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PHARMACOGNOSTICAL ASSESSMENT OF *ALLAMANDA BLANCHETII* LEAVES

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ABSTRACT

The pharmacognostical study of *Allamanda blanchetii* leaves revealed key diagnostic features: dorsiventral lamina with uniseriate epidermis and thick cuticle, Anisocytic stomata on the lower epidermis, and uniseriate trichomes on both surfaces. Mesophyll showed 1–2 layers of palisade cells with oil globules and spongy parenchyma with intercellular spaces. The midrib exhibited collenchymatous support and crescent-shaped bicollateral vascular bundles. Powder microscopy identified rosette, cluster, and prismatic crystals, spiral and pitted vessels, and characteristic trichomes. These anatomical markers provide reliable criteria for identification and quality assessment. Physicochemical parameters including Leaf constant such as stomatal number & stomatal index, Vein islet and vein termination, moisture content, total ash, acid-insoluble ash, water-soluble ash, sulphated ash, extractive values and fluorescence analysis were determined. These findings establish distinctive markers for the identification and differentiation of *Allamanda blanchetii* leaves and provide a valuable reference framework for future phytochemical research.

KEYWORDS: *Allamanda blanchetii*, Morphology, Microscopy, physicochemical parameters.

1. INTRODUCTION

The World Health Organization defines traditional medicine as the accumulated knowledge, skills, and practices that originate from the cultural theories, beliefs, and experiences of various communities. These methods whether scientifically validated or not are employed to sustain health and to aid in the prevention, diagnosis, and treatment of physical and mental conditions.[1] Since ancient times, plants have been used for medicinal purposes. Various plant-derived products, including foods, herbal mixtures, and powders, have been traditionally used to help treat and prevent illnesses, with different levels of effectiveness.[2] *Allamanda* is an angiosperm commonly found in all parts of world. These plants are evergreen shrubs, commonly found in all parts for their beautiful inflorescence. This plant consists of approximately 15 species, namely *Allamanda augustifolia*, *A. blanchetii*, *A. caccicola*, *A. cathartica*, *A. doniana*, *A. laevis*, *A. martii*, *A. nobilis*, *A. oenotherifolia*, *A. polyantha*, *A. puberula*, *A. schottii*, *A. setulosa*, *A. thevetifolia*, *and A. weberbaueri*. The specimen *Allamanda violacea* belongs to the kingdom Plantae, Family Apocynaceae, Sub-family Rauvolfioideae, Genus *Allamanda* and Species *Violaceae*. The leaves of this plant are arranged opposite in whorls. The inflorescence is compound cyme, which contains five loabed sepals. The fruit is schizocarp which contain two and occasionally four.[3] Based on the length and width *Allamanda blanchetii* have the largest flowers (10.6–12.8 × 9.1–11.4 cm). *Allamanda blanchetii* have reddish lavender color flower bud and yellowish pink color flower.[4] The substances plumericin, isoplumericin, and 5, 6-dimethoxy coumarin were isolated using *Allamanda blanchetii* (unckalin). The roots also contain a number of powerful phytochemicals that have been found. *Allamanda blanchetii* shows

antioxidant, cytotoxic, thrombolytic, membrane-stabilizing, and antimicrobial properties.

Brine shrimp lethality bioassay was conducted to identify cytotoxic potential of the extractives. The test samples were also involved in thrombolytic and membrane stabilizing activity assays to evaluate their abilities to promote clot lysis and to stabilize erythrocyte membrane under hypotonic and heat induced conditions. The extractives were involved in disc diffusion assay to measure their ability to give zones of inhibition in cultured bacterial medium.[5] Leaves are Simple, whorled, glossy, entire, elliptic (oval), evergreen having latex, light green, pubescent which are 7-12cm long and arranged in whorls of four.[6]

According to the reports *Allamanda blanchetii* acts as Laxatives, antibiotics treatments for malaria, jaundice, an enlarging spleen, coughing, anti-inflammatory properties, purgative antioxidant qualities, cytostatic and cytotoxic activity, anti-dyslipidemic activities, anti-diabetic activities, thrombolytic activities, membrane stabilizing and antimicrobial activities, the plant is also known to deal with heat and different toxic products it activates blood circulation and diuresis.[7]

Due to the scarcity of scientific data on *Allamanda blanchetii* leaves, this study undertook macroscopical, microscopical, and quantitative analyses. Microscopical examinations involved transverse section (T.S.), longitudinal section (L.S.), and powder drug analysis of the leaves. Quantitative evaluation of the crude dried powder included determination of moisture content, total ash, water-soluble ash, acid-insoluble ash, sulphated ash, fiber length, fiber width, and both water- and alcohol-soluble extractive values.

