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Pharmacognostic Standardization and Monograph Development of *Annona Squamosa* Linn., Leaves.

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A B S T R A C T

This study investigates the pharmacognostical properties of *Annona squamosa* Linn., (custard apple) leaves, a plant traditionally utilized in various medicinal applications. In the pharmacognostical study macroscopic and microscopic examination of the leaves, including histological analysis is carried out to identify key plant constants. The leaves are subjected to comprehensive phytochemical screening, revealing the presence of bioactive compounds such as flavonoids, tannins, saponins, and alkaloids, which contribute to their therapeutic effects. Standard maceration method is employed to extract bioactive compounds using methanol and water as the solvents, to yield around 7.05±0.5 and 9.37±0.5 percentage extracts. Pharmacological study is carried out via literature review that indicates extracts from the *Annona squamosa* Linn., leaves exhibit diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties etc. Further research is warranted to explore the mechanisms of action, isolation, identification and characterization of the constituents that exhibit these underlying effects and to evaluate the efficacy of these extracts under in-vitro & in-vivo studies.

Keywords: Pharmacognostical study, Standardization, Monograph, *Annona squamosa* Linn., Phytochemical screening, Pharmacological study.

1. INTRODUCTION

The survey of literature pertaining to *Annona squamosa* Linn., leaves shows that they have been used in various ayurvedic formulations. Investigations are being carried out regarding its therapeutic properties; but very few attempts have been made in India to investigate these plants as a source of medicine for formulation of various drugs. Hence the present work is focused on the Pharmacognostical study of *Annona squamosa* Linn., leaves. *Annona squamosa* Linn., belonging to family Annonaceae is commonly found in India, cultivated in Thailand, West Indies and South America.

It is mainly grown in gardens for its fruits and ornamental value. It is known as custard apple, sugar apple, in English and Sharifa in Hindi and sitaphalam in Telugu in India[1]. *Annona squamosa* Linn., is a shrub or small tree of around 7 m height and is cultivated throughout India. It's a multipurpose tree with edible fruits and is a source of one of the medicinal and industrial products. It has a long history in traditional Indian medicine for being used to treat several diseases including cancer. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, bacterial infection, dysuria, fever and ulcers. It also has antifertility, antitumour and abortifacient properties[2]. The extraction of different parts of *Annona squamosa* Linn., in different solvents revealed the presences of alkaloids, flavonoids, phenols, carbohydrate, saponin, sterols and tannins[3]. Annonaceous acetogenins are only found in the Annonaceae family. Made of action studies have recently determined that these acetogenins are superb inhibitors of enzyme processes that are found only in the membranes of cancerous tumour cells.[4]

1.1 Scientific classification of plant Annona Squamosa Linn.[5]

Table 1: Scientific classification of plant *Annona Squamosa* Linn.

Kingdom	**Plantae**
Sub kingdom	Angiosperms
Division	Magnoliophyta
Order	Magnoliales
Family	*Annonaceae*

Sub family	Maloideae
Genus	Annona
Species	*Annona squamosa* Linn.

1.2 Vernacular names of Annona squamosa Linn.[6]

Table 2: Vernacular names *Annona squamosa* Linn.

English	Custard-apple, sugar-apple, sweetsop
Hindi	Sharifa
Kannada	Sitaphala
Malayalam	Aathappazham
Tamil	Seetha pazham
Urdu	Shareefa

1.2 GEOGRAPHICAL DISTRIBUTION

Annona squamosa Linn., belonging to family *Annonaceae* is cultivated in almost all tropical and subtropical countries. The original home of *Annona squamosa* Linn., which is also branded as sugar apple or custard apple is unknown. Its considered endemic to tropical America but is widely distributed in tropical and subtropical climates, climate of Africa, Australia, South America, West Indies, countries in Asia such as Malaysia and Indonesia Laos, Thailand and Vietnam and also distributed throughout India. It is mainly grown in gardens for its fruits & ornamental value. [7]

Fig 1: *Annona squamosa* Linn., leaf

Fig 2: *Annona squamosa* Linn., whole plant

Fig 3

Fig 4

Fig 3, 4: *Annona squamosa* Linn., fruit

1.3 PLANT DESCRIPTION:

Annona squamosa Linn., is a small tree with thin grey bark, has flower crown that resembles a flat or round ball. *Annona squamosa* Linn., leaves are green in colour with a width of 3-5 cm and a length of up to 15 cm, this plant dormancy can be caused by fluctuations in temperature, light, or rainfall. *Annona squamosa* Linn., is also a type of plant with bisexual flowers with the groups of 2 to 4 and can reach a length of about 2.5 cm. One of the animals that plays a role in the pollination process of *Annona squamosa* Linn., is the nitulid beetle. After the pollination process is carried out, tuberculous fruit is formed and has an aromatic also sweet taste. Each carpel has a smooth seed, with black or dark brown in colour, and has oval shape.[8]

Annona squamosa Linn., can flower in spring to early summer, but in areas with permanent humidity levels, *Annona squamosa* Linn., can flower throughout the year. The flowers are actinomorphic, protogynous, pedicillate, spirocyclic, bracteates, and bisexual. The *Annona squamosa* Linn., flower has six petals and a degenerated sepal formation.[9]

The stems and branches of *Annona squamosa* Linn., are irregular in shape and gray in color and contains compounds such as roemerolidine, nitroso xylophone, and duguevalline alkaloid.[10]

