



## Pharmacognostical and physiochemical study on the leaves of *Annona squamosa* Linn.

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### ABSTRACT

This paper gives the information about the *Annona squamosa* linn. Pharmacognostical and Phytochemical studies on the leaves of *Annona squamosa* linn were performed. Interest in medicinal plant has increased enormously over the last two-decades. The present work covers the pharmacognostical, phytochemical studies on leaves of *Annona squamosa* linn. which commonly called as ,“Sitaphal” in Hindi It is an important medicinal plant having many traditional uses. Leaves are applied to, antifungal, bacteriostatic skin disease, worms, seed used as an insecticide. In the present study, pharmacognostical, phytochemical and pharmacological investigations on the *Annona squamosa* linn. carried out. It is concluded that, scientific parameters based on taxonomical, pharmacognostical and phytochemical studies are essential to identify a plant. Phytochemical investigation shows the presence of aminoacid, terpenes, lipids, steroids, flavonoids . Many important diagnostic characters such as paracytic stomata found in lower epidermis, Single layer of palisade cells are present below upper epidermis etc will certainly help in identification of drug. These parameters not only help in the standardization of these drugs but also aid in formulating pharmacopoeial standards of drugs. The present study was undertaken to evaluate pharmacognostical characters, phytochemical analysis of leaves of *Annona squamosa* linn.

**KEYWORDS:** *Annona squamosa*, Pharmacognostical, Phytochemical, Annonaceae, Sitaphal

### INTRODUCTION

India has a rich cultural heritage of traditional medicines, which chiefly comprised the two widely flourishing systems of treatments i.e. Ayurvedic and Unani systems since ancient times. The crude drugs being always available easily in abundance comparatively cheaper, with negligible side effects and have frequently been prescribed to patients of all age groups. The multiple therapeutic action and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books and pharmacopoeias <sup>1, 2</sup>. *Annona squamosa* (Hindi- Sitaphal<sup>3</sup>) a small well-branched tree or shrub that bears edible fruits called sugar-apple, species of the genus *Annona* and member of the family Annonaceae more willing to grow at lower altitudes than its relatives *Annona reticulata* and *Annona cherimola* (whose fruits often share the same name) making it the most widely cultivated of these species. *Annona squamosa* L. (Custard

apple) is a plant belonging to the family Annonaceae. It is popularly cultivated in all parts of Thailand, especially in the northeast, as a sweet fruit.<sup>4-6</sup> *Annona squamosa* is a small, semi-(or late) deciduous, much branched shrub or small tree 3 metres (9.8 ft) to 8 metres (26 ft) tall very similar to soursop (*Annona muricata*) with a broad, open crown or irregularly spreading branches and a short trunk short, not buttressed at base. The fruit of *A. squamosa* has delicious whitish pulp, and is popular in tropical markets<sup>6</sup>. Thin leaves occur singly, 5 centimetres (2.0 in) to 17 centimetres (6.7 in) long and 2 centimetres (0.79 in) to 6 centimetres (2.4 in) wide; rounded at the base and pointed at the tip. Pale green on both surfaces and mostly hairless with slight hairs on the underside when young. The sides sometimes are slightly unequal and the leaf edges are without teeth, inconspicuously hairy when young. Leaf stalks are 0.4 centimetres (0.16 in) to 2.2 centimetres (0.87 in) long, green, sparsely pubescent<sup>5-6</sup>. The flowers are

Solitary or in short lateral clusters about 2.5 centimetres (0.98 in) long, 2-4, greenish-yellow flowers on a hairy, slender 2 centimetres (0.79 in) long stalk. Green outer petals, purplish at the base, oblong, 1.6 centimetres (0.63 in) to 2.5 centimetres (0.98 in) long, and 0.6 centimetres (0.24 in) to 0.75 centimetres (0.30 in) wide, inner petals reduced to minute scales or absent.<sup>1</sup> Very numerous stamens; crowded, white, less than 1.6 centimetres (0.63 in) long; ovary light green. Styles white, crowded on the raised axis. Each pistil forms a separate tubercle (small rounded wartlike protuberance), mostly 1.3 centimetres (0.51 in) to 1.9 centimetres (0.75 in) long and 0.6 centimetres (0.24 in) to 1.3 centimetres (0.51 in) wide which matures into the aggregate fruit. Flowering occurs in spring-early summer and flowers are pollinated by nitidulid beetles. Aggregate and soft fruits form from the numerous and loosely united pistils of a flower which become enlarged and mature into fruits which are distinct from fruits of other species of genus (and more like a giant raspberry instead). The round or heart-shaped greenish yellow, ripened aggregate fruit is pendulous<sup>1</sup> on a thickened stalk; 5 centimetres (2.0 in) to 10 centimetres (3.9 in) in diameter with many round protuberances and covered with a powdery bloom. Fruits are formed of loosely cohering or almost free carpels (the ripened pistils). The pulp is white tinged yellow, edible and sweetly aromatic. Each carpel containing an oblong, shiny and smooth, dark brown to black, 1.3 centimetres (0.51 in) to 1.6 centimetres (0.63 in) long seed. *Annona squamosa* is willing to grow at altitudes of 0 metres (0 ft) to 2,000 metres (6,600 ft) and does well in hot dry climates; at much lower altitudes than many of the other fruit bearers in its family<sup>7-8</sup>. Traditionally its used as antifungal bacteriostatic, in skin disease and in intestinal worms. (Figure 1-2)