Taxonomical classification[3]

Kingdom : Plantae
Division : Tracheophytes
Phylum : Magnoliopsida
Class : Equisetopsida
Order : Fabales
Family : Apocynaceae
Sub family: Rauvolfioideae
Genus : *Allamanda*
Species : *Allamanda blanchetii*
Synonyms : *Allamanda violacea Gardn.*
Common name: *Cherry allamanda, Purple allamanda, Violet allamanda.*

Vernacular Names[4]

Kannada : *Allamanda*
English : *Purple Allamanda*
Sanskrit : *Pilaghanti*
Portuguese: *Alamanda-purpura, Alamanda-roxa*
Brazil : *Alamanda-de-Jacobina*
Tamil : *Pilagantiyum*

2. MATERIALS AND METHODS

Collection of plant material

In October 2025, the plant material was gathered from Mandya, Karnataka, India. Dr. V. Rama Rao, Research Officer (Botany), Central Ayurveda Research Institute, Bengaluru, identified and verified the plant. For future use, an herbarium voucher specimen was prepared and preserved in the Pharmacognosy department of the Bharathi College of Pharmacy, Bharathinagara.

Drying and size reduction of the leaves

The collected plant material was shade-dried and subsequently ground into a coarse powder. The leaf powder of *Allamanda blanchetii* was then sieved using sieve number 80 and preserved in an airtight container for further use.

3. EXPERIMENTAL PROCEDURE

Macroscopical studies[9]

Macroscopic evaluation of *Allamanda blanchetii* leaves was carried out to assess their colour, texture, size, shape, fracture, odour, and taste. For this purpose, the crude plant material was placed on a white paper background, allowing direct visual examination of the raw drug with the unaided eye.

Microscopical studies[10]

Free-hand slices of fresh leaves have been used for microscopical studies. After being cleaned with a chloral hydrate solution and then water, thin sections were stained with safranin and examined under a microscope. Additionally, the dried leaves powder was examined under a microscope after being treated with a chloral hydrate solution, followed by water, and stained with safranin. Using a Cat Cam microscope camera that is attached to the microscope, microphotographs were captured.

Physicochemical constants[11-13]

The Indian Pharmacopoeia's standard processes were used to calculate physicochemical constants like the percentage of moisture content, total ash, acid insoluble ash, water soluble ash, sulphated ash, water and alcohol soluble extractives, fluorescence analysis and weight loss on drying.

Preliminary phytochemical studies[14]

Using conventional protocols outlined by Kokate C.K., Purohit A.P., and Gokhale S.B., preliminary phytochemical testing was conducted for the leaves of *Allamanda blanchetii*, and chemical constituents were identified.

