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MACROSCOPIC AND MICROSCOPIC CHARACTERIZATION OF RAW HERBAL DRUG *MAMAJJAKA* [*ENICOSTEMA AXILLARE* SUBSP. *LITTORALE* (BLUME) A. RAYNAL]

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ABSTRACT

Enicostema axillare subsp. *littorale* (Blume) A. Raynal belonging to the family Gentianaceae, known as *Mamajjaka* in Ayurveda, is a medicinally important plant used to treat Madhumeha or diabetes mellitus. The plant has high demand in the Indian herbal drug market and is used in various herbal preparations. The present study was conducted to characterize dried herbal samples of *E. axillare* subsp. *littorale* botanically. The botanical characterization involved macroscopic and microscopic studies on the herbal samples. Qualitative and quantitative microscopic characters were studied, and digital photographs of transverse sections (T.S.) and powder cell structures were taken. In T.S. of the stem, the vascular region characters such as the appearance of xylem tissue, lumen diameter of xylem vessels, and powder characters of aerial parts such as abundance and size of starch grains, prismatic crystals, presence of stomata, and trichomes were studied. The characters identified in the present study can be used to identify the herbal samples of *E. axillare* subsp. littorale in fresh as well as in dry forms.

Keywords: Raw herb, anatomical characters, Chota Chirayta, Mamajjaka, qualitative characters, quantitative characters.

INTRODUCTION

Enicostema axillare subsp. *littorale* (Blume) A. Raynal belonging to the family Gentianaceae, occurs as a weed in diverse natural habitats such as savannas, grasslands, forests to beaches, from wet to very dry habitats, and also survives well in a very saline environment. This species is globally distributed in South America, Africa, and Asia. It is found almost throughout India up to 450 m asl, commonly in coastal areas (Praveena *et al.,* 2014). As per www.theplantlist.org version 1.1 (TPL, 2013), *E. axillare* subsp. *littorale* is known by various synonyms such as *Enicostema littorale* Blume, *Adenema hyssopifolium* G.Don, *Coutoubea verticillata* G.Don, *Ericoila verticillata* Borkh., *Gentiana hyssopifolia* L. ex Spreng., *Gentiana lignosa* Murray ex Steud., *Gentiana octoflora* Vitman, and *Hippion littorale* (Blume) Miq. It is commonly known as 'Bitter tonic,' 'Chota Chirayta,' 'Indian Gentian,' or 'Indian Whitehead.' In Ayurveda, it is known by various names such as Naagjhvaa, Maamajjaka, Naahi, and Tikshnapatra; in Unani, it is known as Naai, and Naahi, and in Siddha, it is known as 'Vellargu' (Khare, 2007).

The plant is reported to be used in Indian Systems of Medicine (ISM), including Ayurveda, Unani, and Siddha. Dried roots and the aerial parts of the plant are used as a laxative, anthelmintic, in curing several health problems such as fever, rheumatism, vata diseases, skin diseases, abdominal problems, obesity, regulating blood sugar levels, and also in snake bite (Kirtikar and Basu, 1935; Saranya *et al.,* 2013). In Ayurveda, it is used to treat diabetes, typhoid, control arthritis, and as a cooling agent (Mishra *et al.,* 2017; Thondaiman and Saha, 2017). The plant is known to have several phytoconstituents and secondary metabolites such as alkaloids, sterols, volatile oils (Natarajan and Prasad, 1972), betulin, a triterpene sapogenin (Dymock *et al.,* 1893), monoterpene alkaloids, flavonoids (Ghosal *et al.,* 1974), catechins, saponins, steroids, sapogenin, triterpenoids, flavonoids, xanthones, and flavone C-glucoside (Verticilliside) (Jahan *et al.,* 2009). The plant is reported to have ophelic acid, which is also present in 'Chiretta' as a hydrolytic product of 'Chiratin' (Khare, 2007). It is considered a potential hypolipidemic plant (Gopal *et al.,* 2004) and used as a substitute for *Swertia chirayita* (Roxb.) Buch.-Ham. ex C.B.Clarke (Nadkarni, 1976; Khare, 2007). The plant is also known to have many pharmacological properties such as hepatoprotective (Dash *et al.,* 2000; Gupta and Singh, 2007; Vaijanathappa *et al.,* 2008; Gite *et al.,* 2010), antimicrobial (Katewa and Arora, 2001; Leelaprakash and Dass, 2012), hypoglycaemic (Ravi *et al.,* 2000; Vishwakarma *et al.,* 2010), antihelminthic (Mishra and Shukla, 2011), antinociceptive (Jaishree *et al.,* 2009), antioxidant (Mukundray *et al.,* 2011; Thirumalai *et al.,* 2011), antiulcer and anti-inflammatory (Roy *et al.,* 2010), antihyperlipidaemic (Gopal and Udayakumar, 2008; Vaidya *et al.,* 2009; Gopal *et al.,* 2011), and anti-diabetic (Shah and Gopal, 1985; Upadhyay and Goyal, 2004).

