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Pharmacognostical Characterization of Kokilaksha (Asteracantha longifolia Nees.) Based on Macroscopic and Microscopic Analysis

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***Abstract*—Introduction**: *Pharmacognostical evaluation is essential for confirming the identity, quality, and purity of medicinal plants used in traditional medicine. Kokilaksha (Asteracantha longifolia Nees.), an important Ayurvedic drug, requires proper authentication to ensure its safe and effective use. Macroscopic and microscopic analyses provide reliable diagnostic features that help differentiate genuine plant material from possible substitutes or adulterants.* **Aim**: *The present study aims to carry out a detailed macroscopic and microscopic evaluation of the fresh stem and stem powder (choorna) of Kokilaksha (Asteracantha longifolia Nees.) to establish characteristic features for authentication and quality assessment.* **Materials and Methods:** *Fresh stems of Kokilaksha were collected, cleaned, and sectioned for microscopic examination. Thin transverse sections were prepared, stained with safranin, and mounted using glycerin. The dried stems were powdered using a pulverizer and sieved through an 85-mesh sieve to obtain uniform choorna. Macroscopic properties of the powder, including colour, texture, odour, and taste, were recorded. Powder microscopy was performed by treating the sample with chloral hydrate, phloroglucinol, and dilute HCl, followed by examination under digital and compound microscopes at 10x and 40x magnifications. Diagnostic features such as epidermal fragments, vessel elements, fibres, and starch grains were observed and photographed.* **Results**: *Fresh stem analysis revealed an erect, soft, sub-quadrangular structure with greyish-brown coloration and swollen nodes. Microscopy of the fresh stem showed cork arranged in 5–10 layers, collenchyma forming 4–8 layers beneath the cork, cortical cells with prominent internal partitions, a single-layered endodermis, narrow phloem, and radially arranged xylem vessels with spiral thickenings. Medullary rays extended into the secondary cortex, and the pith consisted of large, thin-walled parenchymatous cells. Powder microscopy showed characteristic fragments of epidermal cells, xylem vessels, elongated fibres, and simple starch grains. Macroscopically, the choorna appeared brownish, rough, odourless, and bitter.* **Conclusion**: *The study documents key macroscopic and microscopic features of the stem of Kokilaksha, establishing reliable identification markers. These findings provide essential baseline data for the authentication and standardization of Kokilaksha stem, supporting its safe and effective use in Ayurvedic formulations.*

***Keywords*—** *Kokilaksha, Asteracantha longifolia Nees., Authentication*.

I. INTRODUCTION

Pharmacognosy is the scientific discipline that focuses on the study of medicinal plants and the natural products derived from them.[1] It involves the systematic examination of plant materials through macroscopic and microscopic methods to determine their identity, purity, and overall quality. Such evaluations are vital in herbal medicine, where variations in plant sources can greatly influence safety and therapeutic effectiveness. *Kokilaksha* (*Asteracantha longifolia* Nees.) is an important medicinal plant used widely in Ayurveda for various therapeutic purposes. Ensuring its proper identification is therefore essential to avoid adulteration and to maintain the efficacy of Ayurvedic preparations. Pharmacognostical analysis plays a crucial role in verifying the authenticity of plant materials by documenting their structural and diagnostic features. In the present study, both fresh and powdered stem samples of *Kokilaksha* were examined. The *choorna*[2]was prepared according to the method described in the *Sarangadhara Samhita*. Macroscopic features such as colour, texture, odour, and taste were recorded, while microscopic studies were performed to observe key diagnostic characters, including cork cells, cortical tissues, fibres, vessels, and starch grains.[3] The work was carried out in the Pharmacognosy Laboratory of the Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, with additional analysis performed at CARe Keralam Ltd., Thrissur. This evaluation provides essential baseline data needed for the authentication and quality control of Kokilaksha stem used in traditional Ayurvedic formulations.

2.1 Macroscopic evaluation of the fresh plant of Kokilaksha (Asteracantha longifolia Nees.)

a. Aim

To identify the macroscopic features of the fresh sample of *Kokilaksha* (*Asteracantha longifolia* Nees.)

b. Materials

Fresh sample of *Kokilaksha* (*Asteracantha longifolia* Nees.) magnifying lens, and digital camera.

c. Procedure

The fresh sample of *Kokilaksha* (*Asteracantha longifolia* Nees.), was collected from natural habitat, washed thoroughly under running water to remove impurities, and then subjected to identification as the fresh sample.

