Journal of Forestry Research and Management. Vol. 16(2).118-126; 2019, ISSN 0189-8418
www.jfrm.org.ng

Isolation and Anatomy of leaf and stem of *Icacina tricantha* Oliv. (Icacinaceae)

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ABSTRACT

Icacina tricantha is well known to be a handy, household medicinal plant. However, there is a dearth of information on the anatomical study and diagnostic character, which has made it difficult for taxonomical identification. The anatomical structure of the leaves, roots, stem etc of plants contribute to the proper identification of the plant. This study therefore gave a vivid anatomical description of the leaf, stem and nodes of icacina tricantha which is a member of Icacinaeace family. Fresh *Icacina tricantha* plant was collected from a garden opposite Botany Department, Ambrose Alli University, Ekpoma. Fresh leaf, stem and node of *icacina tricantha* were collected. Small sizeable portion of the matured leaf was obtained, the stem was cut transversely and few drops of safranin was applied. They were then microscopically analysed for their anatomical properties. Stomata only occurred on the lower surface of the plant. Two main stomatal types were encountered namely: anisocytic and paracytic stomatal types with anisocytic being predominant. The number of epidermal cells ranged from 80-125 while number of stomata per field view was between 16 and 25. The stomata index ranged between 16.2 to 25.5%. Anatomical description of the stem showed that the epidermis, cortex and vascular bundle were clearly differentiated. Epidermis was composed of wax-coated dermal cells, thick-walled with straight anticlinal cuticles. Vascular bundles were found to be irregularly-scattered in the tissue. Therefore, this description may all serve as useful diagnostic tool; thus, improving or being a basis for proper identification and authentication of *Icacina tricantha* leaf and stem.

Keywords: Anatomy, *Icacina tricantha*, Anticlinal cuticles, stomata, Epidermis

Introduction

Icacina tricantha Oliv is a perennial shrub of the family Icacinaceae, with broadly-elliptic, simple and alternate leaves and large underground tuber (Timothy and Idu, 2011). It is commonly called false yam. The plant is a common weed of field crops, forest re-growths and waste areas (Akobundu and Agyakwa, 1998) and has been reported to become a weed of rice-padis in former Bendel state presently Edo and Delta states. In Nigeria, it is locally referred to as gbegbe by the Yorubas while the Igbos call it Ibugo (Burkill, 1985). The leaf is said to be used as a wrapper for castor oil seeds, but the purpose of wrapping them is not discussed. The thick yam-like root attains a large size. Yorubas say that it is edible alone when dry and pounded to a white powder called gbe-wutu (Yoruba-Ijebu dialect), which is used in making soup. The powder is also added to a food made from the roasted seeds of *Citrullus lanatus* (cucurbitaceae) known as Igalo. Igbos treats it as a famine food eating the flour after prolonged maceration and repeated washing. The tuber is inflammable and when burning gives out so fierce a heat as to be unapproachable (Shagal and Kubmarawa, 2013). The plant is very extensively used in the rural areas and this is supported by the fact that it is regarded as a major handy

household medicine for emergency treatment; hence, virtually all household have tuber in ethanol, which is stored in corked bottles (Asuzu and Abubakar, 1995). The leaf and tuber are used in the treatment of diabetes amongst Lagosians (Gbolade, 2009).

As noted by Byng *et al.,* (2014), the family Icacinaceae has been poorly understood because of lack of diagnostic characters, under collection and frequent misidentification of materials. The present study thus aimed at examining the anatomy of the leaf, stem, and nodes of *Icacina trichantha* in an attempt to providing diagnostic characters to supplement existing taxonomic information about the species and to further aid its identification especially in fragmentary or sterile conditions.

Materials and Methods

Sample collection and identification

Fresh sample, consisting of leaves, stem and nodes of *Icacina trichantha* was collected from a small garden opposite Botany Department, Ambrose Alli University, Ekpoma, Edo State, and was carefully identified and confirmed by a curator in the same department.

Micro-morphological study

Small sizeable portions of the leaves of the species were obtained from matured and well expanded leaves. The mature leaves were scrapped using razor blade so as to get the adaxial (upper surface) and abaxial (lower surface) part of the epidermis. Scrapping was carefully done with razor blade and a brush was used to clean the scrapped part and dipped into water for proper cleaning. After scrapping the epidermis was stained with safranin and excess stained was removed with the aid of a brush. Thereafter, the peeled and stained part was put on a slide containing glycerol droplets with the aid of pin. Glycerine was then added to preserve the specimen. Furthermore, cover slip was used to cover peeled plant in the slide and lady's paint was used to seal it to avoid air from entering into it. Afterwards, it was mounted on a light microscope and viewed under x10 eyepiece and x40 objective lens (Abdulraham *et al*., 2009).