4. RESULTS AND DISCUSSION

Macroscopical studies

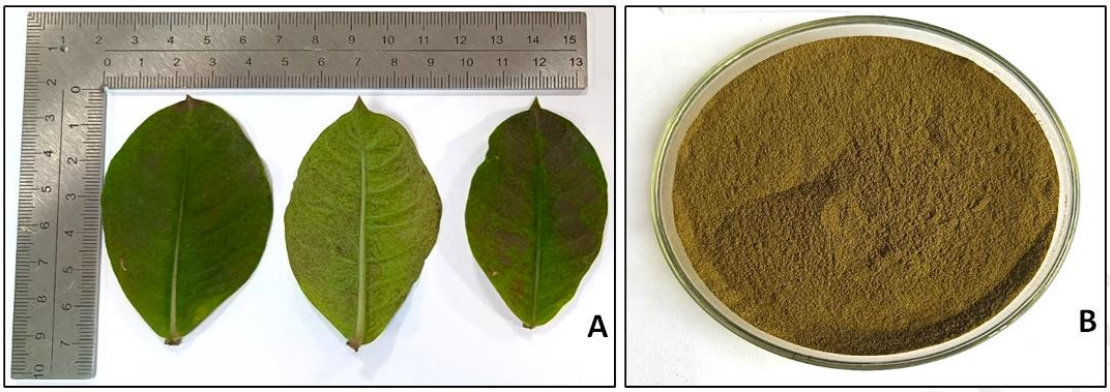


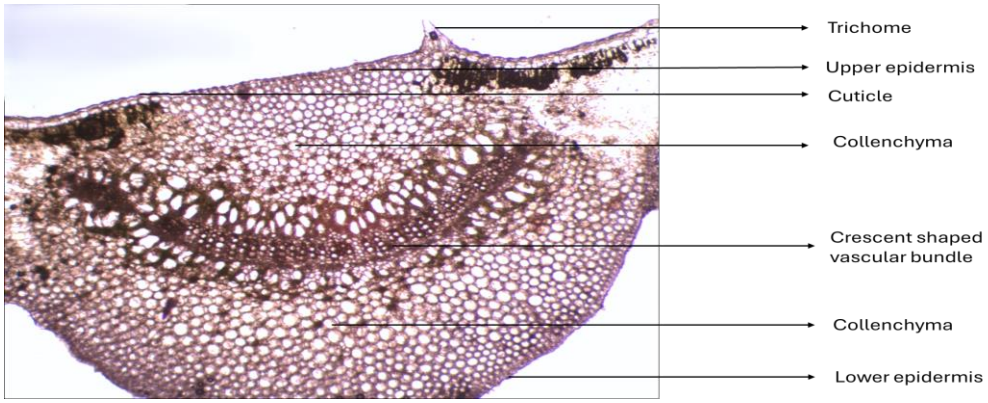
Fig. 1.1 A. Measurement of Fresh Leaves & B. Leaf Powder.

Table 1: Macroscopical character of leaves *Allamanda blanchetii* includes. (Fig. 1)

Colour	Dark Green
Odour	Pungent
Taste	Bitter
Size	Leaf blades range from 10-20cm length
Shape	Oblong-ovate to elliptic
Fracture	leathery
Texture	Slightly fuzzy or pubescent

Microscopical Character

Transverse section of the leaf



TS of Leaf (Lamina)

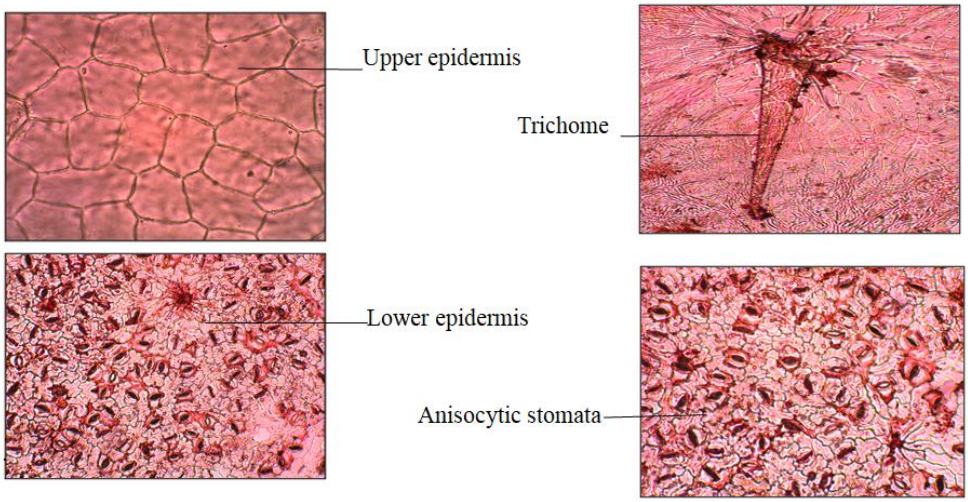


Fig. 1.2. Adaxial and abaxial Surface view of leaf.

TS of Leaf (Lamina)

