

HISTOLOGICAL STUDY AND QUANTITATIVE STANDARDISATION OF PLANT *Allamanda cathartica* Linn.,

SANGEETHA J*, SAI KRISHNA O, DURGADAS R

*Department of Pharmacognosy, Malla Reddy Institute of Pharmaceutical Sciences
Maisammaguda, Dhulapally, Secunderabad – 500100.

Abstract

The present study was carried to determine the plant histological characteristics, quantitative and physico-chemical standards of *Allamanda cathartica* Linn. The histology of leaf midrib, lamina, stem, petiole and root were studied. The quantitative standardisation of plant was carried out with determination of stomatal number, stomatal index, veinlet termination number, vein-islet number and palisade ratio. The anatomical constants and physiochemical parameters such as ash value and extractive value were done to support the study. The histology of *Allamanda cathartica* Linn, leaf shows the presence of anomocytic type stomata in the abaxial epidermis. Apart from regular microscopical description of plant, leaf T.S. shows single layer of palisade cells under the adaxial epidermis which has no stomata, the petiole shows winged protrusion on the upper end, the root shows lignified xylem & multilayered loosely arranged cork cells, the stem shows bunches of pericyclic fibers scattered in the phloem region. The stomatal number and stomatal index were calculated and the average stomatal number and stomatal index were found to be 27 & 19.5 respectively. The palisade cells were also identified and the average palisade ratio was found to be 4.6. The vein islet and vein-let termination number of *Allamanda cathartica* Linn. were identified and reported. The average value was found to be 7 & 12.5. The physico-chemical standardization techniques like ash value, extractive value were performed. The microscopical study and standardization of *Allamanda catharticalinn* can possibly help to differentiate the drug from its species. The pharmacognostic profile of plants presented here may be useful to supplement information with regard to its identification and will be helpful in establishing standardization criteria.

Keywords: Plant histology, leaf constants, Extractive value, Ash value, *Allamanda cathartica* Linn.

INTRODUCTION

Apocynaceae is a flowering family which includes herbs, shrubs, trees, stem succulents, vines etc.[1] The plants usually contain milky exudation when cut in their aerial parts and many are poisonous in nature. The commonly found plants are *Catharanthus roseus*, *Nerium oleander*, *Red frangipani*, *Carrisa carandas*, *Aganosma cymosa*, *Allamanda Sp.* Etc. The family is found to be rich in alkaloids and glycosides.[2] Though the family is mainly used as ornamental plants they are proved to be highly therapeutic and pharmacologically effective in the treatment of various conditions. Herbs, plants and natural resources have become one of the most preferred topics among industrialists and educationists. The reason behind the attraction toward natural resources is due to their overwhelming advantages and fewer side effects in the field of medicine. The use of herbal medicines insists the researcher identify and authenticate the raw materials.[3-6]

Allamanda cathartica Linn. is one among the Apocynaceae family seen widely distributed as imperishable ornamental shrub mostly found in forests, and marshy lands as decorative plants in gardens and lawns. Several chemical constituents have been studied in *Allamanda cathartica* Linn. and were found to have much therapeutic significance. The phytochemicals like plumericin, plumieride, allamandin were found to be rich in leaves. Either the whole plant or individual parts like flowers, stem, leaves, or root extract is used from ancient times for potential pharmacological action. Various reports on pharmacological studies indicate that the roots are used against spleen enlargement, malaria, jaundice etc[7,8]. Microscopical characteristic studies on *Allamanda cathartica* Linn. whole plant are not adequate necessitating the current study. The objective of the work is to contribute to quality evaluation, identification and one of the standardizing parameters for the plant material. Hence, the histological studies and quantitative standardization were done for the whole plant of *Allamanda cathartica* Linn.[9-10]

MATERIALS & METHODS

Collection & Authentication of plant material

Allamanda cathartica Linn. whole plant was collected from in and around Ranga Reddy district, Telangana, India and authenticated by botanist from Osmania University. The collected whole plant was cleared for foreign matters, soil and other adhering materials. Part of the plant material was reserved for histological studies and the remaining crude drug dried in shade under controlled humidity. The dried sample was made into coarse powder for quantitative evaluation.