The stems of the three specimens were cut transversely using cork in order to get thin section of the stems. Each of the stems was cut with a safety blade into various petri dishes which were filled with water. The sectioned stem of the plant were placed on separate slides, containing glycerol droplet. Few drops of safranin were applied and covered with cover slips which were later mounted under light microscope for examination. This was done to view the arrangement of the vascular bundles, the cortex, the epidermis and other part of the stem. Photomicrograpic images of the specimen were taken with a digital camera. The mean value and the standard error were calculated, while method earlier described by Ahmad *et al*. (2010) was adopted in determining the stomata epidermal cells. The cells were manually counted from the viewed specimen. The stomata index (SI) was calculated using the formula described by (Salisbury, 1972) that is

SI=S/E+S*100

Where;

SI=stomata index, S=number of stomata per unit area, and E=number of epidermal cells in the same unit area.

Maceration of wood was done according to the procedure outlined by Gill and Onuja (1988). Thin silver of wood were put in a test tube containing 5ml of Nitric acid and boiled for 5-10mins for maceration materials were washed several times with distilled water. Small quantity of the macerated

materials were placed on a clean slide, gently teased out with a needle and stained with 1aqueous of safranin-glycerol solution. A coverslip was carefully placed on the slide and viewed under a light microscope. This was done to view the vessel and fibres element dimension of stem. Each of this was photographed by a digital camera.

Results and Discussion

The anatomical sections of the leaf, stem, petiole and node of *Icacina tricantha* is shown in plates 1-4. Based on the occurrence of stomata on the leaf surface, the species can be said to be hypostomatic (i.e. stomata occurring on abaxial surface only). Generally, two types of stomata were identified viz- anisoytic and paracytic stomata having a frequency of 20.16, 31.5 and 27.2mm^2 respectively for the abaxial surface. The number of epidermal cells ranged from 80-125 while number of stomata per field of view was between 16 and 25. The stomata index ranged between 16.2 to 25.2% for the abaxial surface. Qualitative and Quantitative features of foliar epidermis was also stated (Table l).

Furthermore, mass of mucilage was found to be condensed at a spot on the adaxial surface of the leaf as shown in Plate1b. Anatomical description of the stem showed that the epidermis, cortex and vascular bundle were clearly differentiated (Plate 2). Epidermis was composed of wax-coated dermal cells, thick-walled with straight anticlinal cuticles. Vascular bundles were found to be irregularly-scattered in the tissue. Each Vascular bundle was surrounded with sclerenchyma cells. The xylem and phloem were found close to the epidermal wall. Cortex was composed of parenchyma cells with primary walls.

