Pharmacognostic studies and anatomical peculiarities in medicinal plant *Enicostemma axillare*

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Abstract. Mulani RM, Dhawle KS, Dhuldhaj UP. 2020. Pharmacognostic studies and anatomical peculiarities in medicinal plant, as *Enicostemma axillare*. Asian J Ethnobiol 4: 23-30. Pharmacognostic evaluations were carried out by plant material collection, preparation of herbarium; organoleptic properties, macroscopic characters, microscopic characteristics. For the physiological studies Transverse Sections (T.S.) of root, stem and leaves were taken and also powder analysis, physicochemical value determination such as total ash, water-soluble ash, acid-soluble and acid-insoluble ash were done. The distinguishing characters of *Enicostemma axillare* (Poir. ex Lam.) A. Raynal stem are the presence of parenchyma. Leaves microscopy indicates presence of anisocytic stomata, minor leaf veins, phloem transfer cells and numerous sclereids. Macroscopic study shows leaves and roots with glabrous-surface, and stem was tall simple to branch and maybe prostrate or ascending. Color variation indicates presence of different types of components and essential oils; determined the percentage of leaf, stem and root: ash values; water-soluble, acid-soluble, and acid-insoluble ash values. This study provides anatomical properties for the proper identification and establishment of standards of *E. axillare* for the medicinal applications to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching, and insect poisoning. This helps in pharmacognostical standardization of drug in crude form and also to differentiate them from adulterations.

Keywords: Adulteration, *Enicostemma axillare*, medicinal plant, organoleptic properties, pharmacognosy

**INTRODUCTION**

Medicinal plants used as a drug in Unani-Tabbi, Ayurved, Siddha, Homeopathy, Allopathy and Naturopathy and Home Remedies. This rate is higher in African countries where up to 90% of the total population relies on the uses of medicinal plants to help in their primary health care needs (Adewale and Oduyemi 2014). Herbal medicines are one of the promising choices for modern synthetic drugs. In Sudan, traditional medicines prepared from medicinal plants showed antiplasmodial activity (Ahmed et al. 2010). The increasing popularity of herbal remedies all over the world suggests rules for their regulations (Ameh et al. 2011). Medicinal plants are playing very much active role in traditional medicines for the treatment of various types of ailments. According to the World Health Organization (WHO 2011), it is estimated that 80% of the whole world’s population, especially for millions of vast rural areas of developing countries people uses medicinal plants for the treatment of various types of diseases as a primary health care needs (Bhargava et al. 2013; Raja et al. 2015).

The green plants provide food, clothing, shelter, and other all needs for human beings (Mathew and Babu 2011). Plant identification is necessary as far as the genuine sample of crude drugs is required for the efficacy of the crude drugs. It includes pharmacognostic studies mainly anatomical and morphological parameters which help in authentication of crude drugs (Shahina and Nampy 2014). In herbal technology, pharmacognostic studies play very essential role as it provides standardization parameters which will help to prevent adulterations in the original plant material and also ensures correct plant identity. This information will help in authentication of the plants and ensures reproducible quality of herbal products resulting in safety use as well as retaining effectiveness of natural products (Shahina and Nampy 2014; Chaudhari and Mahajan 2015). Medicinal plants have been recognized from time immemorial and are also known throughout the world as a rich source of therapeutical agents in the prevention of various diseases (Mathew and Babu 2011). Herbal medicine is very important branch in Ayurvedic Medical Sciences but there is lack of standard identification methods. *Enicostemma axillare* is flower producing herbaceous, perennial plant growing up to 1.5 feet (Shahina and Nampy 2014).

The word ‘*Enicostemma*’ is derived from three words ‘en’ means inside, ‘icos’ means twenty, ‘stemma’ means wreath, referring to the arrangement of about twenty flowers around every node of the stem (Shakya 2016; Upreti et al. 2013; Sanmugarajah et al. 2013). *Enicostemma axillare* occurs in grasslands, sea coasts, peninsular India. It is cosmopolitan and exhibits variations in the habit (Zaheer et al. 2011). It is found throughout India and marine conditions and highly alkaline are favorable for the *Enicostemma axillare* (Poir. ex Lam.) A. Raynal. When growing in good soil, away from the seashore this plant attains larger size with large broad leaves than the one near the sea. The plant is also native to tropical Africa, Southeast Asia, Malaysia; Africa to the Lesser Sunda Islands. The crude drug or plant is commonly called Nai or Kadvi-nayi. The focus of this study was Pharmacognostic...
studies and anatomical peculiarities in *Enicostemma axillare* while there is not much work on pharmacognostic details of this plant.