Annona squamosa Linn., begins to bear fruit when it is 3-4 years old. In India, usually *Annona squamosa* Linn., bear fruit around July-August. Custard apple has a sweet taste like sugar, their ripe fruit is indicated by the sweet aroma of the fruit. *Annona squamosa* Linn., seeds are dark brown to black, and generally 30-40 seeds can be found in one fruit. *Annona squamosa* Linn., is a type of plant that is classified as diploid with 2n-14.[8]

1.4 PHYTOCHEMISTRY

Leaves, roots, bark, fruits and seeds of Annonaceae family contain numerous bioactive chemical substances, such as acetogenins, alkaloids, terpenes, flavonoids and oils. At least some acetogenins have insecticidal, cytotoxic, antitumoral, antifeedant, antibacterial, immuno-suppressant, pesticidal or anthelminthic properties[11]

In custard apple leaves, stem and bark, there are acetogenins that have cytotoxic activity and potential use in cancer treatments[12]. Sugar apple leaves are rich in aporphines[13] and fruits contain diterpenoids. Bark contains acetogenins[14]. Squamotacin (similar to bullatacin) and molvizarin acetogenins have cytotoxic activity against prostate tumour cell lines[15]. Fatty acid composition of seeds is stearic acid (9.3%), oleic acid (37%), linoleic acid (10.9%), arachidic acid (3.3%) and isoricinoleic acid (9.8%). The seeds also contain terpene hydrocarbon essential oils, such as alpha pirene, beta pirene, limorene, beta farnesene and trans orimene.[16]

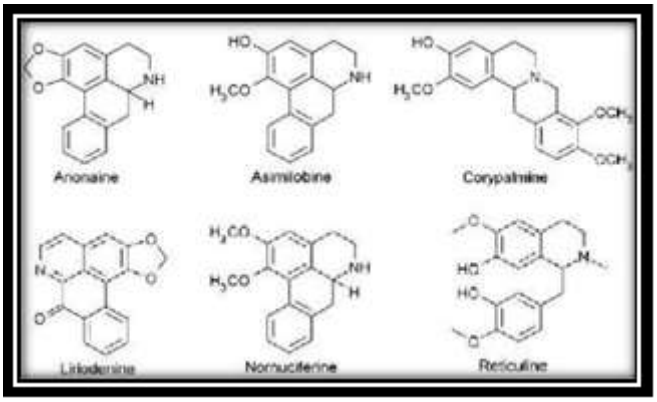


Fig 5: Structures of *Annona squamosa* Linn., phytoconstituents

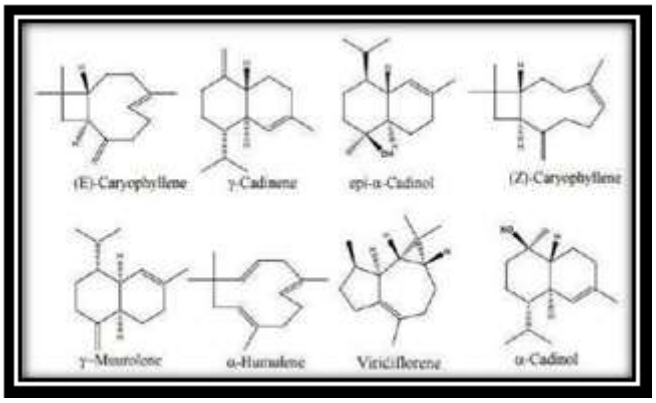


Fig 6: Structures of *Annona squamosa* Linn., phytoconstituents

2, MATERIALS AND METHODOLOGY

2.1 COLLECTION AND AUTHENTICATION OF PLANT Annona squamosa Linn.:

The fully matured fresh leaves of *Annona squamosa* Linn., plant was harvested from the various geographical locations of Mysore city region, Karnataka, South India, that were used for the study. The authentication of the leaves was done by Dr. V. Rama Rao, Research Officer, (Botanist), Department of Botany, Central Ayurveda Research Institute, Govt. of India, Ministry of AYUSH, Central Council for Research in Ayurvedic Sciences, #12 Uttarahalli Manavarathe Kaval, Kanakpura Main Road, Thalaghattapura post, Bengaluru-560109 (Authentication/SMPU/CARI/BNG/2023-24/397).

The leaves were rinsed thoroughly under tap water followed by double distilled water to eliminate dust particles. The leaves were kept in shade at room temperature until the leaves get dried. The dried leaves were made to a coarse powder in a mixer and stored in a dry place.

2.2 MORPHOLOGY

The leaf part of sample was subjected to macroscopical identification based on colour, odour, taste and shape of the drug. Morphological observations are recorded.

2.3 MICROSCOPY

The thin transverse sections of the leaf were taken and examined for microscopic features using chloral hydrate as clearing agent and stained with phloro - glucinol and hydrochloric acid (1:1). It was mounted on a slide, glycerine added, covered with a cover slip and observed under microscope.

Plant leaf constants:[17]

Determination of leaf constants include stomatal number, stomatal index, vein islet, veinlet termination number and palisade ratios.

A) Stomatal number: is average number of stomata per sq. mm of epidermis of the leaf.

