CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://zenodo.org/records/11394459

https://www.iajps.com/volumes/volume11-may-2024/26-issue-05-may-24/

Available online at: http://www.iajps.com *Research Article*

QUANTITATIVE MICRSCOPIC EVALUATION OF *Annona squamosa Linn.* (SITAPHAL) LEAVES

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Abstract:

Plant based crude drugs is an important traditional medicinal practice which still using today throughout the world. Based on the different characteristics of the medicinal plantlets their therapeutic usage. Evaluation process depends on scientifically determine the qualitative microscopy. This study have greater importance for the authentication and phytochemical identification. So that their microscopical constituents have vital role for the safe utilization .The wild source plants like Annona squamosa Linn. leaf continues to draw wide attention for their role in treating diseases like Cardiac ailments, Thyroid-related disorders, Diabetes, and Cancer..etc. Present work focus on quantitative parameters of leaf transfer section and their evaluation studies like Stomatal number, Stomatal Index, Vein Islet number Vein Terminal number and Palisade ratio are reported for the future prospects to increase the standardization of these parameters for the safe utilization of crude drugs are discussed.

Keywords: *Annona squamosa, Traditional Medicinal plants, Phytochemicals,*

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Please cite this article in press ***Kyatham Hemanth*** *et al.,* ***Quantitative Micrscopic Evaluation of Annona Squamosa Linn. (Sitaphal) Leaves****., Indo Am. J. P. Sci, 2024; 11 (05).*

INTRODUCTION:

Annona squamosa, commonly known as Sugar-apple or Custard apple, is a small to medium-sized deciduous tree native to tropical Americas. Its morphology includes alternate, oblong-lanceolate leaves with prominent veins, fragrant greenish-yellow flowers, and compound syncarp fruit with knobby skin and sweet, custard-like flesh. The tree's shallow, spreading root system aids in water and nutrient absorption. Sugar-apple holds cultural significance, appreciated for its delicious fruit and valued in traditional medicine. Annona squamosa L. (Annonaceae), also known as "custard apple," is a tropical, endemic species of the West Indies, South and Central America, Ecuador, Peru, Brazil, India, Mexico, Bahamas, Bermuda, and Egypt [1,2,3]. In India, as reported by the Indian Council of Agricultural Research (ICAR), Annona squamosa is extensively cultivated in various states (Maharashtra, Gujarat, Madhya Pradesh, Chhattisgarh, Assam, Uttar Pradesh, Bihar, Rajasthan, Andhra Pradesh, and Tamil Nadu) with a total area of 40,000 ha [4]. Annona squamosa is known for its edible fruits, and the tree grows as a small sapling, rising from 3 m and reaching up to 8 m, with large, randomly spread branches having brownish or light brownish bark with thin leaves [1]. Annona squamosa has been utilised as a natural medicine and in various other food applications, e.g., its pulp is utilised as a flavouring agent in ice cream, and 50–80% of custard apple fruit is edible and can be pulped as juice. It contains appreciable vitamin C in the range of 35–42 mg per 100 g, and dietary fibre, vitamin B1 (thiamine), and potassium contents are also notably high [5].