Histological standardization

The microscopical characters of various parts of *Allamanda cathartica* Linn. studied by preparing manual cross sections of leaf, stem, petiole and root. The sections were cleared using chloral hydrate, stained with Phloroglucinol and Conc. Hydrochloric acid reagent later mounted on a slide with glycerin. The detailed microscopical characteristics of the plant were supported with the examination of powder microscopical characters.[11-15]

Quantitative Standardization

The stomatal number, stomatal index, vein islet number, vein-let termination number and palisade ratio of the fresh leaf sample were investigated using standard procedure.[16-18]

Physico-chemical standardization

The physical and chemical standards like total ash value, acid insoluble ash value, water soluble ash value, sulphated ash value, water soluble extractive value, alcohol soluble extractive value and ether soluble extractive value were determined according to India pharmacopoeia.[19]

RESULTS

Histology of *Allamanda cathartica* Linn.

Leaf (Midrib & Lamina)

The T.S. of Leaf through midrib and lamina is given in Fig.1 (a-c). The epidermal trichome are unicellular conical thick walled with covering. The limb is adhering to the epidermis. The meristem consists of phloem and xylem. The phloem consists of large and small parenchymatous cells arranged compactly just above the xylem. Meristem consists of radiating xylem parenchyma and vessels which form an arc. The transverse section thorough

midrib shows there are no palisade cells, above and beneath the meristem. The lamina shows adaxial epidermis without stomata. Single layer of palisade cells arranged below upper epidermis. Lamina contains vascular bundles, loosely arranged mesophyll parenchyma cells containing starch grains & calcium oxalate prisms.

Stem

T.S. of the stem *Allamanda Cathartica Linn.* in Fig 1 (d) exhibit thick-walled epidermal cells. The cortex contains simple round parenchymatous cells with cell contents. The phloem consists of polyhedral parenchymatous cells containing unligified pericyclic fibres as discrete bundle. Continuous band of radially arranged 2-3 cell long xylem vessels embedded with xylem parenchyma is found separating medulla and phloem. The xylem parenchyma and xylem vessels both found to be lignified. The cells of pith containing starch mucilage, calcium oxalate. The medulla or pith is found at the centre of the stem and it is parenchymatous cellulosic in nature, they are non-lignified larger cells found in the middle whereas the smaller cells encircle larger cells.

Petiole

The transverse section of petiole in Fig 2 (a-b) shows the epidermal trichomes that are multicellular conical thick walled covering cuticle. The limb of trichome is extension of the epidermal cell. The meristem consists of phloem and xylem. The phloem consists of larger and small parenchymatous just above the xylem. Meristem consists of radiating xylem parenchyma and vessels which form an arc under phloem parenchyma cells. The upper portion of the petiole shows a winged extension of the epidermal and parenchymatous cells on both sides.

Root

T.S. of root of *Allamanda Cathartica Linn.* in Fig 2 (c) shows the presence of several layers of parenchymatous cork cells with about (5-7) alternative bands of smaller cells and larger cells. The cork cells are iso-diametric in shape. Phelloderm consists of 10-12 rows of cellulosic parenchyma present below the cork. Phloem consists of simple parenchymatous cells with sieve tissues arranged alternatively between the parenchymatous cells. Xylem consists of lignified radially arranged parenchyma cells and thick walled vessels. Medullary rays are lignified separating xylem parenchyma and vessels. Abundant starch grains and calcium oxalate crystals were found.

Quantitative standardization

Arrangement of Stomata and Epidermal Cells

The epidermal cell arrangement and stomatal arrangement in *Allamanda Cathartica* Linn. leaf observed under 40X magnification. The Fig. 2 (d) shows adaxial epidermis with no stomata present in the leaf sample. Multiple samples were studied to confirm the absence of stomata. The Fig. 2 (e) shows abaxial epidermis with anomocytic stomata, multiple samples were taken to confirm the type of stomata.

Stomatal number and Stomatal index

The Fig 3 (a) shows the superimposed image of arrangement of stomata and their epidermal cells surrounding them. The average stomatal number and stomatal index were calculated as $n=4$ and listed in Table 1.