Procedure:

- Firstly, clear the middle part containing-piece of the leaf by boiling with a chloral hydrate solution or chlorinated soda solution. Then peel off both epidermises. Separately with the help of forceps, place It on a glass slide and mount it with glycerine water. Set the camera lucida and drawing board for making the drawing to scale.
- Draw a 1 mm square with the help of a stage micro-meter, then place the epidermis containing a slide of cleared leaf on the stage of the compound microscope and trace the epidermal cells and stomata on the paper sheet.
- Count the number of stomata existing in 1 mm square area, record the result of each 10 fields and calculate the average number of stomata in the prescribed area.

B) Stomatal index: it is the percentage which the numbers of stomata form to the total number of epidermal cells, each stoma being counted as one cell.

Stomatal index can be calculated by using the following formula:

Stomatal index (S.I.) = S/(E+S) ×100 Where, S=Number of stomata per unit area

E= Number of epidermal cells in the same unit area.

C) Vein-islet number: It is defined as the number of vein islet per sq.mm of the leaf surface midway between the midrib and the margin.

D) Veinlet termination number: It is defined as the number of veinlet termination per sq. mm of the leaf surface midway between midrib and margin. A termination is the ultimate free termination of veinlet.

E) Palisade ratio: It is defined as the average number of palisade cells beneath each epidermal cell.

2.4 POWDER MICROSCOPY

Powder Characteristics: In Present study, the *Annona squamosa* Linn., leaves were pulverized into fine powder. The powder was investigated for the powder microscopic characteristics. The coarse powder is boiled with chloral hydrate for 5 minutes, then stained with phloroglucinol and HCL (1:1), mounted with glycerine. It was observed for the microscopic features under high power (10x and 45x).

2.5 PHYSICAL EVALUATION:

The authenticated shade dried leaves of plant *Annona squamosa* Linn., subjected to size reduction to get the coarse powder of drug and then passed through sieve no. 45 to get the uniform powder.

The uniform powder was then subjected to Standardization.

Analysis of physicochemical parameters of the powder such as loss on drying, total ash value, pH value, water soluble extractive value, alcohol soluble extractive value, etc were conducted

2.5. I) Moisture content (loss on drying) (LOD)

Moisture content of the powder drug samples was determined by loss on drying method described in Ayurvedic pharmacopoeia of India.

Procedure: 5g of the drug powder were taken in previously weighed petri plate and kept in the oven at 105ºC for 5 hours. The petri plate was then cooled in a desiccator and weighed. The difference in weight was taken. The drying and weighing was continued till, after 30 minutes of heating and cooling until a constant weight was obtained.

Calculation:

$$\%LOD = \frac{[\text{Loss in weight of sample}]}{[\text{Weight of sample taken}]} \times 100$$

2.5. II) Determination of Ash values

The total ash, acid insoluble ash and water-soluble ash values were determined for air dried sample using procedure described in quality control methods for medicinal plant materials.

a) Total Ash Value:

Steps:

- 2g of the ground air-dried sample powder was weighed into previously ignited, tarred silica crucible cooled in a desiccator. The material was spread evenly as a thin layer.
- It was ignited slowly to obtain a carbonized residue and placed in the muffle furnace and the temperature was adjusted to 450-500° C and allowed to ignite until it was white, indicating the absence of carbon.
- Crucibles were removed from muffle furnace, allowed to cool for 30 minutes in a desiccator and weighed without delay.
- Total ash in mg per g of air-dried material was calculated as shown below and results are expressed as % Ash.
- Total Ash Value calculated using formula

$$\%Ash = \frac{[\text{(Wt. of ash)}]}{[\text{(Wt. of crucible and sample} - \text{Wt. of crucible)}]} \times 100$$

b) Water soluble ash

Steps

- To the Silica crucible containing the total ash obtained, 25ml of water was added.
- The insoluble matter was collected on an ashless filter paper by filtration and rinsed repeatedly with hot water.
- The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight in the muffle furnace at 450-500 ºC.
- The silica crucible was removed from the muffle furnace and allowed to cool in a desiccator for 30 minutes, and then weighed without delay.
- Calculated the content of water-soluble ash as %.

$$\%\text{ water soluble ash} = [\text{(Wt. of water insoluble residue)} \times 100$$

[(Wt. of crucible and sample − Wt. of crucible)]

c) Acid insoluble ash

Steps

- To the Silica crucible containing the total ash obtained, 15ml of water and 10ml HCl was added, and covered with a watch glass. It was boiled gently for 10 min. and allowed to cool.
- The insoluble matter was collected on an ashless filter paper by filtration. This filter paper was rinsed repeatedly with hot water until the filtrate is neutral /free from acid.
- The filter paper containing the insoluble matter was transferred to the original crucible dried on a hot plate and ignited to a constant weight in the muffle furnace at 450-500 ºC.
- The silica crucible was removed from the muffle furnace and allowed to cool in a desiccator for 30 minutes, and then weighed without delay.
- Calculated the content of acid insoluble ash as %.

$$\% \text{ of alcohol-soluble extractive value} = \frac{[(\text{Wt. of extract})]}{[(\text{Wt. of sample taken})]} \times 100$$

2.5 III) Foreign organic matter

It refers to any other part of plant except that constituting the drug.

2.6 PHYTOCHEMICAL SCREENING:

The leaves of *Annona squamosa* Linn., were collected, washed and dried at room temperature and after complete drying, it was powdered using mixer grinder and passed through a No. 60. mesh sieve and stored in air tight container.

25g of dried powdered drug was used to prepare extract. The extracts prepared by maceration (using water and methanol) as solvents were used for phytochemical evaluation and study.

Concentration of extracts is done using water bath and dried.