The estimated annual trade of *E. axillare* is between 50-

100 MT (NMPB, 2020). Dried herbal samples do not show many characteristic morphological features useful for identification. Besides, similar and confusing common names create an identification problem in dried herbal samples. For quality assurance of plant samples in herbal medicines, correct identification of raw herbal samples is required (Sahoo *et al.,* 2010). Microscopic characterization of herbal drugs is considered significant for taxonomic identification of fragmented herbal samples (Metcalfe and Chalk, 1957). In the present study, macroscopic and microscopic studies were performed on aerial plant samples of *E. axillare* subsp. *littorale* to develop botanical references, which can be used for correct identification of its samples in both fresh and dried forms.

MATERIAL AND METHODS

Authentic plant material was collected from Chota Udaypur, Rajasthan, and dry herb material was procured from Khari Boali Herbal Market Delhi. Duly identified herbarium specimens were submitted to the internationally recognized Janaki Amal Herbarium (RRLH) at Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu (accession nos RRLH-23379 and RRLH-23380), and dried raw herb samples were submitted to the Crude Drug Repository at CSIR-IIIM Jammu (accession nos CDR-4035 and CDR-4046).

Macroscopic and microscopic studies were performed on dry herbal samples. The macroscopic study involved the study of surface characters, color, texture, and appearance. For the anatomical study, dried samples were rehydrated in water for 24 h, and thin transverse sections (T.S.) were obtained by freehand sectioning using a razor blade. Fine T.S. was stained as per Kumar *et al.,* (2018) with minor modifications. The T.S. were dehydrated in series of alcohol gradients (30%, 50%, and 70% alcohol, each for 10-15 min), stained in safranin stain for 5-7 min, decolorized in 70% alcohol for 5 min, stained in fast green for 3-5 min. The T.S. was again decolorized in 70% alcohol for 3-5 min and then dehydrated in 90% alcohol, followed by absolute alcohol for 5-7 min. The T.S. were cleared in xylene, mounted in Canada balsam, and were examined under a compound microscope (LEICA DM 750) with the associated camera (LEICA ICC50E). Also, a microscopic examination of powder samples was done on the aerial drug powder. The powder was passed through a fine sieve, and the water mounted slides were observed under a compound microscope to study the various cell types and cell contents.

RESULT AND DISCUSSION

The macroscopic and microscopic studies' observations have been given in Table 1 and Figures 1 & 2.

Macroscopic characters

The plant is a perennial herb, 5-30 cm tall with few branches at the base having a cylindrical, glabrous stem, sessile leaves, and pentamerous flowers in axillary clusters (Fig. 1A-1F). Leaves are linear to lanceolate or narrowly oblong, sessile, glabrous, with entire margin, mucronate apex, lamina narrow, lanceolate, and 3-nerved (Fig. 1D). Flowers are pentamerous, sepals 5 with acute apex and fused base; petals 5, fused at base, appear funnel-shaped, five epipetalous stamens, and ovary superior (Fig. 1E & 1F). Flowers white with greenish lines and turn yellowish after drying (Fig. 1B & 1C). The plant is known to produce many seeds but reported with low germination rates in natural conditions. Root suckers are used for vegetative propagation. Dried plant material consists of the light yellowish-brown, quadrangular thin stem (0.1-0.2 cm), with nodes and internodes (Fig. 1A). Nodes bear opposite decussate leaves and inflorescence in leaf axils (Fig. 1B & 1C).

Microscopic characters

The stem's T.S. appeared irregular circular or angular in outline with wing-like protuberances (Fig. 1H & 1I). The outermost zone is formed of the single-layered, cuticularised epidermis with thin-walled, square, or rectangular-shaped cells. Next to the epidermis, the parenchymatous cortex zone (3-8 layered) of variable thickness (46.64 to 111.20 µm) with loosely arranged cells is present. Cortex is followed by a thin, continuous phloem zone (21.29±2.18 µm) present over a broad xylem zone (192.88±10.63 µm). Xylem tissue formed a continuous ring-like structure and appeared nearly circular in outline on the outer side (near phloem region), whereas it appeared irregular to angular (quadrangular) on the inner side. Xylem consisted of thick-walled xylem fibers and few xylem vessels, being present in a linear arrangement. The central part formed of broad hollow pith, mostly with disintegrated cells in dried stem samples (Fig. 1H & 1I). Pith formed a major broad (408.50±35.41 µm) hollow zone in the center compared to other tissue zones. Phloem was observed to form the smallest zone relative to the radius of studied T.S. of the root (Table 1).