Vein Islet and Vein-let termination

The Fig. 3 (b-c) shows vein islet and vein-let terminations arrangement in the leaf sample. The Vein Islet number and vein-let termination number were calculated as $n=4$ listed in Table 1.

Palisade ratio

The Palisade cells arrangement in *Allamanda cathartica* L. leaf can be observed from Fig. 3 (d-e). The palisade ratio were calculated as $n=4$ and listed in Table 1.

Physico-chemical standardization

The physical parameters like total ash, acid insoluble ash, water soluble ash, sulphated ash, moisture content and chemical parameters like water soluble, alcohol soluble and ether soluble extractive values were evaluated and listed in Table 2 & Table 3 respectively.

Table 1: Quantitative Estimation of the Plant *Allamanda cathartica* Linn.,

S.No.	Plant Constants	Range	Average
1	Stomatal number (upper epidermis)	20 – 30	27.5
2	Stomatal Index (upper epidermis)	16.6 – 25	19.6
3	Vein Islet Number	6 – 10	7
4	Vein-let termination Number	8 – 12	12.5
5	Palisade ratio	3.7 – 5.5	4.7

**Table 2: Estimation of Physico-Chemical Parameters of the Plant
Allamanda cathartica Linn.,**

S.No.	Physical Parameters	Leaf (%w/w)	Root (%w/w)	Stem (%w/w)
1	Total Ash Value	9.48±0.5	9.2±0.1	8.86±0.12
2	Acid Insoluble Ash Value	2.93±0.05	5.1±0.2	2.6±0.1
3	Water Soluble Ash Value	4.53±0.2	6.1±0.2	1.53±0.04
4	Sulphated Ash Value	8±0.5	6.9±0.3	7.76±0.3
5	Moisture content	1.23±0.3	0.21±0.04	0.54±0.08

**Table 3: Estimation of Extractive values of the Plant
Allamanda cathartica Linn.,**

S.No.	Chemical Parameters	Leaf (%w/w)	Root (%w/w)	Stem (%w/w)
1	Water soluble extractive value	26.4	14.4	10.4
2	Alcohol soluble extractive value	29.6	22.4	15.2
3	Ether soluble extractive value	9.6	1.2	1.6