The extracts obtained were then subjected to qualitative chemical examination for the identification of various plant constituents.

2.7 CHROMATOGRAPHIC STUDIES:

The Thin Layer Chromatography studies of extracts of *Annona squamosa* Linn., leaves are carried out to confirm the presence of phytoconstituents.

Thin Layer Chromatography

The extract was subjected to Thin Layer Chromatography for the presence of phytoconstituents. In this method, for TLC purpose, the silica gel GF254 was used as an adsorbent and plates Prepared by spreading technique, then air dried for an overnight, activated for one hour at 110°C and were used.

Stationary phase: Chromatography grade Silica gel GF254 **Solvent System: -** 2:8 ration of water and methanol **Detection:** Day light observation and UV light observation.

The Rf values were determined.

Evaluation of Rf: Rf value of various samples was evaluated using the following

Formula:

$$Rf = \frac{\text{(Distance travelled by the sample from the base line)}}{\text{(Distance travelled by the solvent from the base line)}}$$

3. Results

3.1 COLLECTION AND AUTHENTICATION OF PLANT MATERIALS:

Around 20 kg of the fully matured fresh leaves of *Annona squamosa* Linn., plant was harvested from the various geographical locations of Mysore city region, Karnataka, South India, that were used for the study. The leaves were collected during the month of November 2023, and washed, cleaned and

shade dried. Which yielded to around 15kg of dry leaves. The shade dried leaves were pulverized into powder using a mixer-grinder. Around 7kg of powder was obtained which was stored, in a polythene bag in clean, dry conditions and used for the study.

3.2 MORPHOLOGY

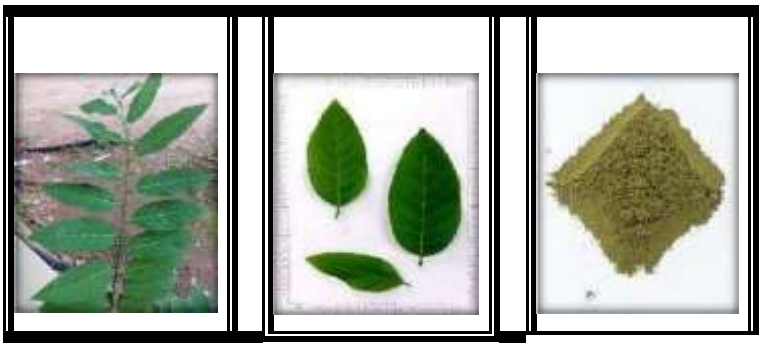


Fig 7 Fig 8 Fig 9

Fig 7 & 8, showing arrangement pattern, size, colour and shape of the leaves and, Fig 9 showing the powder of *Annona squamosa* Linn., leaf respectively.

Table 3: Morphological characterization of parts of *Annona squamosa* Linn., plant

CHARACTERS	**SEEDS**	**LEAVES**	**STEM**	**ROOTS**	**FRUITS**
COLOUR	Black	Green	Green to brown	Light brown to dark brown	Greenish outside, whitish pulpy inside
ODOUR	Odourless	Characteristic Odour	Characteristic odour	Odourless	Sweet
TASTE	Tasteless	Bitter	Sight bitter	Bitter	Sweet

Table 4: Morphological characterization of *Annona squamosa* Linn., leaf

FEATURES	SAMPLE A	SAMPLE B	SAMPLE C	AVERAGE
SHAPE	Simple, elliptical, oblong, entire margin			
LENGTH	13 cm	15 cm	10 cm	12.7 cm
WIDTH	5 cm	5 cm	4 cm	4.7 cm

The leaves are simple, have entire margin, elliptic to oblong with rounded to acute apex (5-15 cm long, 2 - 5 cm wide), have pinnate venation, peculiarly scented.

3.3 MICROSCOPY

LEAF MICROSCOPY

The microscopic characteristics of leaf of *Annona squamosa* Linn**.,** leaf was examined for the microscopical characteristics. The thin transverse sections of the leaf were taken and examined for microscopic features using chloral hydrate as clearing agent and stained with phloroglucinol and hydrochloric acid (1:1).

Fig 10

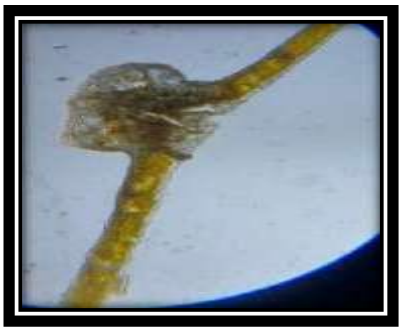


Fig 11

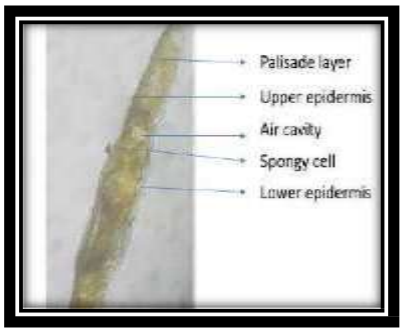


Fig 12:

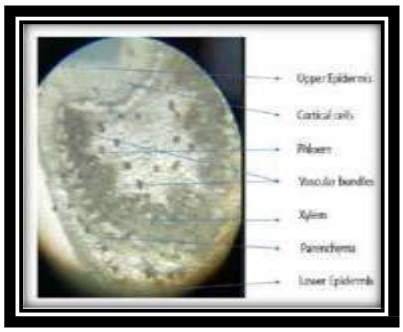


Fig 13:

Fig: 10, 11, 12, 13, 14: Photographs of T.S of leaf of Annona squamosa Linn.,

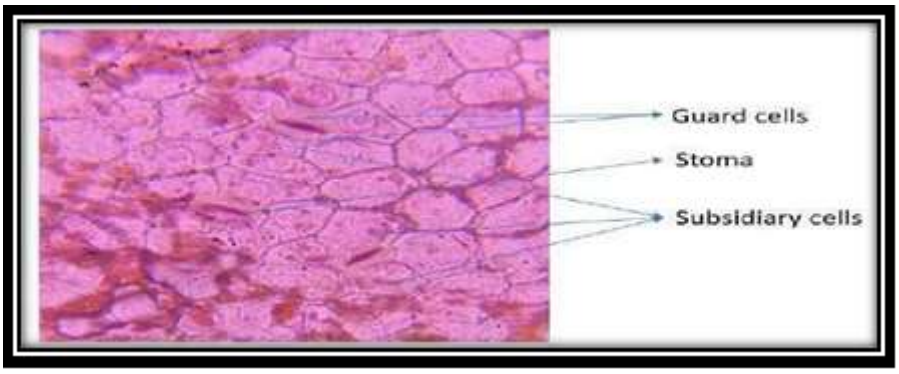


Fig 14: T. S. of *Annona squamosa* Linn., showing stomatal cells

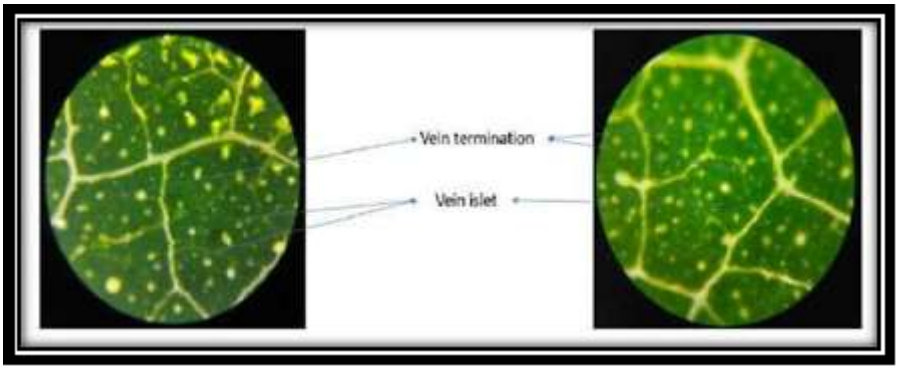


Fig 15

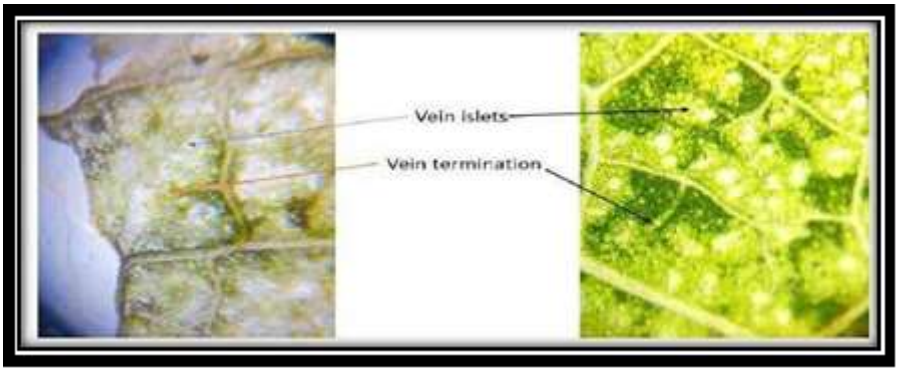


Fig 16

Fig 15 & 16: T. S. of *Annona squamosa* Linn., showing vein islets and vein terminations

LEAF CONSTANTS

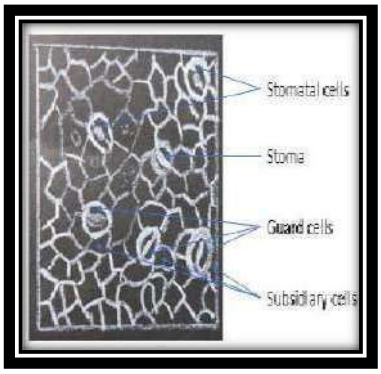
Table 5: *Annona squamosa* Linn., leaf constants

PARAMETER	**VALUE**
Stomatal number	10-13
Stomatal index	12.5
Vein islet number	10.5
Vein termination number	17
Palisade ratio	7.7

Note the values are average of three trials performed

The T.S. of lamina of leaf showed the presence of stomata, single layered epidermal cells, mesophyll differentiated into palisade tissues and spongy parenchyma of 3 to 5 layers. T.S. of midrib showed single layer epidermis on both surfaces, collenchyma cells, followed by thin walled, round or oval parenchymatous cells, vein islets, vein terminations were observed as shown in above images.

