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STANDARDIZATION AND MICROSCOPICAL EVALUATION STUDIES OF *CENTELLA ASIATICA LINN.* (BRAHMI) LEAVES

Kyatham Hemanth[1], S.K. Godasu[2], Sravanthi Kotha[3], Supraja Konakanti[4], Narni Jaya Prakash[5]

[1] Associate Professor, Sri Indu Institute of Pharmacy, Department of Pharmacognosy, Sheriguda, Hyderabad

[2] Associate Professor, Sri Indu Institute of Pharmacy, Department of Natural products, Sheriguda, Hyderabad

[3,4,5] Students of Sri Indu Institute of Pharmacy, Sheriguda, Hyderabad

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Abstract:

Based on trial-and-error experience and folk remedies continue to draw wide attention for their role in the treatment of mild and chronic diseases. We focus on plant research has increased all over the world and a large body of evidence has been accumulated to highlight the immense potential of medicinal plants used in various traditional systems of medicine. Centella asiatica is an important medicinal herb that is widely used in the orient and is becoming popular in India. Triterpenoids, Saponins, the primary constituents of Centella asiatica are mainly believed to be responsible for its wide therapeutic actions. The herb is recommended for the treatment of various diseases like leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhea, fever, amenorrhea and Alzheimer's. The present work employs the standardization and microscopical evaluation of leaf drug to authenticate by methodological parameters ranges of Stomatal number, Vein islet number and Stomatal index of the crud drug. Respective practical and morphological parameters of the drug of Centella asiatica . Various microsopical parameters to evaluate the characteristics are reported, which is imperative for the safe utilization of the herb, are discussed.

Key Words: *Centella asiatica, description, stomata, safe utilization, authentication.*

Corresponding author:

Kyatham Hemanth,
Associate Professor,
Sri Indu Institute of Pharmacy,
Department of Pharmacognosy, Sheriguda, Hyderabad

QR code

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INTRODUCTION:

Centella asiatica (L.) Urban (*Syn. Centella coriacea* Nannfd., *Hydrocotyle asiatica* L., *Hydrocotyle lunata* Lam., and *Trisanthus cochinchinensis* Lour.) is a tropical medicinal plant from Apiaceae family native to Southeast Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia as well as South Africa and Madagascar [1]. *C. asiatica*, commonly known as "Gotu kola, Asiatic pennywort, Indian pennywort, Indian water navelwort, wild violet, and tiger herb" in English, is a tropical plant, which has been also cultivated successfully due to its medical importance in some countries including Turkey, and it has a long history of utilization in ayurvedic and Chinese traditional medicines since centuries [2]. The leaves, which are edible, are in yellowish-green color, thin, alternate with long petioles, and quite characteristic reniform, orbicular, or oblong-elliptic shapes with seven veins [3]. The plant grows horizontally through its green to red stolones which combine to each other and roots in underground. Monographs of the plant describing mainly its wound healing and memory enhancement effects exist in the European Pharmacopeia, Commission E of the German Ministry of Health, and World Health Organization (WHO) [4]. In addition to neuroprotective effect of *C. asiatica*, it has been reported to own a wide range of biological activities desired for human health such as wound healing [5–7], anti-inflammatory [8, 9], antipsoriatic [10], antiulcer [11, 12], hepatoprotective [13], anticonvulsant [14], sedative [15], immunostimulant [16], cardioprotective [17, 18], antidiabetic [19], cytotoxic and antitumor [20, 21], antiviral [22], antibacterial [23], insecticidal [24], antifungal [25], antioxidant [26–28], and for lepra [29] and venous deficiency treatments [30, 31].

The present study is to investigate the Preliminary and Microscopical characteristics of Brahmi leaves. The objectives of the study are selection authentication and procurement of plant, conducting extensive literature survey, to carry out leaf characteristics microscopical methods, to conduct standard methodology procedures in microscopy of leaf drugs, to carryout qualitative analysis of the leaf constituents and standardize with different techniques.

MATERIALS AND METHODS:

Plant collection and identification

The leafs of Brahmi (Centella asiatica) were collected from the Sri Indu Institute of Pharmacy, AYUSHVEDHA Medicinal garden. The native collection of this plant is from Telangana Moolika Vanamu , Himayathsagar, Hyderbad. and it was authenticated by the Botanist, Dr. Venkat Rao, Osmania University, Hyderabad. The leafs are hard fleshy, orbicular to reniform and dentate with small trailling herb. Petiole is long. Smooth on upper surface and hairy below. Fresh and dry leaf powder is used for the research studies.