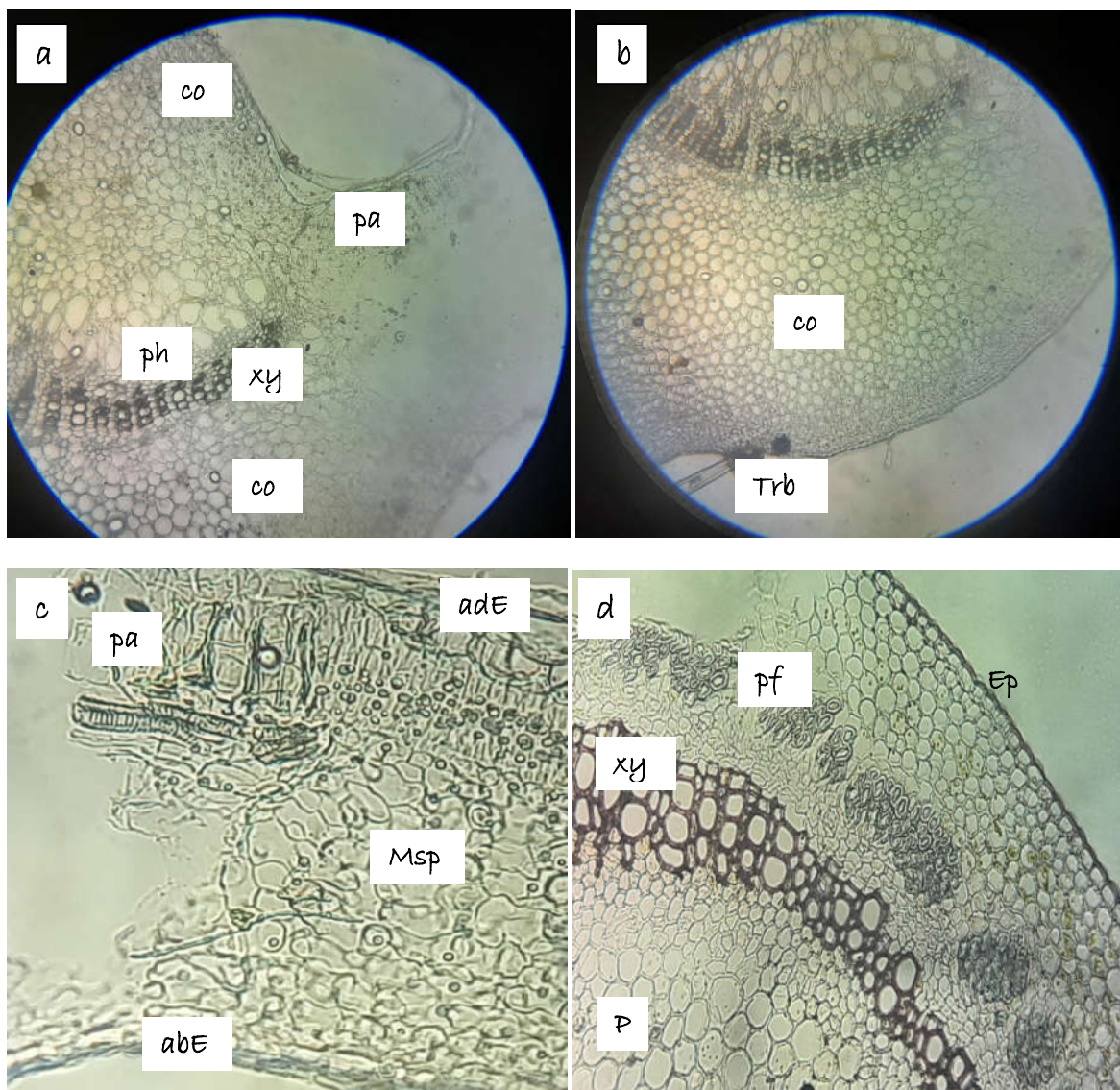


Fig 1 (a-d): 1(a-b): T.S of Leaf (midrib); 1(c): T.S of Leaf (lamina); 1(d): T.S of Stem; abE- abaxial Epidermis; adE-adaxial Epidermis; co-collenchyma; Ep-Epidermis; Msp- Mesophyll parenchyma; P-Pith; pa-palisade cells; pf-pericyclic fibers; ph-phloem parenchyma; Trb-Trichome base; xy-xylem parenchyma