A semi-circle curved shaped vascular bundle consisting of xylem and phloem, present in center, beneath the vascular bundle lies a layer of cortical parenchyma cell followed by lower epidermis



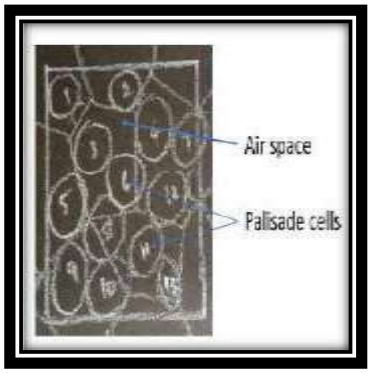


Fig 17 Fig 18

Fig 17 & 18: Sketch of Stomatal cells and Palisade cells of Epidermal peal of *Annona squamosa* Linn as observed under Camera Lucida respectively

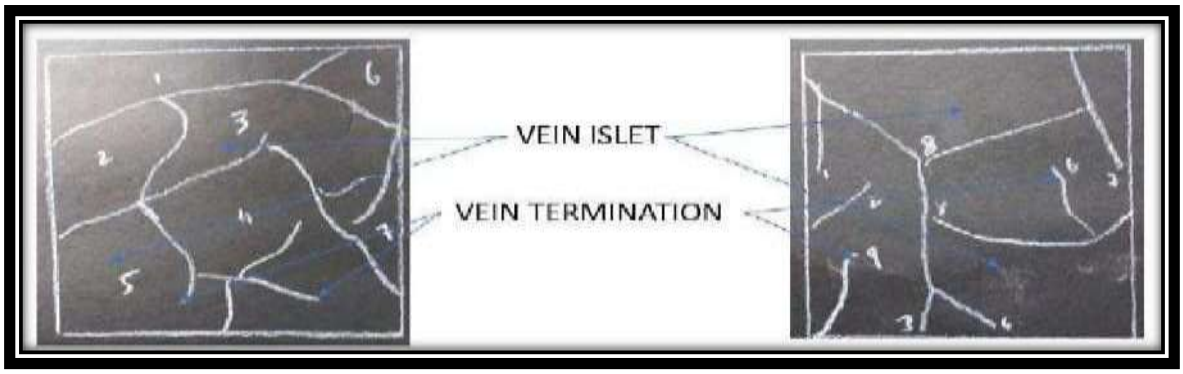


Fig 19: Sketch of Vein Islets and Vein Terminations of T.S. of *Annona squamosa* Linn as observed under Camera Lucida respectively

POWDER MICROSCOPY:

The coarse powder is boiled with chloral hydrate for 5 minutes, then stained with phloroglucinol and HCL (1:1), mounted with glycerine. It was observed for the microscopic features under the microscope.

Powder microscopy shows the presence of, epidermal cell, trichome, leaf attachment, parenchyma cells, vasculature.

Fig 20 Fig 21 Fig 22

Fig 23

Fig 24

Fig 20,21,22,23 & 24: Showing Trichome, Leaf Attachement, Bundle Of Fibre, Vasaculature In Lamina & Layer Of Parenchyma Cells Observed Under The Microscope In Powder Microscopy

PHYSICAL EVALUATIONS:

The authenticated shade dried leaves of plant *Annona squamosa* Linn., subjected to size reduction to get the coarse powder of drug and then passed through sieve no. 45 to get the uniform powder. The obtained powder was used for physical evaluations such as loss on drying, total ash value, pH value, water soluble extractive value, alcohol soluble extractive value, etc.

Table 6: Physical evaluations of *Annona squamosa* Linn., leaf powder

Sl No	**Parameters**	**Values obtained in % w/w on dry weight basis**
1	Moisture content	3.6 ± 0.5
2	Total Ash value	10.5 ± 0.5
3	Water soluble ash	6.3 ± 0.5
4	Acid insoluble ash	2.3 ± 0.5
5	Water soluble extractive	9.7 ± 0.5
6	Alcohol soluble extractive	2.4 ± 0.5
7	Foreign organic matter	0.33± 0.5

PREPARATION OF EXTRACT

The leaves of *Annona squamosa* Linn., were shade dried under normal condition and homogenized to coarse powder and passed via a Mesh 60 No., sieve. 25 gm of powder was used to create extract. The extracts were prepared using methanol and water. The dried powder was macerated with ethanol and water respectively separately and stored for 72 hours in ice cold condition. After that the miscella was filtered off by using Whatmann No.1 filter paper and organic layer was allowed to evaporate. was kept in cold refrigerator for further characterization.

Fig 25: Extraction by maceration using

Fig 26: Extraction by maceration using methanol as solvent water as solvent

Fig 27: Methanolic extract

Fig 28: Aqueous extract

YIELD AND NATURE OF EXTRACTS

TABLE 7: YIELD AND NATURE OF EXTRACTS

SL NO	WEIGHT OF DRUG (gm)	Solvent used	Yield (gm)	% yield	Colour	State of appearance
1	250	Methanol	12	7.05 ±0.5	Greenish	Viscous sticky mass
2	100	Water	15	9.37 ±0.5	Greenish	Viscous sticky mass

PHYTOCHEMICAL SCREENING

TABLE 8: PHYTOCHEMICAL SCREENING OBSERVATIONS

PHYTOCHEMICAL	TEST / REAGENT	WATER EXTRACT	METHANOL EXTRACT
ALKALOIDS	MAYERS TEST	+	+
	WAGNERS TEST	+	-
GLYCOSIDES	LEGALS TEST	+	+
FLAVONOIDS	SHINODA TEST	+	+
TANNINS	FERRIC CHLORIDE TEST	+	+
SAPONINS	FOAM TEST	+	-
OILS	SPOT TEST	+	+
CARBOHYDRATES	BENEDICT'S TEST	+	+
	MOLISCH'S TEST	+	-
PHENOLS	LEAD ACETATE TEST	+	+
STEROLS AND	LIBERMANN-BURCHARD TEST	-	-
TRITERPENOIDS	SALKOWSKI TEST	-	-
PROTEINS	XANTHOPROTEIC TEST	+	-
ACIDS	SODIUM BICARBONATE TEST	-	+