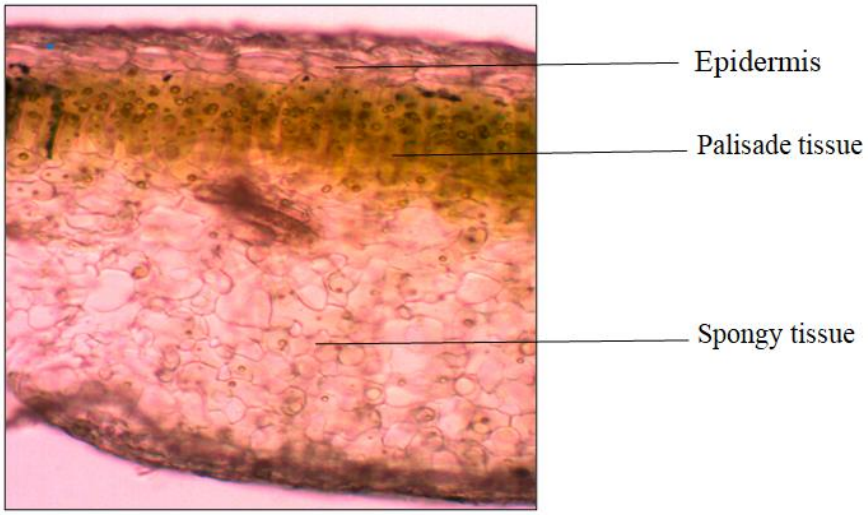


Fig.1.3.1 TS of Leaf (Lamina)

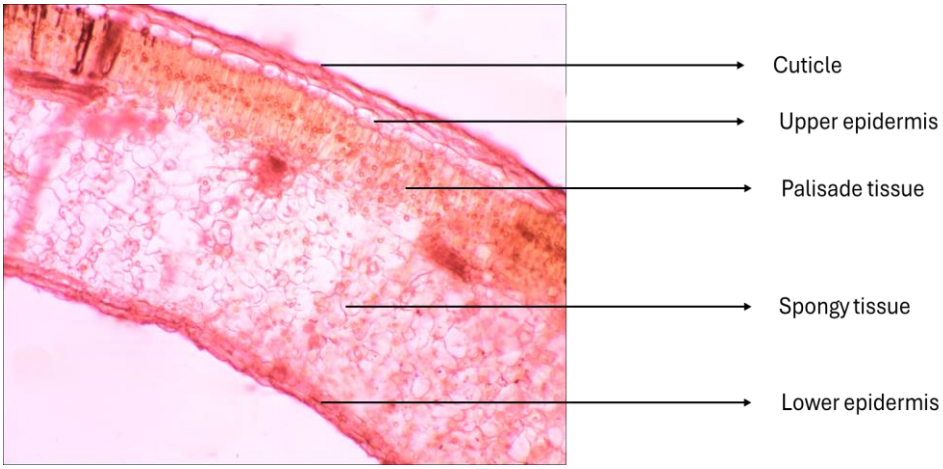


Fig.1.3.2 TS of Leaf (Lamina)

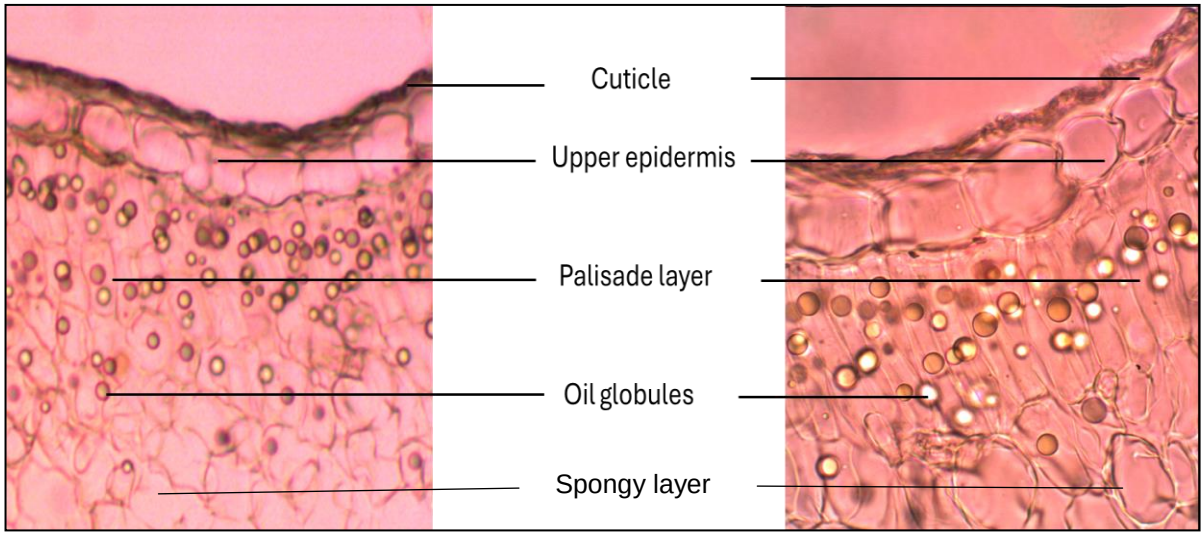


Fig.1.3.3 TS of Leaf (Lamina)