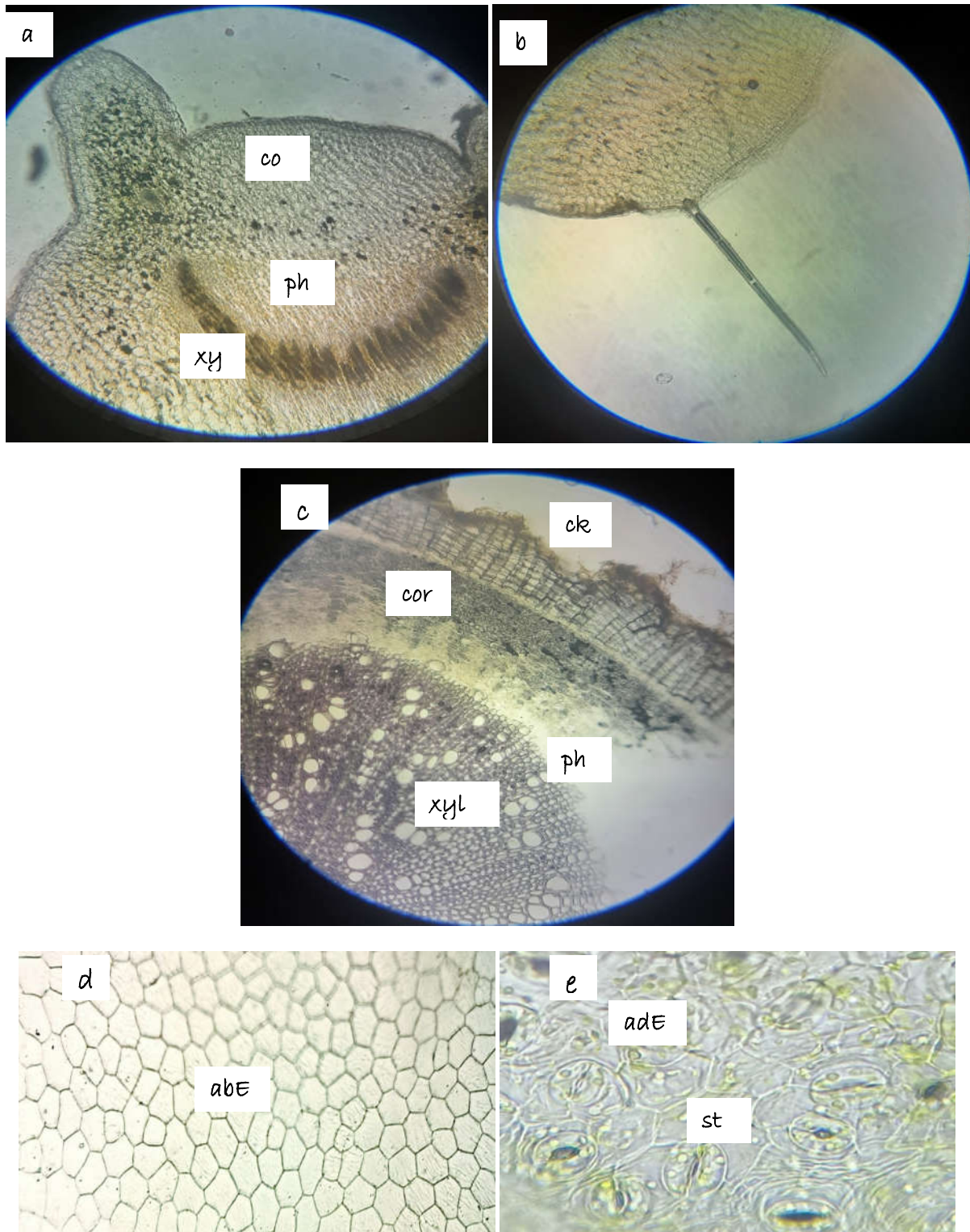


Fig 2 (a-e): 2(a-b): T.S of Petiole; 2(c): T.S of Root; 2(d): Abaxial Epidermis; 2(e); Adaxial Epidermis; abE-abaxial Epidermis; adE-adaxial Epidermis; co-collenchyma; cor-cortex parenchyma; ck-cork parenchyma; ph-phloem parenchyma; st-stoma; xy-xylem parenchyma