**Figure 1: leaves, Fruits, Stems of *Annona squamosa* linn.**



**Figure 2: leaves of *Annona squamosa* linn.**

## MATERIAL AND METHODS

### PLANT MATERIALS

Fresh leaves were collected from the semi-arid region of Orissa (Kraput District), India. Fresh leaves were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles for further use. The powders of dried leaves were used for the determination ash value, extractive value and phytochemical investigation. All chemicals and reagents used for testing were analytical grade obtained from SD Fine Chemicals and Loba Chemicals, Mumbai (India). The plant was authenticated by Bijju Patnaik Plant Garden & Research Center M, S. Swaminahan Research Foundation Jeypor (k), Orissa. A voucher specimen (CR11) has been kept in our research laboratory for future reference.

### Macroscopy

The various parts of fresh herb was subjected to macroscopic studies which comprised of organoleptic characters of the drugs viz., color, odour, appearance, taste, smell, texture, fracture, etc.

### Microscopy

Qualitative microscopic evaluation was carried out by taking transverse sections of fresh leaves of *Annona squamosa*. The arrangement of tissues in transverse and longitudinal sections and types of cells and cell contents are revealed by suitable histological study of a crude drug with the aid of microscope. The vital quantitative microscopic leaf constants like vein islet, vein termination number, palisade ratio and stomatal index were carried out according to standard method<sup>9-11</sup>.

### PHYSICO-CHEMICAL PARAMETERS

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash,

water soluble extractive value, alcohol soluble extractive value, were calculated as per Indian Pharmacopoeia<sup>12-16</sup>.

**PRELIMINARY PHYTOCHEMICAL STUDIES**

The crude extract obtained by successive extraction from Petroleum ether, chloroform, Ethanol and Aqueous extraction were subjected to phytochemical studies<sup>10</sup>. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per Indian pharmacopoeia.

**FLOURESCENCE ANALYSIS OF THE DRUG**

Many crude drugs show the fluorescence when the sample is exposed to ultraviolet radiation. Evolution of crude drugs based on fluorescence in day light is not much used, as it is usually unreliable due to the weakness of the fluorescent effect. Fluorescence lamps are fitted with suitable a filter, which eliminate visible radiation from the lamp and transmits ultraviolet radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation<sup>17</sup>.

**RESULT AND DISCUSSION**

**FLUORESCENCE ANALYSIS OF EXTRACTS**

All the leaf extracts are examined in daylight, short and long UV to detect the fluorescent compounds by thereported method. The observations are given in Table 1.

**Table 1. Consistency, color, and fluorecence character of extracts *Annona squamosa* linn. Leaf**

Parameter	Pet. ether	Chloroform	Methanol
Consistency	Resinous	Sticky	Sticky
Color (day light)	Green	Green	Reddish brown
UV	Green	Green	Black

**MACROSCOPICAL STUDIES**

The macroscopical or morphological description of a drug includes size, shape, nature of outer and inner surfaces, type of fracture, and organoleptic characteristics like colour, odour, taste, consistency, etc.

**Color** - Upper surface dark green and lower surface pale green.

**Size** - 3.8 to 10 cm (l) and 0.6 to 3.9 cm (w) Form - Simple, lanceolate, acute at both ends and narrowed to distinct petiole, stipulate, symmetrical base, mid rib prominent with closely arranged lateral nerves; Venation – pinnately parallel; margin – entire

**Odour** – Odorless.

**Taste** – Sour to bitter.

**QUANTITATIVE MICROSCOPY**

The vital quantitative microscopic leaf constants like vein islet, vein termination number, palisade ratio, Stomatal number and stomatal index were carried out according to the standard method and the results were shown inTable3.

*Annona squamosa* leaf is dorsiventral (Figure 3).Single layer of palisade cells are present below upper epidermis. Stomata are of Paracytic type (Figure 4), found in lower epidermis. Mesophyll consists of 3-4 layers of spongy parenchyma with many intercellular spaces.