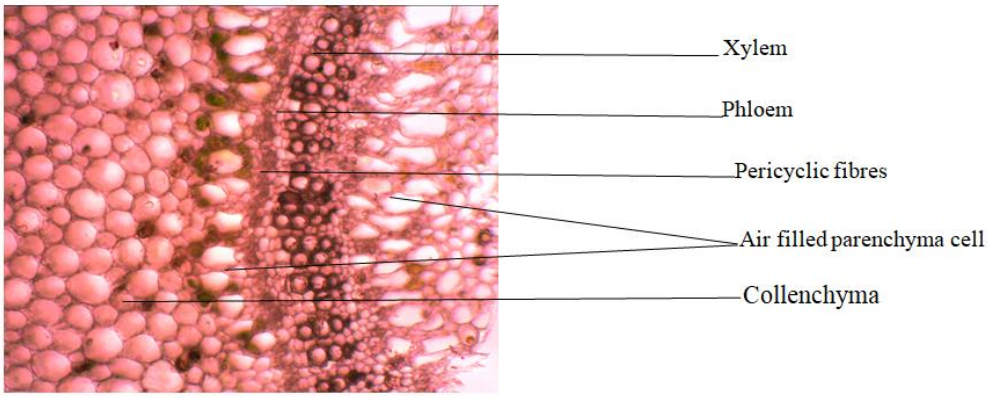


Fig.1.3.4 TS of Leaf (Midrib portion)

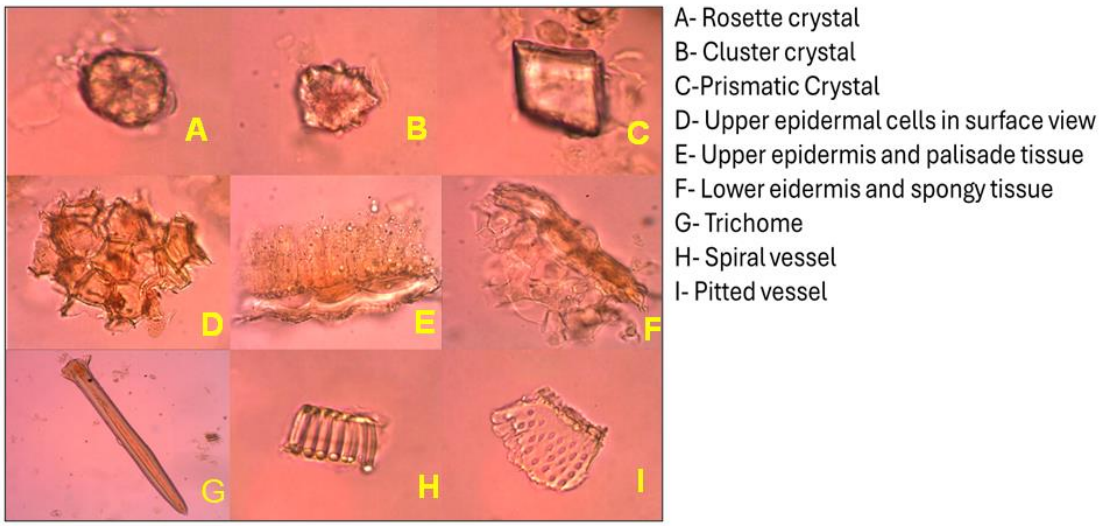


Fig. 1.4 Powder microscopy of Leaf.

Diagnostic characteristics

Dorsiventral leaf, uniseriate epidermis, thick cuticle on upper and lower epidermis, Anisocytic type of stomata present only on the lower epidermis, wavy epidermal cells, presence of uniseriate trichomes (Fig. 1.2), much of the leaf interior is occupied with spongy mesophyll with air spaces.

Lamina- The upper and lower epidermis is uniseriate, consisting of smaller, compactly arranged and heavily cutinised cells. Uniseriate trichomes are present on upper and lower epidermis.

