

Pharmacognostical studies on the leaf of *Annona squamosa* Linn.

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ABSTRACT

The present communication deals with the macroscopical, microscopical & preliminary phytochemical studies on the leaf of *Annona squamosa* Linn. *Annona squamosa* Linn is used for the treatment of various diseases & ailments viz. stimulant, antispasmodic, sudorific, anthelmintic and insecticidal properties etc. No reports are available on the pharmacognostic nature of the leaf, hence, the present study was undertaken to investigate the same. All the parameters were studied according to the WHO & Pharmacopoeial guidelines. The qualitative phytochemical fingerprint of the methanolic extract revealed the presence of alkaloids, terpenoids and phenolics, fats and waxes. The aqueous leaf slurry was found to be safe at the dose level of 2g/kg body weight of mice.

Keywords: *Annona squamosa* Linn., Pharmacognostic, Acute toxicity.

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INTRODUCTION

Annona squamosa Linn. also known as Sitaphala, Custard-Apple, belongs to the family Annonaceae. *Annona squamosa* Linn. is widely cultivated throughout India as an ornamental plant & deciduous tree. Leaves are reported to possess stimulant, antispasmodic, sudorific, anthelmintic and insecticidal properties. The crushed leaves are reported to be applied to the nostrils in hysteria and fits. The poultice of the leaves is used as a cataplasm over boils and ulcers to induce suppuration. It also relieves pain and swellings [1]. The ethanolic extract of the leaves has been found to show activity against L-1210 lymphoid leukemia, sarcoma 180, Walker carcinoma 256, and epidermoid carcinoma of human nasopharynx in tissue culture. The extracts with acid, ether and acetate buffer have shown antibiotic activity against *Micrococcus pyogenes* var. *aureus*. The water-soluble portion of the alcoholic extract of leaf was found to stimulate the isolated heart and relax the isolated duodenum of rabbit, exert spasmogenic effect on the ileum of guinea pig, and increase the blood pressure of the anaesthetized animals; the extract also possessed oxytocic activity on the uterus of rat. The active principle is very similar to adrenaline in its pharmacological action [2].

Herbal medicine is a triumph of popular therapeutic diversity. Almost in all the traditional medicine, the medicinal plants play the major role & constitute the backbone for the same. In order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, safety & efficacy [3]. Keeping these facts into consideration, attempts are made to establish pharmacognostic standards of the plant leaf. *Annona squamosa* Linn. is a large evergreen, straggling shrub or small tree, 7 m in height, introduced into India, found wild and cultivated in various parts, up to an altitude of 900m. Bark thin, grey; leaves oblong-lanceolate or elliptic, pellucid-dotted, peculiarly scented, 5.0–15.0 cm × 1.9– 3.8 cm; flower 1–4, greenish, fleshy, drooping, extra- axillary, more on the leaf shoot than on the older wood, tending to open as the shoot elongates; carpels many, lozenge-shaped, on a central torus, fused into an irregularly globose or heart shaped, tubercled, yellowish green syncarpium, 5–10 cm in diameter; seeds oblong, deep brownish black, aril shining, covered with whitish pulp [2–4].

The present study investigates the macroscopical, microscopical & preliminary phytochemical nature of the leaf of *Annona squamosa* Linn.

MATERIALS AND METHODS

The leaves of *Annona squamosa* Linn. was collected from the local area of Karjat, Maharashtra (India). It was authenticated by Agharkar Research Institute, Pune. The leaves were stored under the normal environmental condition. The macroscopical characters of the leaf were studied as per the procedure given in WHO guidelines & Indian herbal Pharmacopoeia. Physico-chemical parameters such as extractive values, ash values, loss on drying were performed as per the official standard procedures [5–6]. Microscopical investigations were made with transverse section & powder microscopy of the leaf. Transverse section of the leaf was taken for microscopic examination and the powder microscopy was performed according to the prescribed procedure [7–8]. For phytochemical screening 5 gram powdered leaf was subjected for Soxhlets extraction as described by Harborne [9]. To evaluate safety of leaf powder acute toxicity study on Swiss Albino mice was performed as per the detail laid down in the OECD guidelines 420 viz., Fixed Dose Procedure (Evident Toxicity) [10].