Powder characteristics

Aerial plant powder of *E. axillare* subsp. *littorale* is fine textured, light greenish to brown colored with characteristic odor and bitter taste (Fig. 1G). Microscopic examination of aerial powder sample under the compound microscope showed the presence of few xylem vessel fragments, several prismatic crystals of variable size, few fragments of parenchyma cells, few anisocytic stomata, unicellular trichomes, leaf epidermal fragments, and few starch grains (elongated to oval in shape) (Table 1, Figs.

Table 1: Quantitative anatomical characters of the T.S. of the stem of *E. axillare* subsp. *littorale.*

Character	Min.	Max.	Mean (±S.D.)
The thickness of various zones			
The radius of studied T.S.	651.12	778.72	717.96 (±13.15)
Cortex zone thickness	46.64	111.20	67.03 (±6.32)
Phloem zone thickness	10.07	32.14	21.29 (±2.18)
Xylem zone thickness	121.25	236.34	192.88 (±10.63)
Pith radius	364.55	553.71	408.50 (±35.41)
Epidermal cell size			
Length	13.16	26.33	20.11 (±1.17)
Breadth	9.41	21.13	14.18 (±1.10)
Xylem vessel lumen diameter	13.08	35.73	21.34 (±2.49)
Powder characteristics			
Starch grains (Length)	20.61	65.63	35.47 (±4.25)
Starch grains (Breadth)	16.32	34.54	24.70 (±2.16)
Prismatic crystals (Length)	26.78	42.65	35.01(±1.83)
Prismatic crystals (Breadth)	14.57	42.08	24.31 (±2.32)

2A-2I).

Discussion

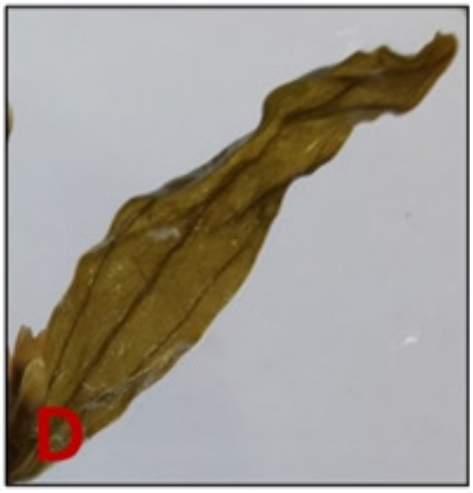
Several earlier studies used qualitative and quantitative anatomical characterization for botanical identification of raw herbal samples. The macroscopic and microscopic study of aerial drug samples, including qualitative descriptions combined with quantitative data, can help dry samples' correct identification. Li *et al.,* (2008) used semi-quantitative and quantitative micromorphological characters to authenticate *Halenia elliptica* (Gentianaceae). Hassan *et al.,* (2015) described the thickness of various tissue zones and T.S.'s total thickness as a quantitative character for the characterization of stem, root, and leaf samples of *Calotropis procera*. Singh *et al.,* (2020) used some quantitative characters such as the size of epidermis, hypodermis, cortex cells, starch grains, number and distribution of vascular bundles, presence or absence of specific cells in cross-section, etc. for the anatomical description of the stem of *F. cirrhosa*. Kumar *et al.,* (2018) observed the shape and size of starch grains, presence, type, shape, and size of calcium oxalate crystals to distinguish closely related Astavarga species.