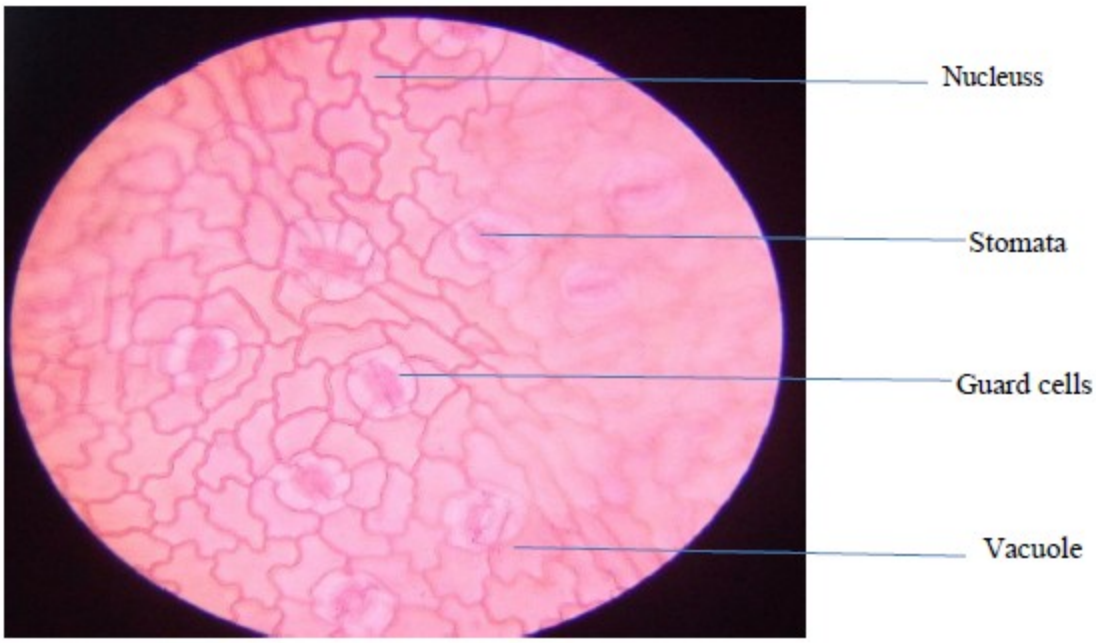

Plate 1a: The abaxial of the Epidermal peel of *Icacina tricantha* leaf (x 10)

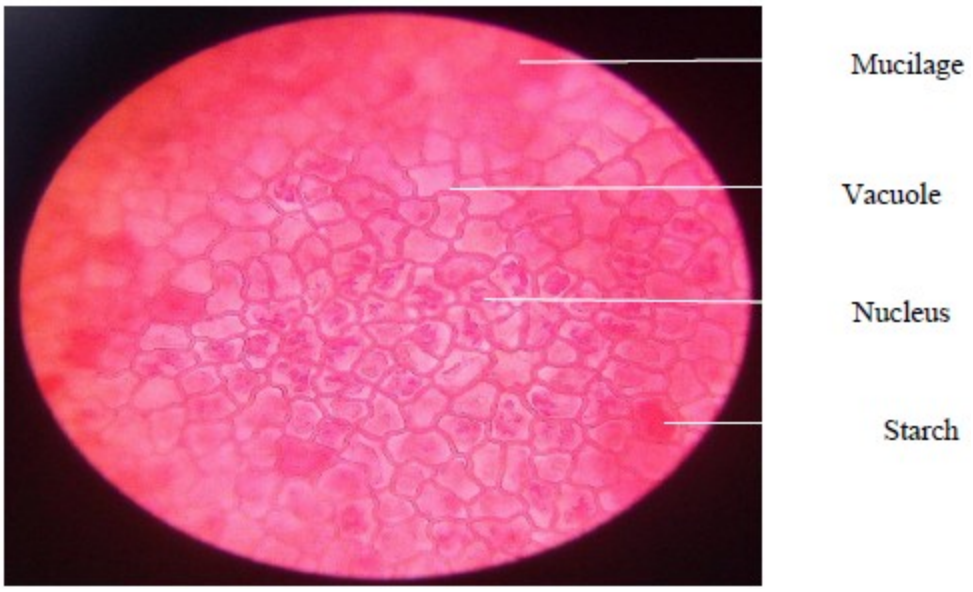

Plate 1b: The Adaxial of Epidermal peel of *Icacina tricantha* leaf (x 10)