RESULT AND DISCUSSION

Macroscopically the leaf was found to be leaves oblong-lanceolate or elliptic, pellucid-dotted, peculiarly scented [Figure 1, Table 1]. The T.S. of lamina of leaf showed the presence of single layered epidermal cells, mesophyll differentiated into palisade tissues and spongy parenchyma of 3 to 5 layers, stomata anomocytic, present on lower surfaces only [Figure 2]. T.S. of midrib showed single layer epidermis on both surfaces, collenchymatous cell, followed by thin walled, round or oval parenchymatous cells, an arc shaped vascular bundle consisting of xylem and phloem, present in centre, beneath the vascular bundle lies a layer of cortical parenchyma cell followed by lower epidermis [Figure 3]. Powder microscopy shows the presence of spiral vessel, epidermal cell, trichome with leaf attachment, trichome, parenchyma cells [Figure 4]. The values of the physical constant like ash values, extractive values, loss on drying were determined [Table 2]. Preliminary qualitative phytochemical screening of the methanolic extract revealed the presence fats and waxes, terpenoids and phenolics, Basic extract-Most alkaloids and Polar extract-Quaternary alkaloids and N-oxide. Chromatographic studies of methanolic extract showed three distinct spots after detection in UV mode at 254 nm with the phytoconstituents separated [Figure 5 and 6, Table 3]. From acute toxicity study carried out on Swiss albino mice the aqueous leaf slurry was found to be non-toxic at dose level of 2g/kg body weight of mice [Table 4].

Table 1: Macroscopical Evaluation of *Cassia fistula* L bark

Sr. No	Features	Observations
1	Color	Upper surface - deep green, Lower surface - paler
2	Odour	Bitter
3	Taste	Bitter
4	Shape	Acute
6	Size	Length: 10 – 15 cm, Width: 3 – 6 cm.

Table 2: Physio-chemical Parameter for *Annona squamosa* Linn.

Sr. No.	Parameters	% Content*
1	Foreign organic matter	0.252 ± 0.014
2	Ethanol soluble extractive	20.2698 ± 0.3326
3	Water soluble extractive	31.3172 ± 0.9060
4	Total ash	6.8467 ± 0.1258
5	Acid-insoluble ash	0.9576 ± 0.0075
6	Water soluble ash	4.4476 ± 0.0712
7	Loss on drying	7.74 ± 0.6863

* Each result is expressed as Mean ± Standard deviation of three readings

Table 3: Percentage of phytochemical Constituents of *Annona squamosa* Linn.

Phytochemical Constituents	% Extract*
Neutral extract - Fats & waxes	2.5033 ± 0.015
Moderately polar extract-Terpenoids and Phenolics	4.19 ± 0.020
Basic extract-Most alkaloids	26.78 ± 0.020
Polar extract-Quaternary alkaloids and N-oxides	1.6226 ± 0.0023
Fibers	60.66 ± 1.5275

* Each result is expressed as Mean ± Standard deviation of three readings.

CONCLUSION

The microscopic characteristics of the leaf powder have been presented for quality assurance at the collection and production stages. A key (anatomical marker) has been developed for leaf powders of *Annona squamosa* Linn. This may enable the detection of adulterants from the genuine raw materials of *Annona squamosa* Linn. Phytochemical profile, foreign organic matter, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive, water soluble extractive, loss on drying, have also been determined. Acute toxicity study of slurry of leaf powder

Table 4: Cage side observations for Acute Toxicity study

Parameters Observed	Observation
Condition of the fur	Normal
Skin	Normal
Subcutaneous slug	Nil
Abdominal distension	Nil
Dullness of the eyes	Nil
Opacity of the eyes	Nil
Discharge from the eyes	Nil
Ptosis of the eyes	Nil
Pupil diameter	Normal
Colour and consistency of faeces	Normal
Condition of teeth	Normal
Breathing abnormalities	Nil
Gait	Normal

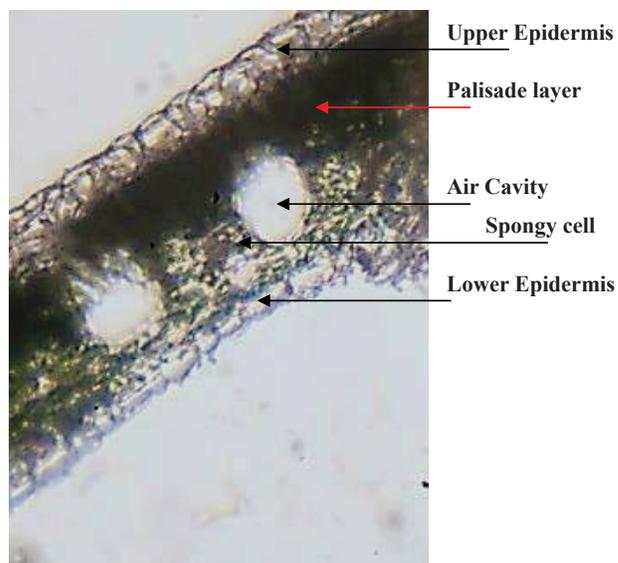
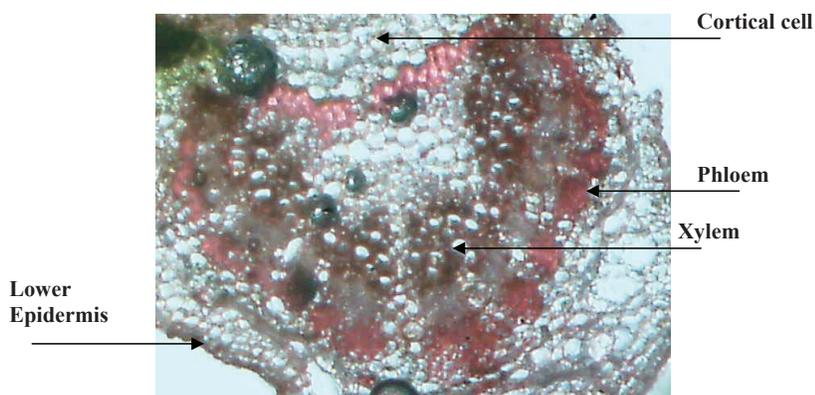