Enicostema axillare subsp. *littorale* is reported to have many pharmacological properties and several important traditional uses. However, its some synonyms and common names are confusing and reported to be interchangeably used with other species of *Enicostema* like *E. verticellatum* (L.) Engl., and also with species of other genera like *S. chirayita*. Distinguishing herbal drug samples of species with confusion in botanical characters requires a reference standard. Sanmugarajah *et al.,* (2013) performed Phyto-physicochemical standardization for correct identification and authentication of whole plant samples of *E. axillare*. In some botanical studies, macroscopic and microscopic characters of fresh leaf, stem, and root samples were studied (Laxman *et al.,* 2010; Praveena *et al.,* 2014). In an anatomical study of the stem, Laxman *et al.,* (2010) observed around ten vascular bundles in the stem, however, Praveena *et al.,* (2014) observed vascular region in T.S. of the stem as a single continuous ring-like structure. In an anatomical study, Praveena *et al.,* (2014) observed T.S. of the stem as four-angled with the short stumpy wing-like structure on four edges, few stomata in the epidermal region, and a central broad, parenchymatous pith. In the present study, a single continuous ring-like vascular bundles were observed in T.S. of the stem with compact, deformed parenchyma cells of the cortex and the pith region's disintegrated cells giving pith a hollow appearance. For botanical identification of dried herbal samples, parenchymatous cells may be prone to disintegration and may not be suitable for species characterization in dried form. However, the vascular region cells were observed as intact and can be considered suitable characters for species distinction. The anatomical characters of the vascular bundle (the type of xylem cells, arrangement of xylem cells, and lumen diameter of xylem vessels) were found to be of taxonomical value.


A
NATARAJ® 621 SCALE


B


C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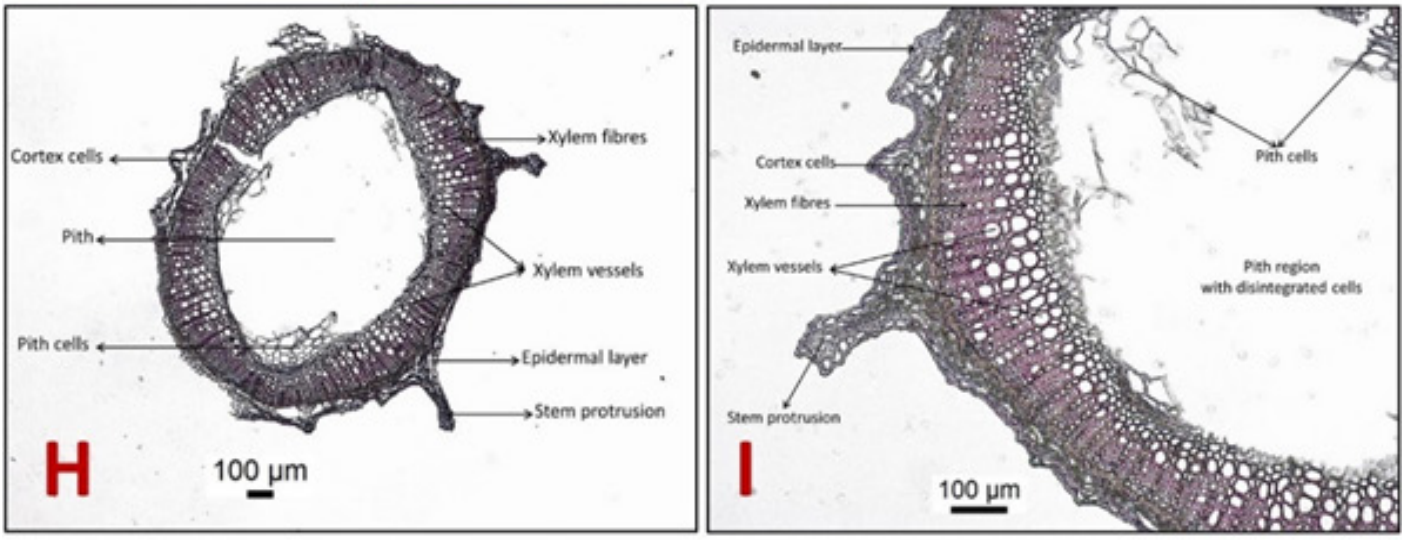

Figure 1: *Enicostema axillare* subsp. *littorale*, A). Dry aerial herb sample, B). Aerial part, (C), Flowers in clusters, D). A 3-nerved leaf, E). Inner pentamerous flower view showing stamens and pistil, F). Outer pentamerous flower view showing sepals and petals, G). Powder of aerial part, H). T.S. of the stem, I). T.S. of stem- an enlarged portion.

CONCLUSION

In the present study, qualitative and quantitative microscopic characters were studied, and digital photographs of transverse sections (T.S.) and powder cell structures were taken, which can be used as a reference for identifying samples of *E. axillare* subsp. *littorale* in both fresh and dried forms. Powder study of aerial parts also revealed some organoleptic and microscopic features such as shape and size of starch grains, prismatic crystals, type of stomata and trichomes, and abundance of other cell structures, which are useful for species identification.

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