2.2 Microscopic evaluation fresh stem cutting of Kokilaksha (Asteracantha longifolia Nees.)

a. Aim:

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To study the microscopic structures of plant parts to identify and understand the basic structure of the cells and tissues.

b. Materials:

Fresh cutting of *Kokilaksha* (*Asteracantha longifolia* Nees.)., safety razor blade, dissecting needles, watch glasses, petri dishes, glass slides, cover slips ¾ circles (No. 2 thickness), camel hair brush (medium size), dropper, safranine stain, glycerine, digital microscope, compound microscope and digital camera.

c. Procedure:

i) Preparation of plant parts for microscopic evaluation:

Wash the plant parts with water to remove any dirt or debris. Cut the plant parts into small pieces using razor blades. Hold the plant part between the first finger and the thumb of the left hand. Cut the material in such a way as to avoid oblique cut sections. Cut a number of thin sections. Sweep the sections with the help of fine camel hair brush and transfer to a watch glass containing water (keep for 3min).

c) Staining of sample section:

Add a few drops (1-2 drops) of safranine stain to the water containing thin sections.When the section is sufficiently stained (keep for 5min), take it using a hair brush and transfer it on a clean slide.

d) Mounting the plant parts:

Place a small piece of plant tissue on a microscope slide. Add a few drops of clearing agent (glycerol) to the tissue to make it transparent. Gently press a cover slip on the slide at the left-hand edge of the glycerol drop. Place the cover slip gently on the drop with the help of a dissecting needle avoiding air bubble and ensuring uniform spread of drop.Remove excess glycerine and water using tissue paper.

e) Microscopic evaluation:

Place the slide under a microscope with the mount lying in the centre. Fix the slide in place with the help of two clips on the stage. Screw down the body tube by means of coarse adjustment until the lens of low power (4x objective lens) comes close to the cover slip. Focus the light to the mounted slide using the mirror. View through the eye piece and screw the tube upwards until the object comes into focus. Control the light with the iris diaphragm, Use the fine adjustment to get the object in sharp focus. Focus on the tissue using the lowest magnification objective lens (4x objective lens) first and then higher magnification (10x objective lens). Observe the stained tissue under the microscope and recorded the observations.

2.3. Powder macroscopic evaluation Kokilaksha (Asteracantha longifolia Nees.)

a. Aim

To identify the characteristic features of *Kokilaksha choorna* (powder of dried stem of (*Asteracantha longifolia* Nees.) with the help of powder macroscopic features.

b. Materials

Kokilaksha choorna (powder of dried stem of (*Asteracantha longifolia* Nees.), magnifying lens, white paper, digital camera.

c. Procedure

Preparation of plant part for powder microscopy

Fresh stem of *Kokilaksha* (*Asteracantha longifolia* Nees.) was collected and kept under sunlight for drying and frequent turnings were done for uniform drying. Dried stem was subjected to powdering. Powdering was done by using pulverizer and obtained *choorna* (powder of *Kokilaksha* (*Asteracantha longifolia* Nees.) was then sieved using a mesh size of 85

Evaluation

The *Kokilaksha choorna* (powder of dried stem of (*Asteracantha longifolia* Nees.) was placed on white paper and viewed using magnifying lens and naked eye. The color, texture, odor, and taste of the powder were evaluated through macroscopic analysis. Color was observed with the naked eye, and the texture was assessed by feeling the powder with the fingers to determine its fineness and uniformity. The powder was then smelled and tasted to assess its odor and taste. Photographs of the powdered drug were taken using a digital camera.