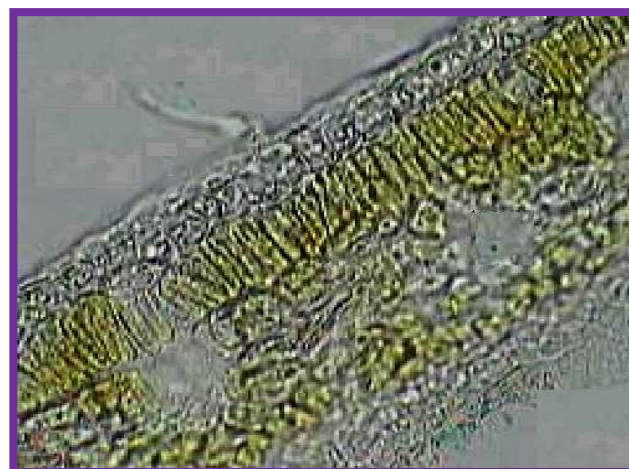
**PRELIMINARY PHYTOCHEMICAL STUDIES**

The crude extract obtained by successive extraction from Petroleum ether, chloroform, methanol and aqueous extraction was subjected to phytochemical studies. The extracts were dried and weighed. The presence or Absence of different phytoconstituents viz. Alkaloids, carbohydrates and glycosides, phytosterols, fixed oil and fats, phenolic compounds and tannins, saponins, flavonoids, etc were detected by usual prescribed methods(Table 2).

**Table 2. Qualitative phytochemical analysis of *Annona squamosa* linn.leaf extracts.**

Test for	Pet. ether extract	Chloroform extract	Methanol extract	Water extract
Alkaloids	-	+	+	-
Carbohydrates and glycosides	+	-	+	+
Phytosterols	+	+	+	-
Fixed oil and Fats	-	-	-	-
Phenolic compound and Tannins	-	-	+	+
Saponins	-	-	+	+
Flavonoid	-	-	+	+
Proteins and Amino acids	-	-	+	+
Gums and Mucilage	-	-	-	+
Volatile Oils	+	-	-	-

+ = Present; - = Absent



**Figure3: T.S of leaf through**

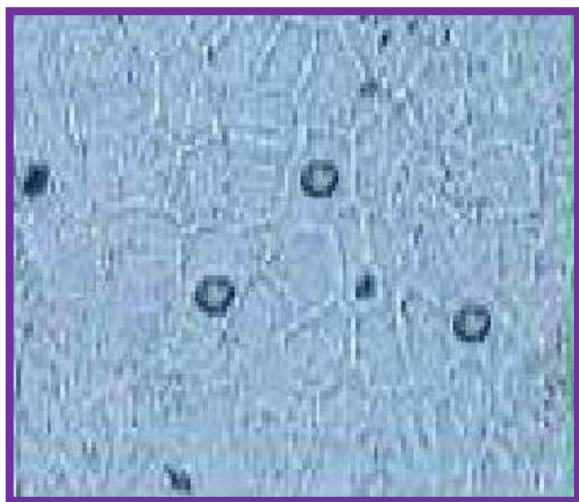


Figure4: Paracytic stomata

Table3. Leaves constant of *annona squamosa linn*

Leaf Constant	Values
Stomatal index	5.77-6.0
Vein islet number	4-6
Vein termination number	10-14
Palisade ratio	5-7

**PHYSICO-CHEMICAL PARAMETERS**

Moisture content, percentage of total ash, acid-insoluble ash and water soluble ash were calculated as per the Indian Pharmacopoeia. Different extracts of the leaves were prepared for the study of extractive values (Table 4, 5).

Table 4. Moisture content of the leaves of *annona squamosa linn*.

Fresh weight (g)	Dry weight (g)	Loss on drying (g)	Moisture content (% w/w)
2.00	1.87	0.13	6.5

Table 5. Ash values of the leaves of *annona squamosa linn*.

Type of Ash values	% w/w (Mean ± SEM)
Total ash	6.09 ± 0.69
Acid insoluble ash	0.25 ± 0.06
Water soluble ash	1.45 ± 0.24

Table 6. Extractive values of the leaves of *annona squamosa linn*.

S. No.	Type of solvents	% w/w (Mean ± SEM)
1	Petroleum ether 60-80o	2.38 ± 0.21
2	Chloroform	2.18 ± 0.18
3	Methanol	9.0 ± 0.21
4	Water	13.0 ± 0.39

**DISCUSSION**

In the last two decades of the century, the scientists are sincerely trying to evaluate many plant drugs used in

traditional system of medicine. The pharmacognostical study is one of the major criteria for identification of plant drugs. The present work covers the pharmacognostical and phytochemical studies on leaves of *Annona squamosa linn* which commonly called as, “Sitaphal” in Hindi. It is an important medicinal plant having many traditional uses. Leaves are applied to, antifungal, bacteriostatic skin disease, worms, seed use as a insecticide. The present study on pharmacognostical characters of *Annona squamosa linn*. Leaves will be providing useful information about its correct identity and help to differentiate from the closely related other species of *Annonaceae*. The other parameters observed may be useful for the future identification of the plant. Pharmacognostical evaluation like morphology, and quantitative microscopy study of family *Annonaceae* is essential for standardization of the plants. Physico-chemical evaluations like ash value, extractive value, moisture content, are the parameters for the standardization of the plants. These parameters not only help in the standardization of these drugs but also aid in formulating pharmacopoeial standards of drugs.

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