CHROMATOGRAPHY

The phytoconstituents from the extract sample got separated out in different colour bands

FIG 29

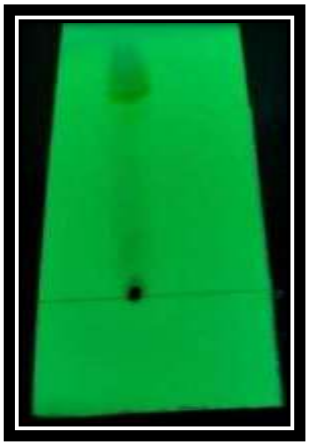


FIG 30

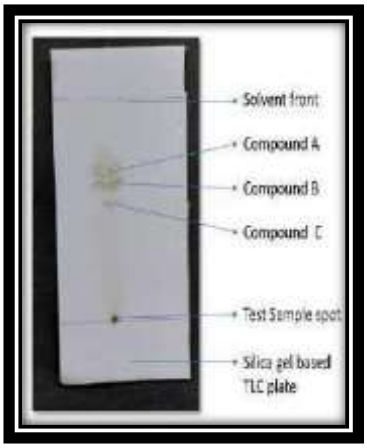


FIG 31

Fig 29, 30, 31 Showing Chromatographic Developments Observed In The Chamber, Under UV Light And Under Day Light Respectively

Distance travelled by solvent = 7.6 cm Distance travelled by compound A = 5.2 cm Distance travelled by compound B = 4.6 cm Distance travelled by compound C = 4.1 cm

The TLC analysis of the methanolic extract of *Annona squamosa* Linn leaves with rf values of 0.54, 0.61 and 0.68 may correspond to the following active constituents:

- rf 0.54: This value may represent saponins or tannins, contributing to the plant's medicinal properties[9].
- rf 0.61: This value may correspond to flavonoids or phenolic compounds, likely quercetin or kaempferol, known for their antioxidant properties[15].
- rf 0.68: This could indicate the presence of alkaloids such as anonaine or isocorydine, which have various biological activities[16].

These compounds collectively contribute to the pharmacological potential, of *Annona squamosa* Linn leaves including antioxidant, anti-inflammatory activities and cytotoxic activities.

TABLE 9: Thin Layer Chromatography study readings

Sample	**Distance travelled in cm**	**Rf value**
Solvent	7.6	1
Compound A	5.2	0.68
Compound B	4.6	0.61
Compound C	4.1	0.54

4. Monograph of *Annona squamosa* Linn.

Sitaphal leaf

Annona squamosa Linn.,

Sitaphal leaf consists of dried leaf of *Annona squamosa* Linn., belonging to Annonaceae family, is a well branch semi-deciduous fruit tree, native of tropical Africa, cultivated in India, Mexico etc.

Synonyms: Sita phala, sita pandu seethapalam, seethapandu, **Category:** Anticancer, antimicrobial, antioxidant, anthelmintic, antimalarial, anti-head lice, hepato-protective etc

Description: Green coloured, characteristic odour, bitter in taste.

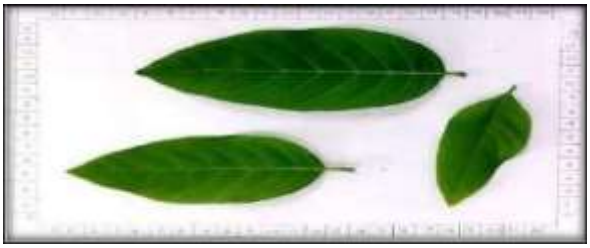
Identification:

The *Annona squamosa* Linn., is a deciduous tree, reaching a maximum of 6.0 m in height, with many lateral branches. The leaves are arranged alternatively, oblong elliptical in shape, peculiarly scented dark green above and light green below with petioles measuring upto 0.5-1 cm in length.

Leaves having a length of about 5-15 cm and 2-5 cm wide, with an obtuse apex.

Flowers having a length of around 2 cm. A round, ovate, heart shaped, knobby fruit, being 5-6 cm in diameter and 8-10 cm in length, weighing around 100-150 gm.

There are around 30-40 seeds, being 1-1.5 cm in length and around 0.5-1.0 cm wide.



Powder: The powder of the leaves is green coloured with distinct odour. On examination under microscope, using chloral hydrate solution, characters such as epidermal cells, trichomes, stomata etc. **Determination by TLC:** where coating the plate with Chromatography grade Silica gel GF254.

Mobile phase: mixture of 2 volume of water and 8 volumes of methanol.

Test solution: Boil 1mg of coarse powder with 10ml of methanol for 5 minutes and filter. Evaporate the filtrate to 1 ml.

Apply the test solution to the plate. Allow the mobile to run $3/4^{th}$ of the plate. Dry the plate and observe the plate under UV light and day light. The chromatographic profile developed is observed.

rf value: 0.54, 0.61 and 0.68

Phytoconstituents: the identified phytoconstituents may be saponins, tannins, alkaloids, flavonoids or phenolic compounds. **Tests:**

Foreign organic matter: not more that 2.0%

Total ash: not more than 11-12%

Storage: store the shade dried powder protected from heat, moisture and light.