2.4. Powder microscopic evaluation of Kokilaksha choorna (powder of dried stem of (Asteracantha longifolia Nees.)

a. Aim

To identify the powder characters of *Kokilaksha choorna* (powder of dried stem of (*Asteracantha longifolia* Nees.) with the help of powder microscopic features.

b. Materials

Kokilaksha choorna (powder of dried stem of (*Asteracantha longifolia* Nees.), watch glass, glass slide, cover slips (no: 2 thickness), camel hair brush (medium-sized), dropper, spatula, blotting paper, chloral hydrate, glycerine, phloroglucinol, HCl, digital microscope, compound microscope and digital camera.

c. Procedure

i) A pinch of *kokilaksha choorna* (powder of dried stem of (*Asteracantha longifolia* Nees.) was taken and placed on glass slide. Then spread the powder evenly over each slide to create a thin layer. Applied a few drops of the chloral hydrate solution onto the powder sample on the slide. Gently heat the slides from below using a Bunsen burner. After the chloral hydrate treatment, remove excess solution from the slides if necessary, using a blotting paper. Then applied a few drops of phloroglucinol solution to slide, covering the powder. Allowed the phloroglucinol to react with the sample for a few minutes. Applied a few drops of dilute hydrochloric acid (HCl) onto the slide, covering the powder sample. Placed a coverslip gently over the treated powder sample using a needle to avoid formation of air bubbles. Examined the slides under a digital microscope, starting with low magnification (10x) and gradually increasing to higher magnifications(40x) as needed. Images were then taken using digital camera.

ii) A pinch of *kokilaksha choorna* (powder of dried stem of (*Asteracantha longifolia* Nees.) was taken and placed on a glass slide. Few drops of water were added to the powder and mixed with a hair brush. This mixture was then spread throughout the glass slide to overcome the overlapping of constituents of various structures. A cover

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slip was placed on the glass slide and it was then viewed using compound microscope under 10X powers. The presence of cell components in the powder of rhizome were identified under 10X and 40X in powder microscopy. Images were then taken using digital camera.

The same procedure was repeated with safranine and glycerin instead of water.

II. Results And Discussion:

A.Organoleptic evaluation of fresh stem of Kokilaksha (Asteracantha longifolia Nees.)

Table 1: Organoleptic evaluation of fresh stem of *Kokilaksha (Asteracantha longifolia* Nees.)

Nature	Woody
Branching	Unbranched
Texture	Soft
Type	Erect
Shape	Sub-quadrangular
Colour	Greyish brown
External features	Fasiculated, swollen at nodes
Modification	N.A

Picture No:1 *Kokilaksha* (*Asteracantha longifolia* Nees.)

B. Microscopic evaluation of fresh stem of Kokilaksha (Asteracantha longifolia Nees.)

Cork: Consists of 5-10 rows of rectangular, radially arranged, moderately thick walled, brownish cells. Collenchyma is 4-8 layered consisting of isodiametric cells. A few thick-walled, isolated cells found scattered in this zone.

Cortical cells thin-walled, round, oblong, variable in size with a number of large air cavities. A special feature of these cells is the formation of tangential and radial walls within the cell dividing it into 4-5 or more parts.

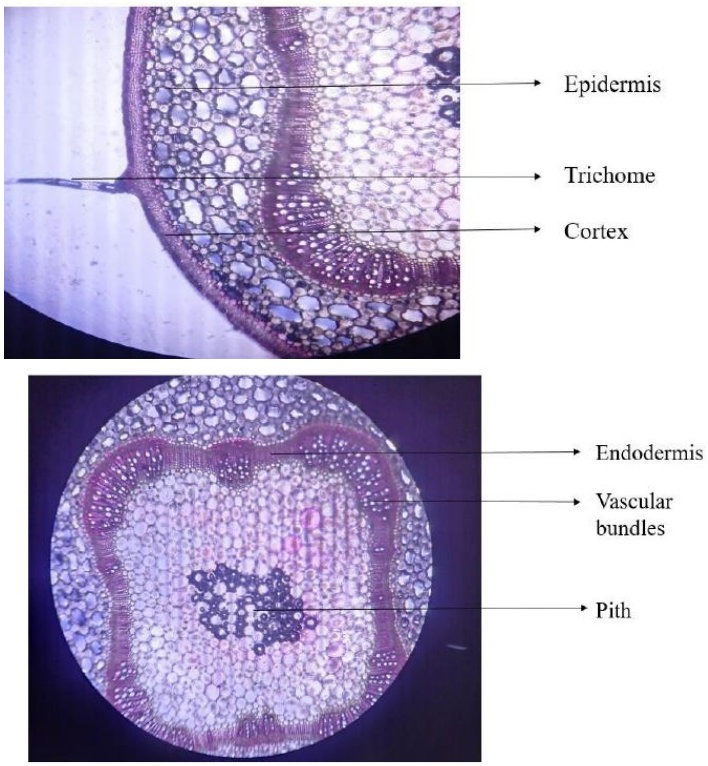
Endodermis: It is single layered, composed of transversely elongated thin-walled cells.