Recent articles have demonstrated that various plant byproducts, such as fruit or vegetable pomace, bran/husk/seed coat, seeds, peel, and leaves, are important source of phytochemicals and can be utilised as innovative ingredient in foods [6,7,8,9,10,11,12,13,14,15,16,17]. Extracts obtained from various sections of the Annona squamosa plant, such as its bark, roots, leaf, stem, fruit, peel, and seeds, have been utilised in traditional pharmacological applications in different countries to cure a variety of diseases, such as dysentery, epilepsy, haemorrhage, fever, and tumours [2]. Annona squamosa seed powder is utilised to abolish lice, leaf extract is used to pacify boils and treat ulcers, and the fruit acts as a sedative in cases involving heart ailments and can be used to alleviate vomiting and treat tumours [18]. Phytochemical studies have revealed that custard apple contains numerous phenol-based compounds, e.g., proanthocyanidins, with 18 different phenolic compounds, mainly alkaloids or flavonoids [19]. Apart from fruit, large amounts of leaves are generated during pruning, which causes complications related to their disposal for farmers. Annona squamosa leaves (ASLs) possess valorisation potential owing to their extensive pharmacological properties and biological activities, such as antioxidant, antimicrobial, antidiabetic, antiviral, anticancer, and hepatoprotective activities. These activities are caused by the presence of glycosides, phytosterols, carbohydrates, oils, saponins, tannins, alkaloids, phenols, flavonoids, peptides, and various acetogenin compounds [5,18,19,20]. Phytochemical assessments have emphasised that numerous active compounds, such as acetogenins and flavonoids, present in Annona squamosa also give rise to plant cytotoxic, antimalarial, antidiabetic, and immunosuppressive activities.

Present study concentrate on quantitative microscopic evaluation of *Annona squamosa linn.* (sitaphal) leaves

METHODOLOGY:

AUTHENTICATION OF CRUDE DRUG:

The leaves of Sitaphal *(Annona squamosa Linn.)* were collected from the Sri Institute of Pharmacy, AYUSHVEDHA Medicinal garden. The native collection of this plant is from Telangana Moolika Vanamu Himayathsagar, Hyderabad, Telangana State. and it was authenticated by the botanist, Dr. Venkat Rao, Osmania University, Hvderabad. Leaves occur singly, 6-17 x 3-6 cm, lanceolate or oblong lanceolate, pale green on both surfaces and glabrate or nearly so , sides sometimes slightly unequal, edges without teeth, inconspicuously hairy, at least when young, minutely

dotted on examination with a lens, thin and dull green to dark green on top surface.

MICROSCOPICAL EVALUATION OR QUANTITATIVE MICROSCOPY

Microscopic evaluation helps in more detailed examination of a crude drug .It helps In identification of organized drugs i.e. those drugs which contain cellular structure, by their well defined histological characters. It is necessary that the drug or material should be well prepared before examination through a microscope. This can be done by size reduction of drug which Means powdering of the drug or by cutting thin sections of the drug or by preparing an extraction by any extraction method like maceration. Microscopes have special role in the microscopy of tiny drug i.e. powder microscopy or quantitative microscopy Microscopes can also be used for quantitative evaluation of drugs and evaluation of adulterated powdered drugs, Quantitative microscopy is done by counting a specific histological feature e.g. Lycopodium Spore method, Stomatal Number, Stomatal Index, Vein- Islet number, Veinlet Termination Number and Palisade Ratio.

LEAF CONSTITUENTS:

1. Stomatal Number:

The average number of stomata present per square millimeter of the epidermis is known as Stomatal number.

Example:

Table-1

S.No.	**Drug**	**Stomatal Number**	
		Upper Epidermis	**Lower Epidermis**
1	Atropa belladona	07-10	77-115
2	Datura metel	147-160	200-209
3	Ocimum sanctum	64-72	175-250

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 epidermis. Separately with the help of forceps, place It on a glass slide and mount it with glycerin 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 micrometer, 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.

2 Stomatal Index:

Stomatal number is the percentage proportion of number of stomata to the total number of epidermal cells, Stomatal number can vary with the age of the leaf, but stomatal index is relatively Constant for a given species.

$$I = \frac{S}{E+S} \times 100$$

Where:

I= Stomatal index
S= Stomatal number per unit area
E= Number of epidermal cells in the same area

Example:

Table 2:

S.No	Drug	Stomatal Index
1	Senna	14-20
2	Atropa belladona	20.2-23.0
3	Vinca rosea	-0.7
4.	Datura stramonium	11-14

Procedure:

Firstly, clear the middle part containing a piece of leaf by boiling with a chloral hydrate solution or chlorinated soda solution. Then peel off both epidermis (i.e. upper and lower) separately with the help of forceps, place it on glass slide and mount it with glycerine water. Set the camera lucida and drawing board for making to scale. Draw a I mm square with the help of stage micrometer. Then place the epidermis containing a slide of cleared leaf on the stage of the compound microscope and trace the epidermal cells in each field. Then calculate the stomatal index by using the above mentioned formula. In this case determine the values for the upper and lower surface of epidermal separately.