**MATERIALS AND METHODS**

Plant materials

*Enicostemma axillare* is a small plant species growing at various sites in SRTM University campus, Nanded, Maharashtra, India. The plant species occur naturally throughout the year wherever moisture is there generally in open places amongst the grasses. It is a tropical plant that grows well in 22-25 °C. Fresh healthy plant materials for the study were collected from various places during summer season (May 2019) from the same campus of latitude 19°06’27.3”N and longitude 77°17’22.1”E with altitude 1500 ft and brought to the laboratory. The climate conditions of the SRTMU Campus in the wet seasons are hot and humid and in dry seasons are uncomfortably hot and mostly clear with annual average temperature of 13 °C to 42 °C. The herbarium specimen was made and submitted in Botanical Depository of SRTMU Nanded. The submitted plant species was identified and authenticated by the expert Prof. (Dr.) Ramjan M. Mulani, Department of Botany, School of Life Sciences, SRTM University, Nanded with detailed taxonomic study and given the herbarium voucher number SRTMU/SLS/Bot/Herb/116/RMM/2019-20.

Morphological and anatomical observation

The remaining plant material was processed for anatomical characterization. In macroscopic evaluation, various parts were checked externally for the length, breadth of the leaves and stem, and roots. The microscopic evaluation was done on qualitative basis. The anatomy of leaf, stem, and root is studied using fresh samples. Stems were sampled at the 2nd and 3rd internodes. The qualitative microscopic study was done by using freehand transverse sections of washed leaves, stems, and roots and from epidermal peelings.

Physicochemical observation

Color variations were observed in the crude powder of different parts of *Enicostemma axillare* such as leaves, stems, and roots. Fluorescence analysis of the whole plant powder was carried out using standard method (Akbar et al. 2014; Pathan et al. 2018). The analysis was done by treating the plant powder with different solvents including both acidic and basic. After treatment, they were exposed to both the UV light (short wavelength of 254 nm and long wavelength of 365 nm) as well as were observed in daylight (Akbar et al. 2014). Color variation is to be analyzed and observed in the crude powdered extracts i.e. leaves, stems, and roots materials of the *Enicostemma axillare* under ordinary light, short ultraviolet wavelength (254 nm), and long ultraviolet wavelength (365 nm), after treatment with following 14 different types of organic and inorganic reagents.

**Organic reagent:** Picric acid, acetic acid, lactic acid, ferric chloride, Sudan III, methanol.

**Inorganic reagent:** Conc. H₂SO₄, conc. HNO₃, HCl, iodine, NaCl, NaOH, ammonia, water.

**Physical constant determination:** Total ash: To measure total ash empty silica crucible ignited to red hot and allowed it to cool down. Weighed it and tear zero. Accurately weighed 2 gm of grounded crude drug of *Enicostemma axillare* leaves, stem, and roots were taken into previously weighed silica crucible and weighed it. Further, drug is taken in crucible heated with increasing heat up to 450 °C, till the powder becomes red hot. The obtained ash appearance of white coloration transferred to desiccators and weighed again with silica crucible and further the percentage of Ash value were calculated (Momin and Kadam 2011).

Water-soluble ash: The above obtained total ash boiled with 25 ml of distilled water for 5 min and insoluble ash material separated with the help of ashless filter paper and washed with hot water and heated, further let it to cool down and weighed again. Subtract the weight of insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The ash value of the drug and the percentage of water-soluble ash with reference to the air-dried drug was calculated as mentioned below:

\[
\text{Initial weight} - \text{Final weight} = \text{Initial weight} \times 100
\]

Acid insoluble ash: The above obtained total ash boiled with 25 ml of dil. HCl for 5 min and insoluble ash material separated and washed with hot water and heated, further let it to cool down and weighed again. With reference to air-dried ash value of the drug calculated as mentioned below:

\[
\text{Initial weight} - \text{Final weight} = \text{Initial weight} \times 100
\]