SUMMARY

In this work an attempt was made to perform Pharmacognostic standardization for *Annona squamosa* Linn., leaves. The current study can be summarized as, the authenticated leaves were studied for their morphological characters, microscopic characters and physicochemical evaluations as well as phytochemical screening. The morphological characters identified the shape, colour, size of the leaves. In microscopy, plant constants were identified and constants such as stomata, trichomes etc were reported. Powder microscopy also was performed for the identification of various characters such trichomes, leaf attachment etc. The physicochemical evaluations such as moisture content, ash value, pH, extractive values have been studied.

CONCLUSION

The leaves of *Annona squamosa* Linn., commonly known as custard apple, contain a variety of bioactive compounds that contribute to their medicinal properties. Studies have identified several active components, including alkaloids and acetogenins. Pharmacognostical study was successfully carried out including seasonal collection, authentication, morphological, microscopical and phytochemical analysis. Two different extracts were developed and analyzed. Future studies should focus on isolating specific compounds responsible for these effects and evaluating their efficacy and safety in clinical settings. The findings of the current study will be a significant resource for formula development, quality control, and standardization of *Annona squamosa* Linn. The developed monograph shall behave as an essential diagnostic tool for the identification, authentication, and establishment of quality characteristics for the species is provided by this current report on the preliminary Pharmacognostical characterization of *Annona squamosa* Linn., of Mysuru region. The data can also be used as a reference for future studies on *Annona squamosa* Linn.

REFERNCES

1. Pandey N, Barve D. Phytochemical and pharmacological review on Annona squamosa Linn. International Journal of research in pharmaceutical and biomedical sciences. 2011 Oct;2(4):1404 -12.
2. Babu C, Devi PR, Kombiah P. Screening of biological actives extracts from Annona squamosa.
3. Agarwal S, Vilvanathan S, Shanthakumar S. Adsorption kinetics, isotherms, thermodynamics studies on the removal of Congo red dye using Annona squamosa (custard apple) peel. Journal of Urban & Environmental Engineering. 2019 Oct 1;13(2).
4. Jacobo-Herrera N, Pérez-Plasencia C, Castro-Torres VA, Martínez-Vázquez M, González-Esquinca AR, Zentella-Dehesa A. Selective acetogenins and their potential as anticancer agents. Frontiers in Pharmacology. 2019 Jul 18;10:783.

5. Gajalakshmi S, Divya R, Deepika VD, Mythili S, Sathiavelu A. Pharmacological activities of Annona squamosa: A review. Interna tional Journal of Pharmaceutical Sciences Review and Research. 2011;10(2):24 -9.
6. Kalidindi N, Thimmaiah NV, Jagadeesh NV, Nandeep R, Swetha S, Kalidindi B. Antifungal and antioxidant activities of organic a nd aqueous extracts of Annona squamosa Linn. leaves. Journal of food and drug analysis. 2015 Dec 1;23(4):795 -802.
7. Kumar M, Changan S, Tomar M, Prajapati U, Saurabh V, Hasan M, Sasi M, Maheshwari C, Singh S, Dhumal S, Radha. Custard apple (Annona squamosa L.) leaves: Nutritional composition, phytochemical profile, and health -promoting biological activities. Biomolecules. 2021 Apr 21;11(5):614.
8. Vyas K, Manda H, Sharma RK, Singhal G. An update review on Annona Squamosa. IJPT. 2012 Feb;3(2):107 -18.
9. Safira A, Widayani P, An-Najaaty D, Rani CA, Septiani M, Putra YA, Solikhah TI, Khairullah AR, Raharjo HM. A review of an important plants: Annona squamosa leaf. Pharmacognosy Journal. 2022;14(2).
10. Johns T, Windust A, Jurgens T, Mansor SM. Antimalarial alkaloids isolated from Annona squamosa. Phytopharmacology. 2011;1(3): 49-53.
11. Adeniran SA, Kadiri AB, Olowokudejo JD. Taxonomic significance of some leaf anatomical features of the species of Annona L.(Annonaceae) from Nigeria. Ife Journal of Science. 2020 May 26;22(1):101 -12.
12. Kyatham Hemanth, S.K Godasu, D.Varun, Kurva Ameresh, Sangepu Gayathri, Gavini Madhuri, Hanumandla Manasa. QUANTITATIVE MICRSCOPIC EVALUATION OF Annona squamosa Linn. (SITAPHAL) LEAVES. Zenodo; 2024. 115.
13. Gusakov AV, Kondratyeva EG, Sinitsyn AP. Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. International journal of analytical chemistry. 2011;2011(1):283658.
14. https://www.coleparmer.in/tech-article/understanding-tss-method-and-procedure
15. Ginting B. Antioxidant activity and phytochemical identification of Annona squamosa leaves methanolic extracts. Pharmacognosy Journal. 2021;13(6s).
16. Vikas B, Akhil BS, Remani P, Sujathan K. Free radical scavenging properties of Annona squamosa. Asian Pacific journal of canc er prevention: APJCP. 2017;18(10):2725.
17. Hemanth K, Godasu SK, Kotha S, Konakanti S, Prakash NJ. PHARMACEUTICAL SCIENCES.