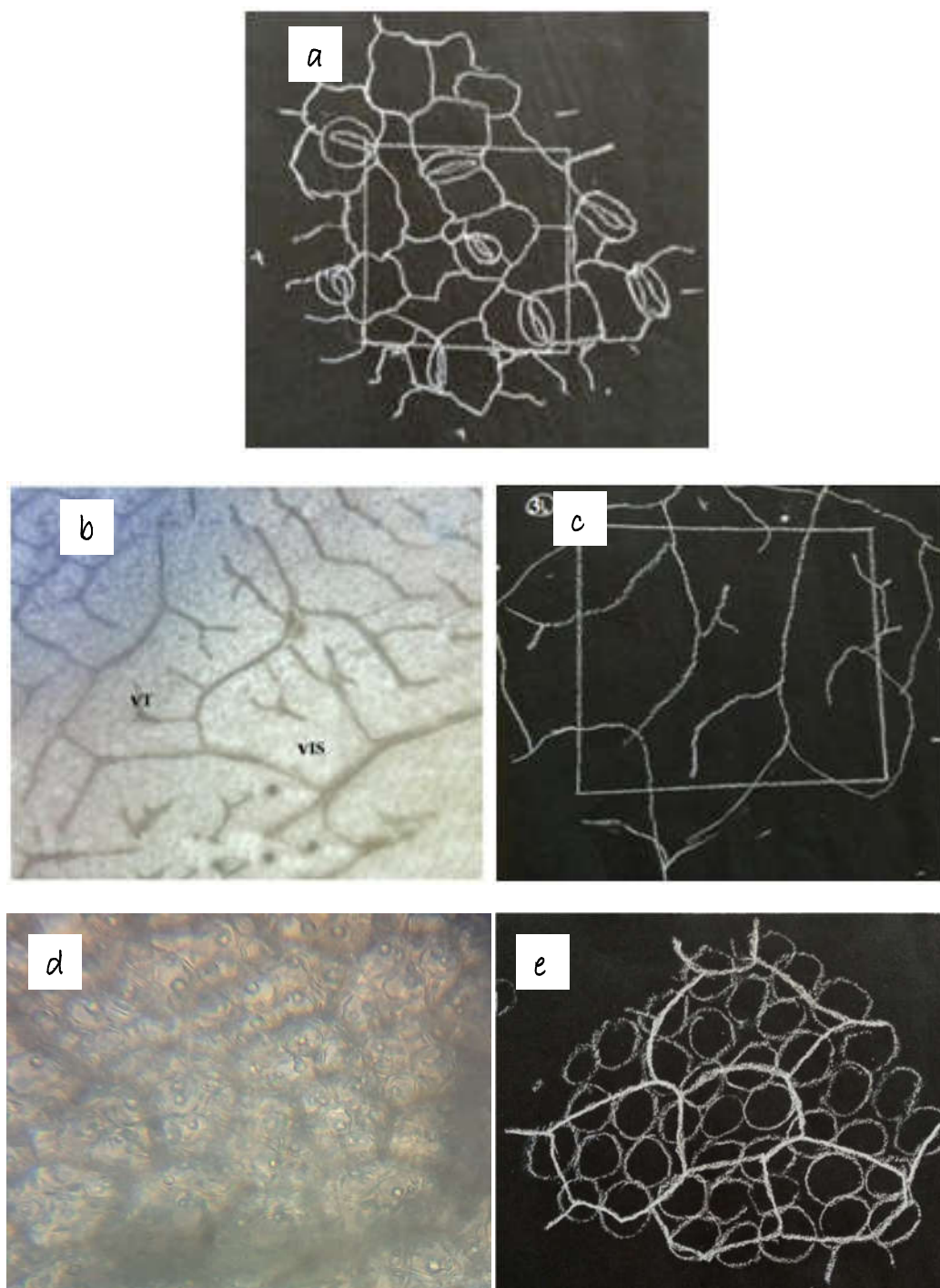


Fig 3(a-e): a, c & e: Diagrammatic representation of Stomata, Epidermis, Vein Islets, Vein let terminations & Palisade cells (super imposed figure drawn using camera lucida); b & d: Microscopic image of Vein islet and palisade cells resp; VIS-vein-islets; VT-vein-let terminations

DISCUSSION

The present study on microscopical characters & physic-chemical standards of *Allamanda cathartica* Linn. is essential to overcome proper identification of the plant. The availability of many species in *Allamanda* result in contamination of the plant within the species. The microscopical characters such as polyhedral parenchyma, lignified pericyclic fibres, multiple loosely arranged cork parenchyma, multicellular covering trichomes, anomocytic stomata, upper palisade parenchyma cells, vascular bundles are important diagnostic features observed. The powder of *Allamanda cathartica* Linn. showed the presence of starch grains, lignified fibers, epidermis with stomata, calcium oxalate prisms & crystals, xylem vessels, cork cells as the important diagnostic features found. The observations were studied based on the WHO guidelines and botanical standards. The physiochemical parameters like ash values, extractive values, moisture content were taken to set the standards for *Allamanda cathartica* Linn. which remain useful for identification of the plant material. The total ash value, acid insoluble ash value, water soluble ash value & sulphated ash value sets the limit for the total minerals, silica, water soluble mineral components & non-volatile inorganic minerals respectively. The moisture content in *Allamanda cathartica* Linn. found to be within the limit specified by international pharmacopeia. The lower moisture content prevents auto-oxidation & fermentation of drugs. *Allamanda cathartica* Linn. is even though an ornamental plant the benefits in alternative system of medicine is huge. The studies will definitely aid proper identification & authentication of the species.

