrib. The lamina of the leaf shows three distinct regions *viz*; upper epidermis, lower epidermis and the mesophyll. The upper epidermis is single layered with straight walled cells which are more or less rectangular in shape. It is continuous and is coated by a wavy cuticular thickening with simple or stellate hairs. Stomata are absent on the upper epidermis. The lower epidermis is continuous and has a wavy cell wall, interrupted by the presence of stomata frequently. Simple or stellate hairs are also found on the lower epidermis. However, the cuticular thickening is not very well represented. Some of the cells of the epidermis both on the upper and lower surfaces contain single stellate, prismatic, square or cluster crystals. In comparison, the epidermal cells of the adulterant, *Annona reticulata* are larger and broad. A distinctive feature is that each upper epidermal cell of this species contains not less than one cluster crystal of calcium oxalate.

The mesophyll tissue consists of palisade and spongy parenchyma. The upper palisade layer is made up of two layers of vertically arranged compact tubular palisade cells containing chloroplastids. Few starch grains are also seen in the palisade cells. The lower bulk of the mesophyll tissue is made up of spongy tissue, which is comprised of oval and circular spongy parenchyma cells, enclosing

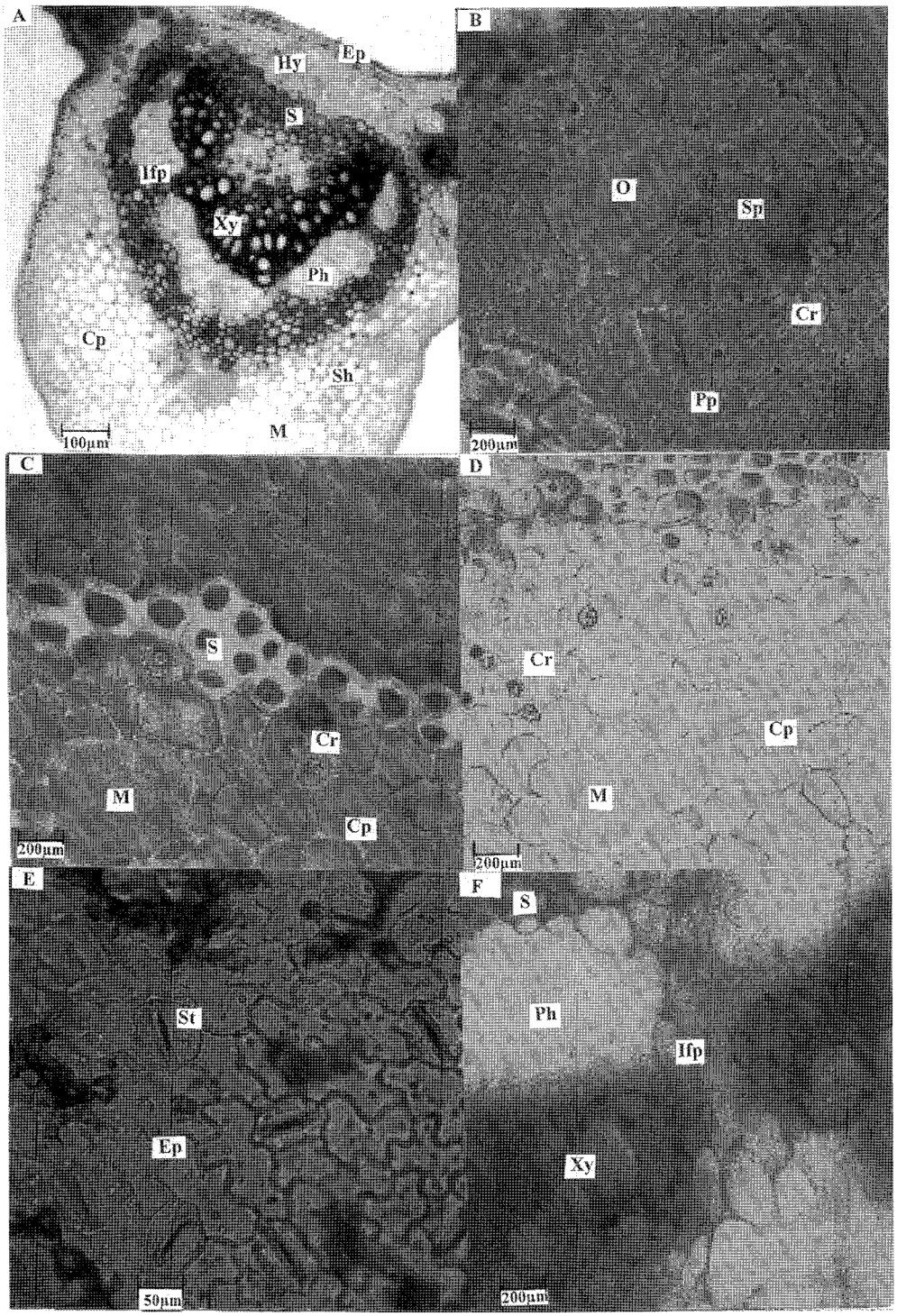

Fig. 2. Microscopy of *folium Annona squamosa* **Fig. 2A**-Photomicrograph showing transverse section of *Annona squamosa* **Fig. 2B**-A portion of lamina (enlarged) **Fig. 2C**-Section showing a sheath of stone cells **Fig. 2D**-Photomicrograph of the cortex region of *Annona squamosa* **Fig. 2E**-A section showing stomata **Fig. 2F**-A section showing interfascicular parenchyma **Cr**-Crystals, **Cp**-Cortical parenchyma, **Ep**-Epidermis, **Hy**-Hypodermis, **Ifp**-Interfascicular parenchyma, **M**-Mucilage cell, **O**-Oil sac, **Ph**-Phloem, **Pp**-Palisade parenchyma cell, **S**-Stone cells, **Sh**-Starch, **Sp**-Spongy parenchyma cell, **St**-Stomata, **Xy**-Xylem.

intercellular spaces filled with air. In the spongy region there are scattered groups of sclereids with thick cell walls, which provide mechanical stability to the leaf. The spongy parenchyma cells also contain chloroplasts and

starch grains. Present are vascular strands with closed collateral bundles which get distributed in a reticulate fashion in the lamina. These specialized structures conduct water and food to the leaf. Unlike the double layered palisade of *Annona squamosa*, the palisade cells of *Annona reticulata* are single layered and therefore serve as an important distinguishing character.