3. Vein-islet Number:

The vein islet number is the area of photosynthetic tissues circled with conducting stands. Vein-islet number is defined as the number of vein-islets in an area of 4 sq mm of the central part of leaf surface between midrib and margin. It is generally constant as a species of the plant. It is irrespective of age factor.

Example:

Table-3:

S.No	Drug	Vein- islet number
1	Senna	25-29.5
2	Bacopa monniera	6-13
3	Eucalyptus globules	8-13.5

Procedure:

For the determination of vein-islet number, mainly clear the piece of leaf boiling with chloral hydrate solution for about 30 minutes. Then set the camera lucida and drawing board for making the drawing to scale. Draw a l mm square with the help of a stage micrometer under 16 mm objective. Set the paper for the visibility of the square in the eyepiece. Then place the cleared leaf containing slide and trace and count the veins which are included in the square completing the out lines of those islets which overlap to adjacent sides of the square. Take the average number of vein Islets from the four adjoining squares to get the value for Isq mm.

4. Veinlet Termination Number:

The number of Veinlet termination per sq mm of the leaf surface midway between midrib and margin is called the Veinlet termination number. Veinlet terminations are the ultimate free termination.

Example:

Table-4:

S.No	Drug	Vein- Termination number
1	Atropa belladona	6.3-10.3
2	Atropa acuminata	1.4-3.5
3	Aloe vera	65

Procedure:

For the determination of the Veinlet termination number, the procedure is the same as determination of the Vein Islet number counts the veinlet termination number included in the square. Take the average of veinlet termination numbers from the four adjoining squares to get the value for l sq mm.

5. Palisade Ratio:

Palisade ratio is the average number of palisade cells beneath one epidermal cell, using four continuous epidermal cells for the count. This study is mainly performed on powdered drugs with the help of camera lucida.

Example:

Table-5:

S.No	Drug	Palisade Ratio
1	Senna	7.5
2	Atropa belladona	05-70
3	Digitalis Lanata	2.5-6.5

Procedure:

Clear a piece of leaf by boiling with chloral hydrate solution or chlorinated soda solution. Set the camera lucida and drawing board for making the drawing to scale. Trace the outlines of four cells of the epidermis on the paper by using a 4mm objective and focus down the draw tube of the microscope on the palisade layer and trace the cells. Count the palisade cells under the four epidermal cells. Calculate the average number of cells beneath a single epidermal cell i.e. palisade ratio. Repeat the determination for five epidermal cells from different parts of the leaves. Calculate the average of the findings for the five groups which is said to be the palisade ratio of the leaf.

Camera Lucida:
It is an instrument used for tracing any object in a magnified image formed under the microscope with the help of camera lucida. It is possible to correctly record the position, dimension of cells and other cell structures on a drawing paper. The most commonly used models of camera lucida in the laboratory are :

1. Abbe's Model
2.Swift Ives Model

1. Abbe's Model:

In this model, a prism is fitted over the eyepiece of the microscope. Along with this, a side arm which carries a mirror is also fitted which is supported in vertical position on the tracing paper. The working principle of this model is that when it is in working position, the light from the drawing board is reflected by the mirror fitted on the side arm into the prism which further reflects into the observer's eye. By this, the observer can see the tracing paper and the marker. The prism fitted in the model has a Small central opening through which the observer can see the image of the object. As a result both images appear to be superimposed on each other and can be traced by the observer easily.