CONCLUSION

The current study reveals the pharmacognostic, quantitative and physico-chemical standards of *Allamanda cathartica* Linn. The microscopical characteristics justify the purity and proper identity of the species when we find various other plants under same genus. The physico-chemical property of the plant shows the physical property and chemical constituents in the plants.

REFERENCES

1. Kirtikar K.R. and Basu B.D. Indian Medicinal Plants. Vol.-II. 2nd Edn. Dehradun. International Book distributors. 2006; 1556-1587.
2. The Wealth of India, Raw Materials, Vol.VIII: Publications and Information Directorate, CSIR, New Delhi. 1969; 163.
3. Alolika & Biswas, Swagata & Biswas, Maitrayee & Ghosh, Pranabesh & Chatterjee, Sirshendu. *Allamanda cathartica* Linn. Apocynaceae: A mini review. 2019; 7. 29-33.
4. Akah R.A., Offiah V.N. Gastrointestinal effects of *Allamanda cathartica* L. leaf extracts. Pharm. Biology. 1992; 30(3): 213-27.
5. Singh A., Singh S.K. Reversible Anti-fertility effect of leaf extract of *Allamanda cathartica* L. in male lab mice. Andrologia. 2008; 40(6): 337-45.
6. Iyengar M.A. Bibliography of Investigated Indian Medicinal plants (1950-1975), 1976; 41: 98.
7. Tiwari, Tarkeshwar Nath & Pandey, V & Dubey, N. Plumieride from *Allamanda cathartica* as an antidermatophytic agent. Phytotherapy research. 2002; 16: 393-4. 10.1002/ptr.967.
8. Peter A. Akah & Veronica N. Offiah. Gastrointestinal Effects of *Allamanda cathartica* Leaf Extracts. International Journal of Pharmacognosy. 1992; 30(3), 213-217, DOI: [10.3109/13880209209054001](https://doi.org/10.3109/13880209209054001)
9. Coppen, J. J. Iridoids with algicidal properties from *Allamanda cathartica*. Phytochemistry. 1983; 22(1): 179-182.
10. Vera Petricevich, Rodolfo Abarca-Vargas. *Allamanda cathartica*: A Review of the Phytochemistry, Pharmacology, Toxicology, and Biotechnology. Molecules. 2019; 24(7):1238.
11. Roma Mitra, Bibliography on Pharmacognosy of Medicinal plants, Economic Botany information service, National Botanical Research Institute, Lucknow-22600. India. 1985; 143: 416-21.
12. Gurudeva Magadi R and Yoganarasimhan S.N. Bibliography of medicinal plants of India (Pharmacognosy & Pharmacology). 2009; 722-728.
13. Fahn A. Plant anatomy. Third Edition Pergamon Press. Oxford. 1980.

14. Majid N, Saduf Nissar, Weekar Younus Raja, Nawchoo IA, Zulfikar Ali Bhat. Pharmacognostic standardization of *Aralia cachemirica*: a comparative study. *Future Journal of Pharmaceutical Sciences*. 2021; 29:7(1).
15. Rubeena M, Divya KG, Sunil Kumar KN. Comparison of the leaf drug *Ruta graveolens* and its substitute *Ruta chalepensis*. *Int J Res Ayurveda Pharm*. 2018; 9(6):100-110.
16. Kokate C.K. "Practical Pharmacognosy". Vallabh Prakashan. New Delhi. 2005;108-112.
17. Harbone J B. Method of extraction and isolation. In: *Phytochemical methods* (Chapman & Hall, London). 1998; 60-66.
18. Evans WC. *Trease and Evans Pharmacognosy*. (Saunders Ltd, London). 2003; 15th ed. 545-547.
19. Pradeep A, Bharathi SV, Ali MS. Preliminary phytochemical, microscopic analysis and metabolite profiling of *Phoenix pusilla* root. *J Appl Pharm Sci*, 2020; 10(03):128-134.