Figure 2: Transverse section of leaf of *Annona squamosa* Linn. (under 10x).



Lower epidermal peel showing epidermal cells and stomata (under 10x).

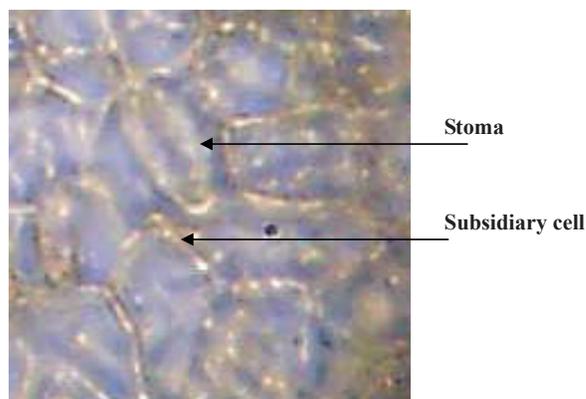


Figure 3: Transverse section leaf of *Annona squamosa* Linn. passing through mid-rib region of (under 10x).



Trichome



Parenchyma Cells



Epidermal cell

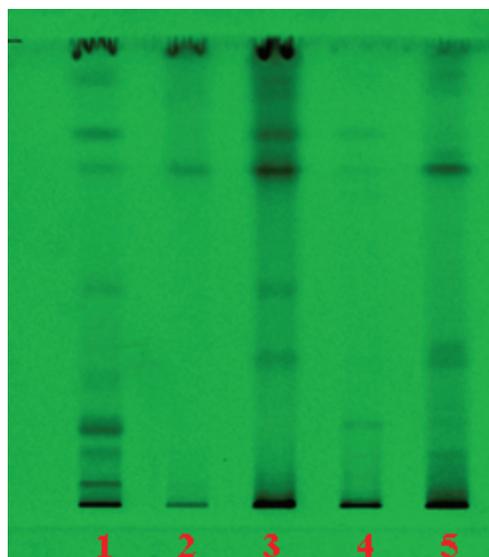


Trichome with leaf attachment



Spiral vessel

Figure 4: Microscopic Analysis of leaf powder of *Annona squamosa* Linn (under 10x).



Track 1 – Methanolic extract of *Annona squamosa* Linn. (Leaf)

Track 2 – Methanolic extract of Fats & waxes

Track 3 – Methanolic extract of Terpenoids and Phenolics

Track 4 – Methanolic extract of Basic Alkaloids

Track 5 – Methanolic extract of Quaternary alkaloids and N-oxides

Figure 5: Chromatographic plate of *Annona squamosa* Linn. with its phytoconstituents.

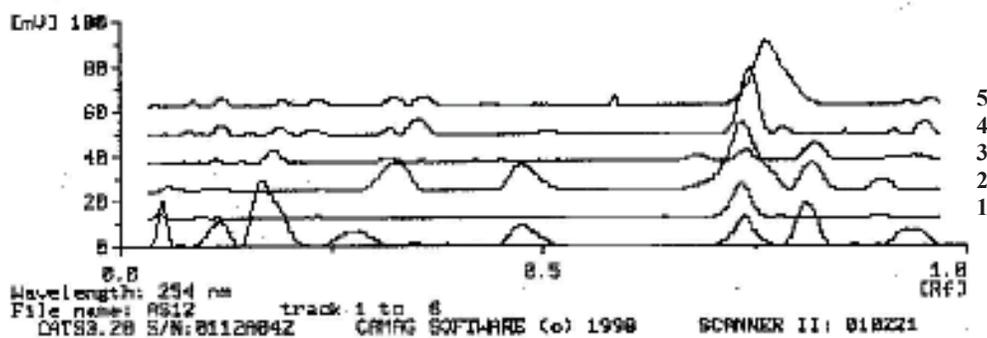


Figure 6: Chromatographic overlay of *Annona squamosa* Linn. with its phytoconstituents.

was evaluated on the basis of mortality, daily food, water intake, body weight and general behavioral changes.

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