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 any drug i.e. powder microscopy or quantative 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	*Datura metel*	141-160	200-209
2	*Ocimum sanctum*	64-72	175-250
3	*Atropa belladonna*	07-10	77-115

Procedure:
Firstly, clear the middle part containing piece of leaf by boiling with **chloral hydrate** solution or chlorinated soda solution Then peel of both epidermis. Seperately with the help of forecep, place it on glass slide and mount it with glycerin water. Set the camera lucida and drawing board for making the drawing to scale. Draw a 1mm square with the help of stage micrometer, then place the epidermis containing slide of cleared leaf on stage of 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 he average number of stomata in 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	Vein-islet number
1.	Cassia angustifolia	19-23
2.	Cassia acutifolia	25-30
3.	Digitalis purpurea	2-6

Procedure:
Firstly, clear the middle part containing piece of leaf by boiling with chloral hydrate solution orchlorinate 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 1mm square with the help of stage micrometer, then place the epidermis containing slide of cleared leaf on stage of 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 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 4sq mm of the central part of leaf surface between midrib and margin. It is generally constant in as species of the plant. It is irrespective with age factor .

Example:
Table 3 :

S.No	Drug	Veinlet Termination number
1.	*Cassia acutifolia*	25-30
2.	*Cassia angustifolia*	25-33
3.	*Digitalis purpurea*	2-5

Procedure:
For the determination of vein-islet number, mainly clear the piece of leaf boiling with chloralhydrate solution for about 30 minutes. Then set the camera lucida and drawing board for making the drawing to scale. Draw a 1 mm square with the help of stage micrometer under 16 mm objective. Set the paper for the visibility of square in the eyepiece.Then place the cleared leaf containg 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 average number of vein

islets from the four adjoining squares to get the value for 1sq mm.

4. Veinlet Termination Number:

The number of Veinlet termination per sq mm of the leaf surface midway between midrib and margin is called as

S.No	Drug	Stomatal index
1.	*Atropa belladonna* (lower surface)	20-23
2.	*Cassia acutifolia* (both surfaces)	17-19
3.	*Cassia angustifolia* (both surfaces)	17-20

Veinlet termination number. Veinlet terminations are the ultimate free termination.

Example:

Table 4:

Procedure:

For the determination of Veinlet termination number,the procedure is same like determination of Vein Islet number count the veinlet termination number included in the square. Take the average ofveinlet termination numbers from the four adjoining squares to get the value for 1 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.	*Atropa belladonna*	6-10
2.	*Datura strawmonium*	4-7
3.	*Solanum nigrum*	2-4

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 4mm objective and focus down the draw tube of microscope on 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 palisade ratio of the leaf.

Camera Lucida:

It is an instrument used for tracing of any object in 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 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 side arm into the prisim 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 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 correct position to avoid distortion.
(c) Place a stage micrometer under objective lens of microscope and 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 microscope and trace the divisions. Then replace stage micrometer and place the object containing slide on stage and trace it under 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 4mm (4000µm) and magnification value is 200, then actual diameter will be

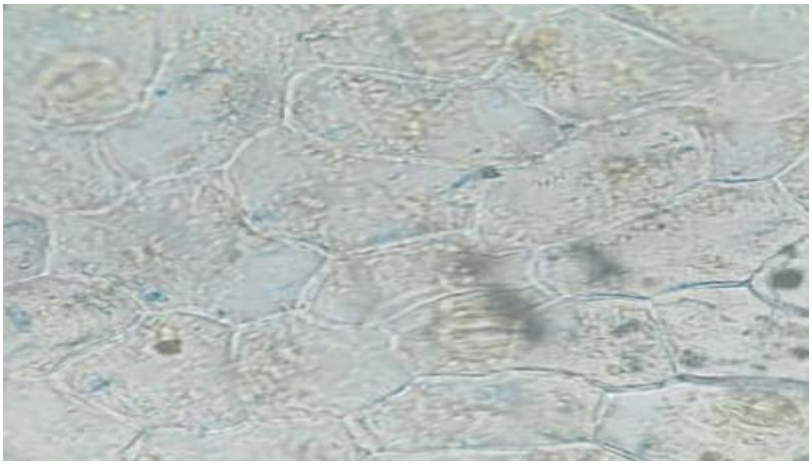
4000 divided by 200 equal to 20 mm ($\frac{4000}{200} = 20\mu m$).