Phloem: Narrow, consisting of round to polygonal cells, peripheral ones larger, inner cells are smaller.

Xylem: Present in a ring vessels with spiral thickenings, arranged radially. It is transversely elongated in secondary phloem.

Medullary rays: Uni to multi seriate extend upto secondary cortex, consist of thin walled, radially elongated in secondary xylem.

Pith: Large, composed of polygonal, thin-walled parenchymatous cells, having intercellular spaces.



Picture No:2 Microscopic evaluation of fresh stem of *Kokilaksha (Asteracantha longifolia* Nees.)

C. Powder macroscopic evaluation of Kokilaksha choorna (powder of dried stem of (Asteracantha longifolia Nees.)

Powder macroscopic features such as colour, texture, odour and taste of *kokilaksha choorna* (powder of dried stem of (*Asteracantha longifolia* Nees.) were tabulated as follows.

Table No: 2 Powder macroscopy of *kokilaksha choorna* (powder of dried stem of (*Asteracantha longifolia* Nees.)

Observations	**Powder (*choorna*) features**
Colour	Brownish
Texture	Rough
Odour	Not distinct
Taste	Bitter

Picture No: 3 Powder macroscopy of powder microscopy of dried stem of *Kokilaksha (Asteracantha longifolia* Nees.)

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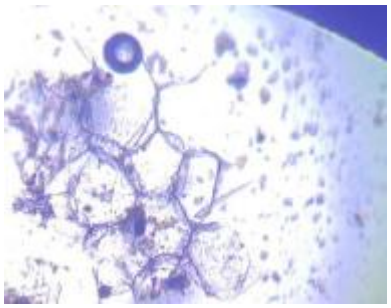
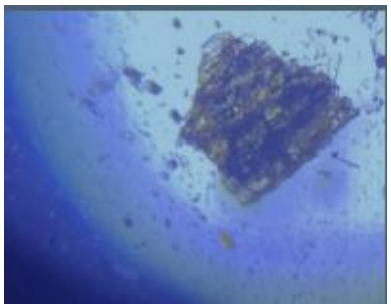
D. Microscopic evaluation of powder microscopy of dried stem of Kokilaksha (Asteracantha longifolia Nees.)

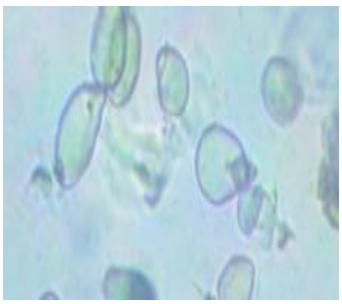
Powder microscopy evaluation of powder of dried stem of *Kokilaksha (Asteracantha longifolia* Nees.) shows fragments of epidermal cells, vessels,starch grains and elongated fibres.



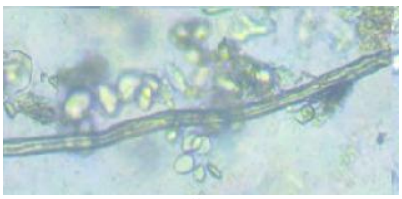
Fragments of epidermal cells



Fragments of vessel



Starch grains



Fibre

Picture no.4 : powder microscopy of dried stem of *Kokilaksha (Asteracantha longifolia* Nees.)

III. CONCLUSION

The detailed macroscopic and microscopic evaluation of powdered dried stem of *Kokilaksha (Asteracantha longifolia* Nees.) highlights its characteristic features essential for identification and authentication. The macroscopic analysis provided insights into its physical attributes, such as color, texture, odor, and taste, while the microscopic evaluation revealed cellular structures, including cork cells, starch grains, fibers, trichomes, and vascular elements. These findings underscore the importance of comprehensive pharmacognostical studies in ensuring the quality, purity, and efficacy of herbal products. The study contributes to the scientific validation of *Kokilaksha (Asteracantha longifolia* Nees.) as a reliable component in traditional medicine.

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Authors Contributions

All the authors contributed equally in design and execution of the article.

Conflicts of Interest

Nil

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