2. Swift Ives Model:

In this model, a small right angled prism is fitted which replaces the plane mirror. This type Camera- lucida is small in size and can be fitted on the eyepiece of the microscope with the help of a screw. It is the most widely used model in laboratories because of its smaller size,less weight and it puts less strain upon the microscope. Some precautions which should be kept in mind while drawing the magnified image of any object using camera lucida are follows:

a.) It is necessary to match the illumination of both object and paper to see the image and Marker equally clearly.

b.) Manage the drawing board in the correct position to avoid distortion.

c.) Place a stage micrometer under the objective lens of a microscope to trace the divisions on the paper then measure the distance between two divisions.

If they are not at equal distance, slightly tilt the board and repeat the same process until all divisions are at equal distance.

d.) For the measurement purpose, place the stage micrometer on the stage of the microscope and trace the divisions. Then replace the stage micrometer and place the object containing the slide on stage and trace it under the same objective lens and eyepiece. The size of any object can be calculated by dividing the imaginary length i.e., value of size on tracing paper by magnification value for Example, if mean diameter of object tracing is 4 mm (4000µm) and magnification value is 200, Then actual diameter will be

4000 divided by 200 equal to 20 mm $\left(\frac{4000}{200} = 20\mu m\right)$.

RESULTS:

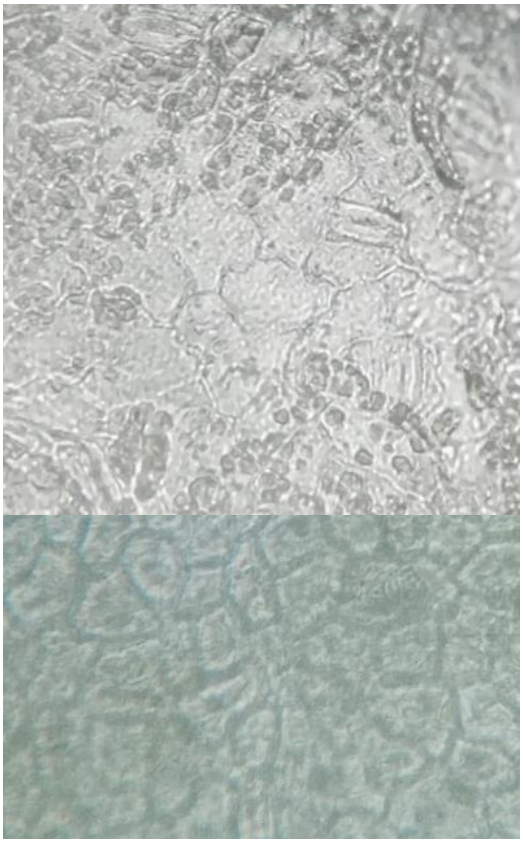
1. STOMATAL NUMBER AND INDEX OF *Annona Squamosa*

Principle:

Annona Squamosa is a plant of family *Annonaceae*. Stomatal number is the average number of Stomatal is the percentage which the number of stomata formed to the total number of epidermal cells, each stomata being counted as one cell.

The stomatal index can be calculated by using the following equation.

$$\text{Stomatal Index (SI)} = \frac{S}{S+E} X\ 100$$



Calculation:

4.6=6 divisions
10cm=?