The mesophyll is differentiated into the upper palisade and lower spongy zone. The palisade mesophyll consists of 1-2 layers of densely packed, elongated, columnar parenchyma cells with abundant oil globules. The irregularly shaped, parenchyma cells of the spongy mesophyll have very few chloroplasts and compactly arranged with intracellular spaces (Fig. 1.3.1, 1.3.2, 1.3.3).

Midrib- Single layer of upper and lower epidermal cells with cuticle; upper and lower epidermis contains uniseriate trichomes with acute apex. The upper epidermis is followed by 6-7 layers of collenchyma tissue, which is also continued as a band above the lower epidermis. The vascular tissue is resolved into crescent shaped ring with bicollateral vascular bundles. A layer of air-filled parenchyma cells is present around the vascular tissue (Fig.1.3,1.3.4).

Powder microscopy: Leaf powder is in dark green colour (Fig1.1 B). Powder microscopy of the leaf showed the presence of Rosette crystals; Cluster crystals; Prismatic crystal; upper epidermal cells in surface view; upper epidermis and palisade tissue; lower epidermis and spongy tissue; uniseriate trichome with acute apex; spiral vessel and pitted vessel. (Fig. 1.4).

Physicochemical Parameters

The findings are presented in Tables 2 and 3. Determination of leaf constants is an important part of Pharmacognostical evaluation of crude drug. Leaf constant includes parameters such as stomatal number and stomatal index, Vein islet and vein termination. Determining the moisture content is vital for maintaining pharmacopeial standards and provides evidence of the drug's stability. Likewise, total ash values are critical in evaluating crude medicines, as they help assess purity by indicating the presence of foreign inorganic materials such as silica or metallic salts. When comparing

solvents, alcohol yielded a higher extraction value (15.2% W/W) than water (12% W/W). The extractives soluble in alcohol and water serve as indicators of the overall solvent-soluble constituents. The Fluorescence analysis is used to identify and authenticate crude drugs based on their characteristic fluorescence under Ultraviolet Light. It also helps in detecting adulteration and ensuring the quality and purity of herbal drugs.

LEAF CONSTANTS[8]

Stomatal number & stomatal index

a) Stomatal number: The average amount of stomata per square millimetre of epidermis is termed the stomatal number.

b) Stomatal index: The ultimate divisions of the epidermis of a leaf percentage which have been changed into stomata is termed the stomatal index.

- Stomatal index was calculated as per the following formula,

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

Where,

I= Stomatal index.

S= Number of stomata per unit area.

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

Vein islet and vein termination

c) Vein-islet number: Vein-islets per sq. mm calculated from four contiguous squares in the central portion of the lamina, midway in the middle of the midrib and the margin.

d) Veinlet termination number: The quantity of veinlet terminations was determined per sq. mm of the leaf surface.

CALIBRATION

Calibration of one division of eye piece micrometer

1 division of stage micrometer = 0.01mm ~10µ.

5 divisions of eye piece micrometer =4 divisions of stage micrometer

The value of one division of eye piece micrometer is calculated as follows

Therefore, 1 division of stage = 0.01mm ~ 10µ.

Now, 5 division of eye piece = 4 divisions of stage i.e., 40µ.

1 division of eye piece micrometer = $\frac{40}{5}$ = 8µ.

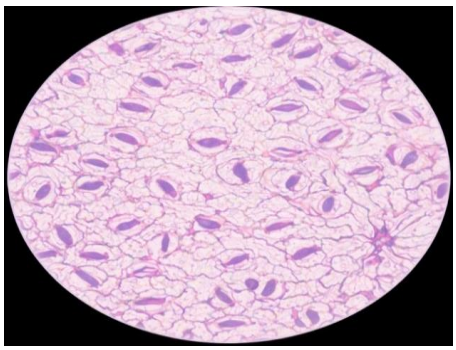


Fig no. 1.5: Microscopic image of stomata.

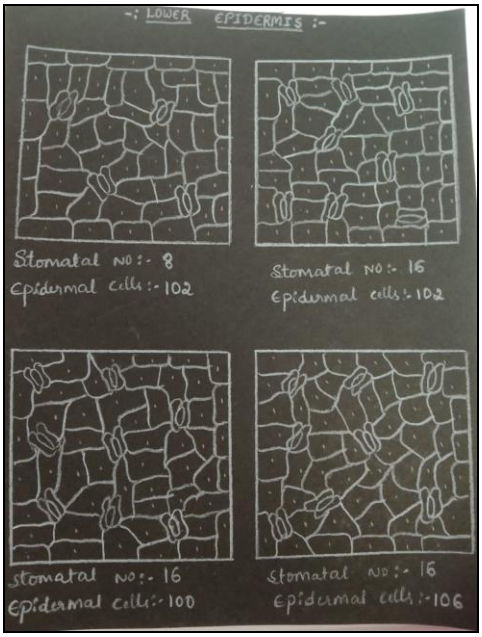


Fig No. 1.5.1 Lower epidermis.