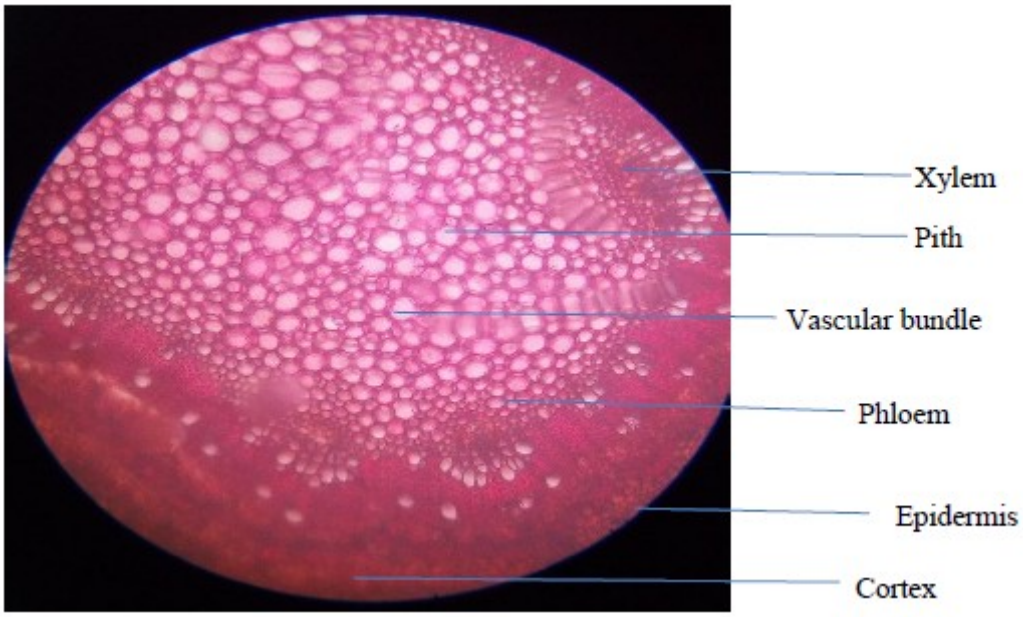

Plate 2: Anatomy of *Icacina tricantha* Stem (x 10)

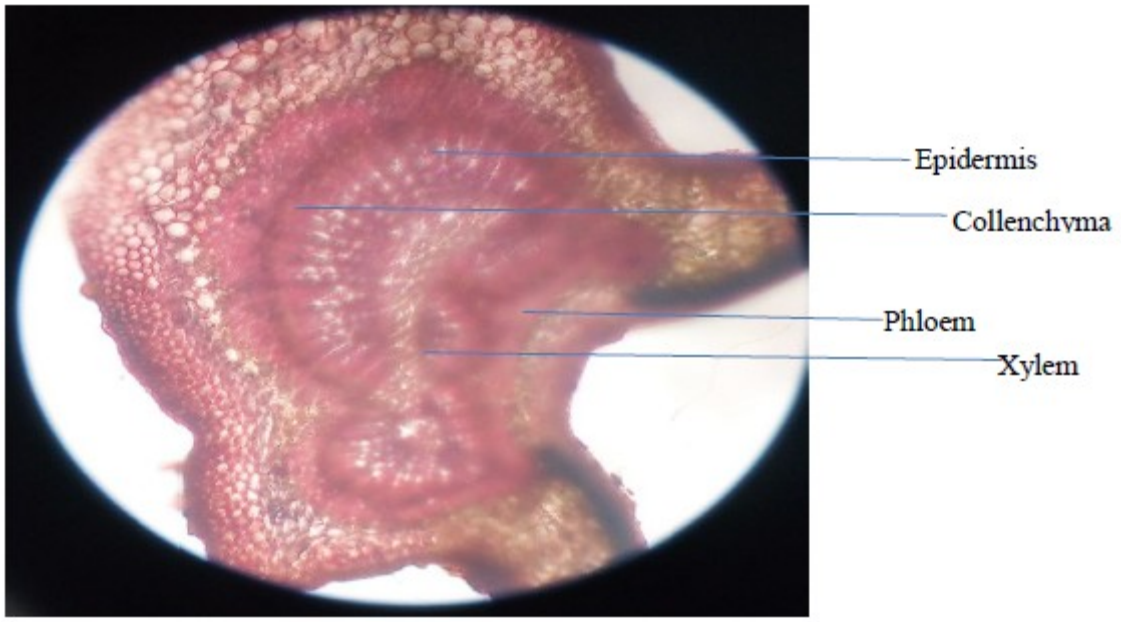

Plate 3: Anatomy of *Icacina tricantha* Petiole (x 10)