RESULTS AND DISCUSSION:

1. STOMATAL NUMBER AND INDEX OF *CENTELLA ASIATICA*

Principle: *Centella asiatica* is a plant of family Apiaceae.Stomal number is the average number of Stomatal index 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 following equation.

Stomatal index $= \frac{S}{S+E} \times 100$



Calculation:

4.6=6 divisions

10cm=?

$=\frac{10\times6}{4.6} = 13.4$cm

1mm=100 divisions

=13.04

y^2=$\frac{13.04}{100}$ = 0.1304 mm

Area=y^2=$(0.1304)^2$=0.134 sq.mm

Number of stomata=4

Number of epidermal cells=12

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

=$\frac{4}{0.017}$ = 235.29

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

=$\frac{12}{0.017}$ = 705.88

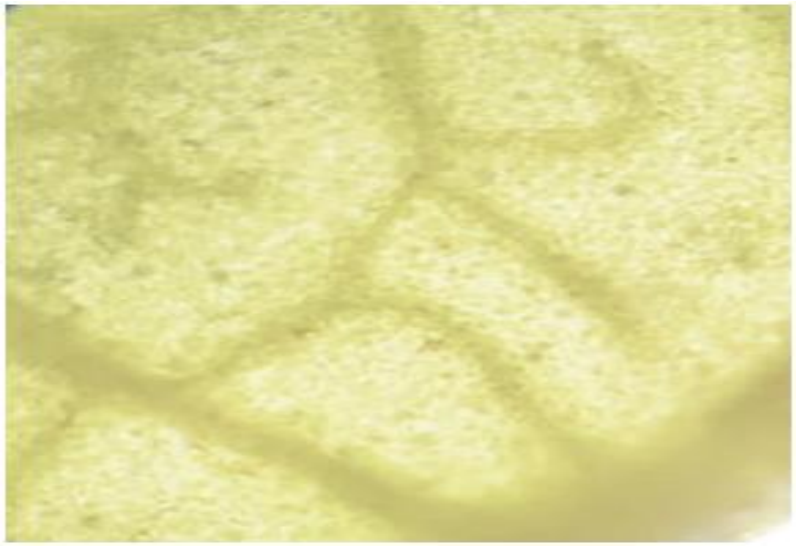
Stomatal index (SI) = $\frac{235.29}{235.29+705.88} \times 100 = 24.99$

2. DETERMINATION OF THE VIEN ISLET NUMBER AND VEIN TERMINATION NUMBER OF *CENTELLA ASIATICA*

Principle:

Vein islet number: The vein islet number is the averagw number of veinlet per sq mm of leaf surface.It is determined by counting the number of veinlets in a area of sq mm of central part of leaf between the midrib and the margin.

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



Calculation:

1.8 cm=10 divisions

10 cm=100mm

x= $\frac{10\times10}{1.8}$=55.55

1 mm=100 divisions

X =55.55

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

Area=l^2=$(0.55)^2$=0.305

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

=$\frac{4}{0.0302}$ = 13.24

Vein termination number=$\frac{\text{Vein termination}}{\text{Area}}$ = $\frac{11}{0.302}$ = 36.42

3. DERTERMINE THE PALISADE RATIO OF *CENTELLA ASIATICA*

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 *Centella asiatica* is 4-7 and can be successfully applied for the studies of several medical importance of leaf.

Calculation:

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

=$\frac{48}{4}$ = 12

4. SUMMARY OF THE RESULT:

Table 6:

S. No	Microscopical Standard Parameter	Values
1.	Stomatal Index	24.99
2.	Vein Islet No. and Terminal No	13.24 & 36.42
3.	Palisade Ratio	12

CONCLUSION:

The therapeutic potential of this plant in terms of its efficacy and versatility of their traditional utilization of crude drug in Ayurvedic system is quite common. Present work deals with standard parameters of particular methodological evaluations of leaf Brahmi (*Centella asiatica)* authenticated with various resultant value. The determination values of particular species as follows Stomatal index 25, Vein islet number 13, Vein terminal number 36 and Palisade Ratio 12 are discussed and further research work to be extended for specific crude drug Pharmacognostic studies are to be developed in future prospects.

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