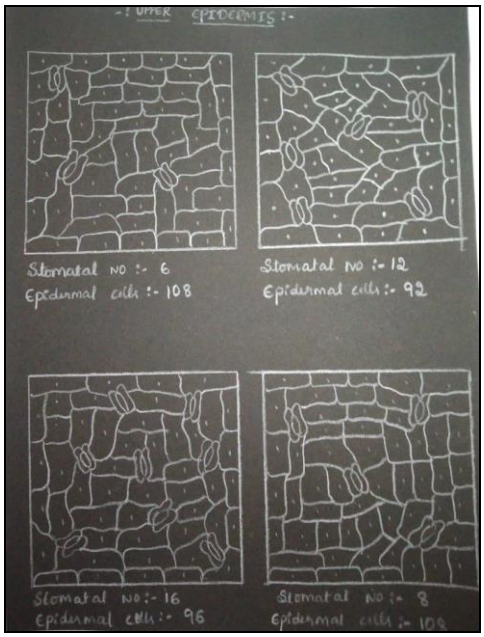
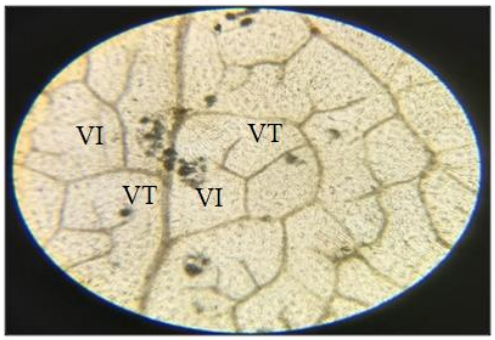


Fig No. 1.5.2 Upper epidermis.

Table 2: Leaf constants of *Allamanda blanchetii.*

LEAF CONSTANT	VALUE PER SQ. MM
Stomatal Number- Upper epidermis	10.5
Lower epidermis	14
Stomatal Index- Upper epidermis	9.475
Lower epidermis	11.93
Vein Islet Number	7.33
Vein Termination Number	3.33



Where,

VI= Vein-islets

VT= Vein terminates

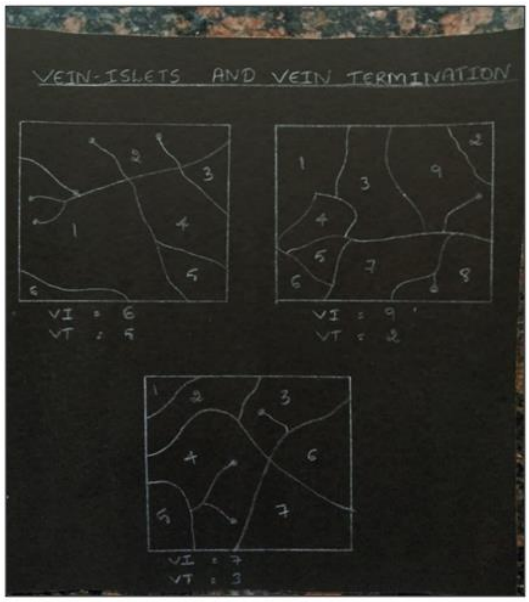


Fig No. 1.5.3 Microscopic image of VI &VT. **Fig No. 1.5.4 VI & VT drawn using camera lucida.**

Table 3: showing results for quantitative evaluation of leaves *Allamanda blanchetii*.

SI NO	EVALUATION PARAMETER(%W/W)	LEAF (%W/W)
1.	Moisture content Fresh sample Powdered sample	 55.5 10.6
2.	Total ash	8.76
3.	Acid insoluble ash	2.7
4.	Water soluble ash	3.3
5.	Sulphated ash	2.8

Table 4: Extractive Values of leaves *Allamanda blanchetii*.