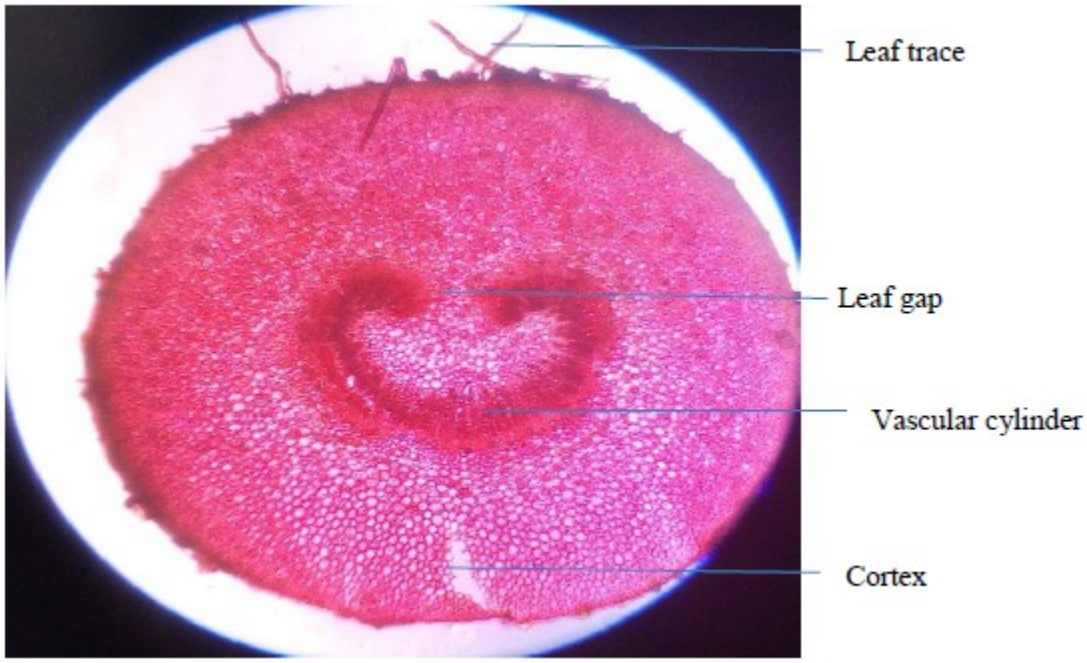

Plate 4: Anatomy of *Icacina tricantha* Root Node (x 10)

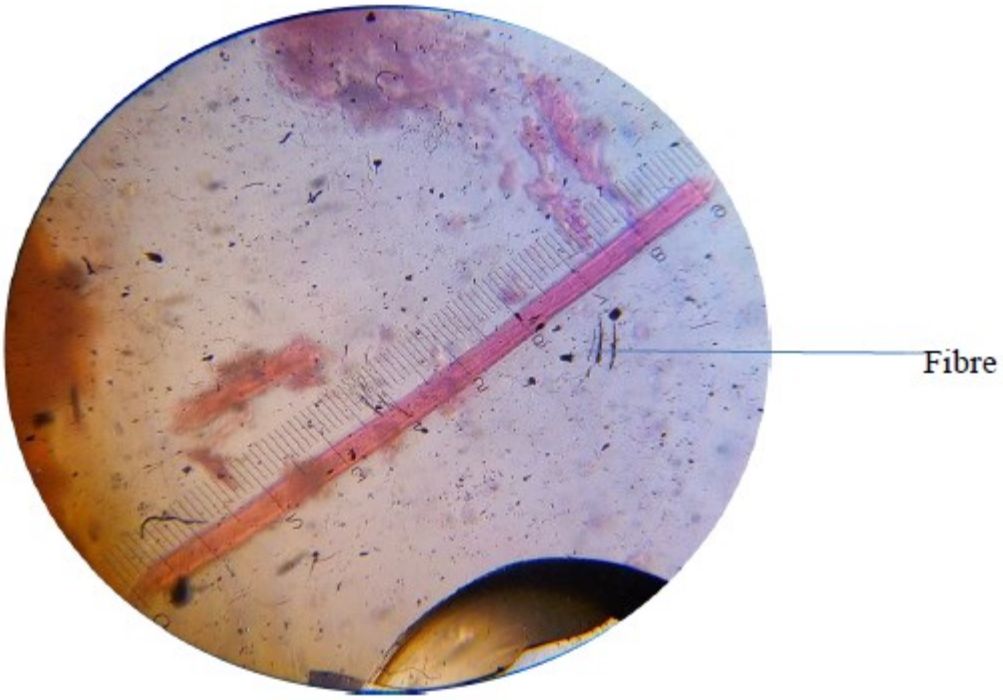

Plate 5: Macerated stem (Fibre) (x 10)