In the midrib, which is prominent, below the upper epidermis and above the lower epidermis there are one or two layers of collenchyma cells, which constitute the hypodermis. Below the upper collenchyma and above the lower collenchyma there are cells of medulla, which are parenchymatous enclosing lysigenous secretory cavities which help in the storage of mucilaginous content. There are groups of sclereids in the upper part of the medulla, which also provides mechanical strength. The vascular bundles are distributed in the form of an arc open towards the upper epidermis. The number of vascular cylinders varies from 8-12. The vascular bundles are collateral, closed, exarch with xylem pointing towards the lower side, having vessels with reticulate thickening. The phloem is towards the lower epidermis and consists of sieve tubes, companion cells and phloem parenchyma. Below the phloem there are sclerenchymatous fibre strands capping each vascular bundle. They also provide mechanical support to the leaf. The central part of the vascular bundle is enclosed by parenchyma, which has starch grains in plenty. The vascular bundles are radially traversed by interfascicular or intervascular parenchyma connecting the central part of the medulla to the outer part of the midrib.

Study of powder (Fig. 3) – The leaf powder is grayish green or dull green in colour with an unpleasant odour and mucilaginous taste. On microscopical examination the powder showed numerous sickle shaped unicellular and multicellular warty and covering trichomes which are lignified, sometimes stellate, broken and rarely collapsed. Paracytic or rubiaceous type of stomata with irregularly shaped epidermal cells are seen. Palisade cells are seen in association with mucilage ducts and veins. Prismatic and cluster calcium oxalate crystals are present in sheath of cells around the fibres. A number of stone cells, which are lignified are seen isolated or in groups. Concentric starch grains are also seen. Apart from these characters, oil sacs are seen at intervals. There are also a few lignified xylem vessels and non-lignified phloem cells. On preliminary phytochemical screening (Table 1) the powder showed the presence of alkaloids, flavonoids, terpenes, volatile oil, resins and phytosterols.

Physico-chemical constants – Ash values of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 2) of the powdered *Annona squamosa* leaf revealed a high concentration of sulphated ash (9.80%), whereas acid insoluble ash was found to be very low since the drug was collected afresh.