$\frac{10x6}{4.6}$ = 13.4cm
1 mm=100 divisions

$y2 = \frac{13.04}{100}$ = 0.1304 mm

Area=y^2 =$(0.1304)^2$= 0.0170 sq.mm

Number of stomata- 4

Number of epidermal cells= 22

$S = \frac{\text{Number of stomata}}{\text{Unit area}}$

$= \frac{4}{0.0170}$ = 235.29

$E = \frac{\text{Number of epidermal cells}}{\text{Unit area}}$

$= \frac{22}{0.0170}$ = 1294.11

$$\text{Stomatal Index (SI)} = \frac{235.29}{235.29+1294.11} X\ 100 = 15.38$$

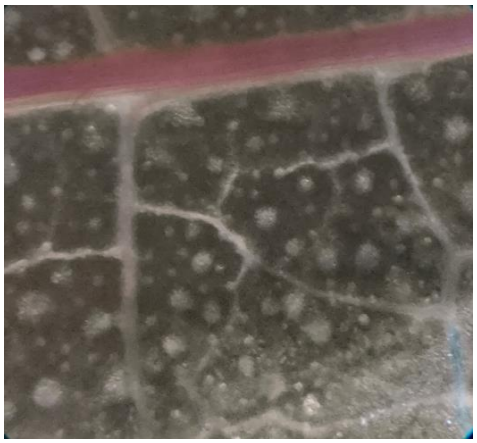
2. DETERMINATION OF THE VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER OF *Annona Squamosa*

Principle:

Vein Islet number :

The vein islet number is the average number of vein lslets per sq mm of leaf surface. It is determined by counting the number of Vein lslets in an area of sq mm of central part of af between the midrib and the margin.

Vein termination number :



It is defined as the number of veinlet terminal per sq mm of leaf surface. Midway between midrib of leaf and its margin. A vein termination is the ultimate free terminals of veinlet. These are constituents for a given species of the plant and are used as a characteristics for identification of allied species.

Calculation:

1.8 cm=10 divisions

10 cm=100mm

1 mm=100 divisions

$\frac{10x10}{1.8}$ = 55.55cm

X=55.55

$X = \frac{55}{100}$ = 0.55cm

Area=X^2 =$(0.55)^2$ =0.3025

Vein islet Number= $\frac{\text{Number of vein islets}}{\text{Area}}$

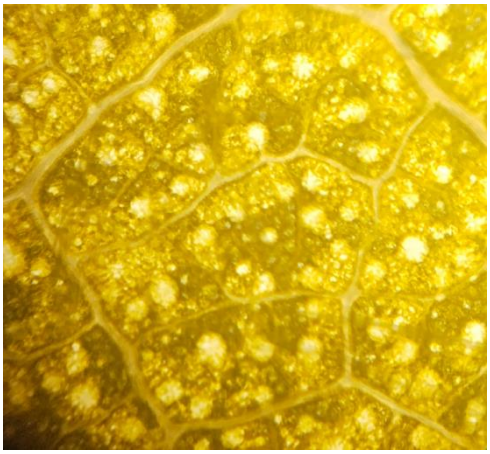
$= \frac{8}{0.302}$ = 26.49

Vein Terminal Number = $\frac{\text{Vein Terminal}}{\text{Area}} = \frac{10}{0.302}$ = 33.11

3. DETERMINE THE PALISADE RATIO OF *Annona Squamosa*

Principle:

The average number of palisade cells beneath are epidermal cells of the leaf is termed as palisade ratio. It is determined by counting palisade cells present beneath the few continuous epidermal cells. Standard palisade ratio furnished important data for drug evaluation of *Annona Squamosa* is 6-9 and can be successfully applied for the studies of several medical importance of leaf.



Calculation:

Palisade Ratio= $\frac{\text{Number of Palisade cells}}{\text{Number of vein Islets}}$

$= \frac{64}{8} = 8$

4. SUMMARY OF THE RESULT:

Table 6:

S. No	**Qualitative Microscopical Parameter of** *Annona Squamosa Linn.* Leaves	**Values**
1	Stomatal Index	15.38
2	Vein Islet No. and Termination No.	26.49 & 33.11
3	Palisade Ratio	8

CONCLUSION:

The traditional usage of this plant crude drugs in terms of its efficacy and versatility of their therapeutic utilization. In ayurvedic system is quite common usage in the dosage forms. Present work focus with qualitative standard parameters of particular methodological evaluations of leaf Sitaphal (*Annona squamosa linn.*) authenticated with various resultant values. The determination values of particular species as follows Stomatal Index 15.38, Vein Islet number 26.49, Vein Termination number 33.11 and Palisade Ratio 8 are discussed and further research work to be extended for specific crude drugs, pharmacognostic studies are to be developed in future.

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