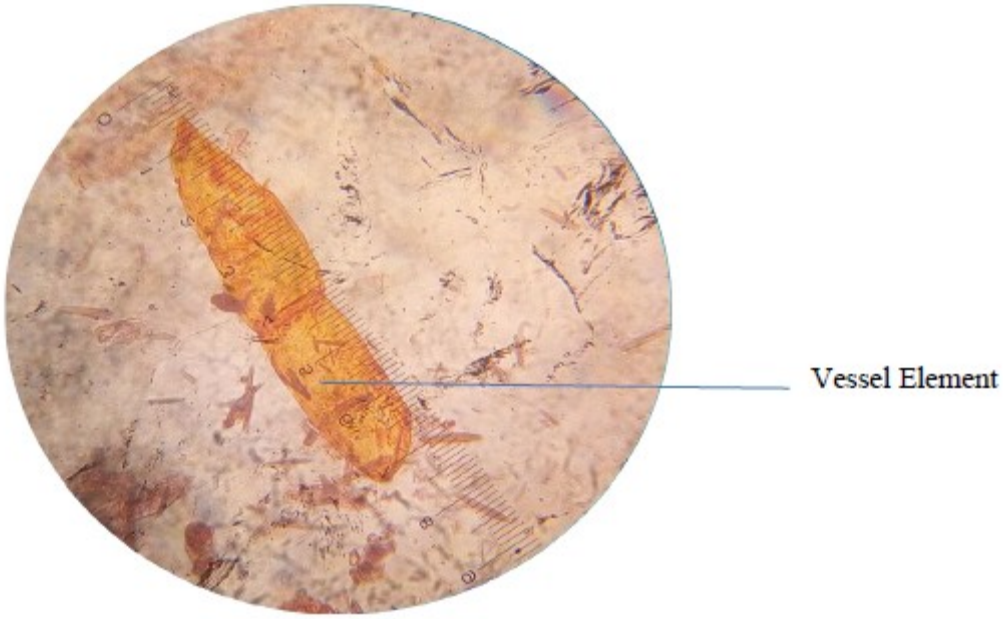

Plate 6. Macerated stem (Vessel) (x 10)

Table 1: Qualitative and Quantitative Features of Foliar Epidermis of *Icacina tricantha*

Leaf surface	Stomatal Complex Types	Number of Epidermal cell per view	Stomatal density	Frequency (mm^2)	Stomatal Index (I= S x 100)
Abaxial	Anisocytic	80	16	20.16	16.2
	Anisocytic	125	25	31.5	25.2
	Paracytic	110	22	27.72	22.2
Adaxial	-	-	-	-	-

Table 2: Macerated stem of *Icacina tricantha*

Vessel Elements and Fibres	Vessel Elements	Fibres
Length	3.3	8.85
Width	1.3	0.35

Discussion

This study evaluated the anatomical description of the leaves and stem of *Icacina tricantha.* A combination of different diagnostic features (quantitative and microscopic parameters such as stomata and trichome types with sizes, shapes and sizes of epidermal cells and so on) has been used for taxonomic distinction and recognition in the angiosperm family (Sonibare *et al.*,2005;Abere *et al.*, 2009;Yasmiri *et al.*, 2013). In this study two types of stomata complex (anisocytic and paracytic) were anatomically identified on the abaxial section of the leaf *of Icacina tricantha* while there was absence of stomata on the axial section, Although there is paucity of information on the anatomical description of the leaf, stem, petiole and mode of *Icacina tricantha* plant, in a study conducted by Asuzu and Nwosu (2017) on the morphology and anatomy of *Strychnos spinosa* Lam.(Loganiaceae)

The stomata density ranged from 16-25 while the stomata index ranged from 16.2 to 25.2%. Many workers have considered the stomatal density as a useful character for distinguishing species when comparable areas of leaf are used (Okeke *et al.*, 2008). The stomata index which indicates the proportion of stomata relative to leaf surface is also a reliable taxonomic character. This is because it is independent of the changes in epidermal cell size brought about by environmental factors (Metcalfe and Chalk, 1988). As also shown in Plate1a, the stomata guard cells found on both abaxial parts of the leaf are essential to keep the water inside the cell intact. However, they may also allow the gaseous exchange essential for photosynthetic activity (Abdulrahaman *et al.*, 2009). From plate2, the anatomy of the stem showed similar description with report

of Ayensu (1972) and Metcalfe and Chalk (1983) and these included a central pith consisting of intercellular spaces. Collateral and open vascular bundles in the stem. Stem cells exhibited variable sizes and starch grains. Epidermis, cortex, and vascular bundle were clearly differentiated. Vascular bundles were found to be irregularly-scattered in the ground tissue with phloem and xylem units. Thus, the observed anatomical description for the stem of *Icacina tricantha* may be a unique trait for members of the *Icacinaceae* family.

Conclusion

In general, the important microscopic features of the leaf and stem of *Icacina tricantha* such as anisocytic stomata types especially in the leaf, presence of mucilage within epidermal cell as well as large vascular bundles, differentiated epidermis, cortex and vascular bundles and even mucilage in the stem may all serve as useful diagnostic tool; thus, improving or being a basis for proper and authentication of *Icacina tricantha*

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