EVALUATION PARAMETER(%W/W)	LEAF (%W/W)
Alcohol soluble extractive value	15.2
Water soluble extractive value	12

Table 5: Fluorescence analysis of leaf powder *Allamanda blanchetii.*

SI NO	SOLVENTS	DAY LIGHT	254nm	366nm
1.	Distilled water	Light green	Light black	Grey
2.	Concentrated HNO_3	Orange red	Black	Light purple
3.	1% KOH	Yellowish green	Light purple	Grey
4.	1N aqueous NaoH	Yellowish green	Light purple	Grey
5.	50% H_2SO_4	Light green	Grey	Greenish black
6.	50% HNO_3	Yellowish green	Dark purple	Light black
7.	50% HCL	Light green	purple	Light black
8.	Methanol	Dark green	violet	Orange red
9.	Benzene	Dark green	Light red	Dark red

EXTRACTION OF LEAF

Preparation of leaf extracts by Soxhlet apparatus using different polar and non-polar solvents such as petroleum ether, ethyl acetate, chloroform, methanol.

$$\text{Percentage yield} = \frac{\text{Weight of the extract}}{\text{weight of packing material}} \times 100$$

Table 6: Percentage of leaf extract of *Allamanda blanchetii*.

SI NO	SOLVENTS	PERCENTAGE YIELD
1.	Petroleum ether	7 %
2.	Chloroform	7.71 %
3.	Ethyl acetate	1.82 %
4.	Methanol	13.99 %

Fig No. 1.6.1. Leaf extracts.

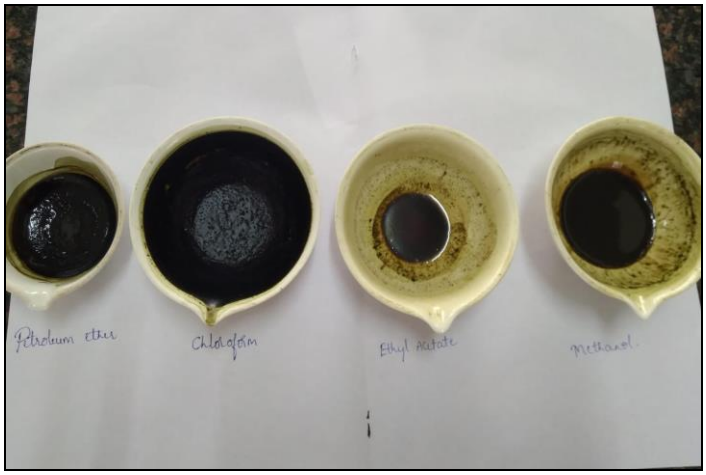


Fig No. 1.6. Soxhlet apparatus.

Preliminary Phytochemical Studies[15]

The preliminary phytochemical investigation of the petroleum ether, chloroform, ethyl acetate, methanol extract of stem bark of *Allamanda blanchetii* showed the presence of alkaloids, glycosides, saponin, flavonoid, carbohydrate, fat and oil, proteins and amino acid, steroid, triterpenoid presented in Table 5.

Table 5: Qualitative Analysis of Phytochemicals in leaves *Allamanda blanchetii*.

PHYTOCONSTITUENTS	PE	CL	EA	ME
Alkaloids	-	-	-	++
Glycosides	-	-	-	++
Saponin	-	-	-	++
Flavonoid	-	-	-	++
Carbohydrates	+	-	-	++
Fats and oil	++	-	-	-
Proteins and amino acid	-	-	+	++
Steroid and Triterpenoid	-	-	-	+

Note: (+) = Present; (-) = Absent

PE= Petroleum ether, CL= Chloroform, EA= Ethyl acetate, ME= Methanol

5. CONCLUSION

Standardization of crude drugs has become a central concern in pharmacognosy, as it ensures their precise identification and consistent quality. The majority of standard parameters are established through the study of a plant's physicochemical properties and microscopic features. Such standards must be defined before a plant can be incorporated into the Pharmacopoeia, since they serve as the foundation for guaranteeing both the quality of crude drugs and the effectiveness of the formulations derived from them. The present study is directed toward developing pharmacognostical standards including macroscopic and microscopic characteristics as well as physicochemical constants to strengthen Ayurvedic medicine and enhance the credibility of traditional therapeutic practices. Establishing these benchmarks

may encourage dedicated researchers to critically investigate and validate the traditional claims associated with medicinal plants.

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CONFLICTS OF INTEREST: No conflicts of interest.

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