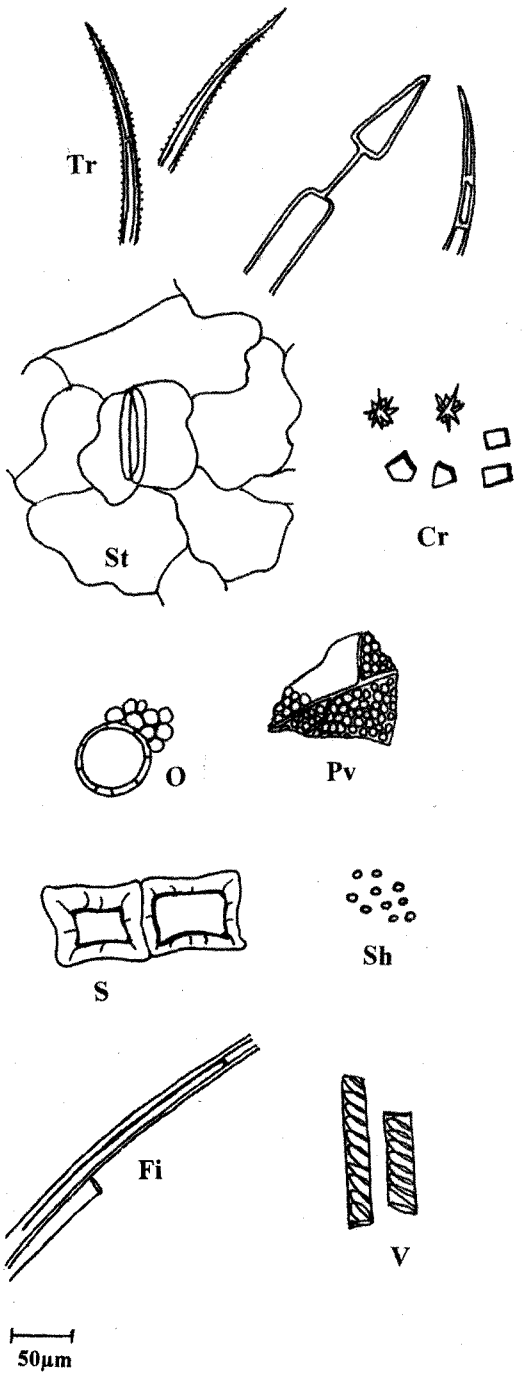

Fig. 3. Powder microscopy of *folium Annona squamosa* **Cr**-Crystals, **Fi**-Fibres, **O**-Oil sac, **Pv**-Parenchymal cells with veins, **S**-Stone cells, **Sh**-Starch, **St**-Stomata, **Tr**-Trichomes, **V**-Vessels.

Table 1. Preliminary phytochemical screening of the leaf powder of *Annona squamosa*

Test	Hexane	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids			+		+	+
Carbohydrates					+	+
Phytosterols	+				+	
Fixed oils and fats	+	+				
and fats						
Saponins						+
Phenolic compounds and tannins					+	+
Proteins					+	+
Gums and mucilages						+
Volatile oil						+
Flavonoids					+	+

+ denotes the presence of the respective class of compounds

Table 2. Ash values of the leaf powder of *Annona squamosa*

Parameters	Values %w/w
Total ash	8.78
Acid insoluble ash	0.52
Water soluble ash	2.03
Sulphated ash	9.80
Carbonated ash	6.88
Nitrated ash	6.10

Table 3. Extractive values of the leaf powder of *Annona squamosa*

Parameters	Values % w/w
Hot extraction	17.92
Cold maceration:	
a) water soluble extractive	18.62
b) Ethanol soluble extractive	08.75
c) Nonvolatile ether soluble extractive	03.28

The extractive values which is of equal importance for the quality control of any crude drug is primarily useful for the determination of exhausted drug. The water soluble extractive (Table 3) was high in *Annona squamosa*.

Moisture content of the leaf was found to be 14%; foaming index < 100; swelling index 18; mucilage content 15%. No bitterness was detected. The leaf contained 2% v/w of volatile oil (Table 4).

Leaf constants – The leaf constants *viz*; the vein islet number, vein termination number, palisade ratio and stomatal index are presented in Table 5.

Successive solvent extractive constants – Of the total extractive (Table 6) the major portion was found to be aqueous extract (11%) followed by hexane, benzene, ethanol, acetone and chloroform.

Table 4. Physico-chemical constants of the leaf powder of *Annona squamosa*

Parameters	Values
Bitterness value	Nil
Foaming index	Less than 100
Swelling index	18.0
Mucilage content	15.26 % w/w
Volatile oil content	2.0 % v/w

Table 5. Leaf constants of *Annona squamosa*

Parameters	Values
Vein islet number (1 mm^2 leaf surface)	16
Vein termination number (1 mm^2 leaf surface)	28
Palisade ratio (under 1 epidermal cell)	8.5
Stomatal index (5 mm^2 leaf surface on lower epidermis)	14.2

Table 6. Successive solvent extractive constants of the leaf powder of *Annona squamosa*

Solvents	Consistency and colour	Average value of extractive (%w/w)
Hexane	Non-sticky solid/greenish black	10
Benzene	Sticky semisolid/greenish black	2.79
Chloroform	Sticky semisolid/brownish black	0.52
Acetone	Sticky semisolid/dark brown	1.66
Ethanol	Sticky semisolid/blackish green	1.95
Water	Solid/blackish brown	11.0

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