**RESULTS AND DISCUSSIONS**

*Enicostemma axillare* collected from the campus of the Swami Ramanand Teerth Marathwada University and identification of the plant is done by depositing herbarium sheet of the plant to the depository of Department of Botany, School of Life Sciences, SRTMU, Nanded, India and herbarium sheet number of the plant is SRTMUN/SLS/BOT/HERB/116/RMM/2019-20 obtained. This plant can be used in the treatment of fever, rheumatism, skin diseases, abdominal disorders, snake bite, obesity, malaria, and diabetes. Species of *Enicostemma*
have special importance in Indian medicine as they also have antimicrobial, antilucre, hepatoprotective, anti-inflammatory, hypoglycemic, and hypolipidemic activities (Saranya et al. 2013; Zaheer et al. 2011).

**Organoleptic evaluation/macroscopic evaluation**

In macroscopic evaluation, we found that there is difference between fresh plant parts and dry plant parts like leaves, stems, and roots on the basis of color, shape, and size (Table 1). Fresh leaves of *Enicostemma axillare* are simple, opposite, sessile-sub sessile; sometimes narrowed into a petiole like base, longer than the internodes, pale-dark green in color, spiral, absence of petioles, rough in texture, strongly aromatic with characteristic odor and bitter taste (Figures 1-2). Lamina base is sessile, linear to lanceolate or narrowly oblong (5.0-8.0 x 0.3-1.0) cm² long, lamina indentations, entire, obtuse, acute-mucronate at the apex, somewhat narrowing towards the base, 3-nerved from the base, texture leathery, possesses minor leaf veins and phloem transfer cells (Figure 3). Dried leaves are dark green, characteristically odored with bitter taste, crooked, diameter (4.0-7.0 x 0.2-1.0) cm². Dried leaves powder is course in touch (Figure 2).

Fresh greenish-yellow stems are terete or quadrangular i.e. 4-angled; cylindrical, 15-45 cm or 1.5 ft tall that may be prostrate to ascending, long, green, pungent in odor, highly bitter in taste, simple or branched, internodes 0.5-1.2 cm long, herbaceous greenish-yellow in nature (soft), glabrous with a decurrent ridge below each leaf. Dried stems are yellowish-green with strong pungent odor, taste is very much bitter, no change in shape and size (Figures 1-2). Dried yellowish green stem powder is course in touch.

Roots are whitish-yellow in color, odor - Pungent, highly bitter in taste, cylindrical or branched-profusely branched, length 15-20 cm, and diameter 2.5 cm without hairs. Dried roots are light brown with Pungent, bitter in taste, crooked, length 15-20 cm, and 2 cm in diameter. Root hairs were absent (Figure 2). Dried light brown powder of roots is course in touch (Figure 3).

**Anatomical peculiarieties:**

Epidermis is single-layered having cells irregularly shaped, Outer cortex broadly parenchymatous with many-layered fibers arranged between the cortical parenchyma and the secondary phloem. Secondary xylem is followed by secondary phloem. Secondary xylem is having significant arrangement of xylem that is metaxyylems are towards outer side and protoxylems are towards inner side. Secondary xylem is followed by primary phloem. Inside of primary phloem, there is presence of xylem region and absence of root hairs (Figure 4).

Both fresh and dried stems showing terete or quadrangular outline, angles are having multicellular wing-like structure, epidermis is single-layered. Compactly arranged unicellular cells are round with parenchymatous chlorophyll without intercellular spaces, covered with thick cuticle. Hypodermis is of 2-layered, collenchymatous. Cortex is of two-layered thick parenchymatous having chlorophyll, presence of subepidermal sclereids, Sphaeraphides are seen occasionally into the pith region.

Vascular cambium having Phloem both external and internal called as secondary included phloem (Included phloem: phloem tissue lying within the secondary xylem in the continuous band above the xylem band). Protoxylem present towards inner region and metaxyylem towards outer region forming conjoint vascular bundle with presence of outer cambium just beneath to the secondary phloem and inner cambium below to the secondary xylem. There is an initial secondary growth with the addition of secondary xylem towards the inner side. Vascular cylinder consists of secondary xylem, secondary phloem, and Vascular cambium with pith into the center (P) (Figure 5).

Whole leaf showing minor leaf veins with phloem transfer cells; midrib region is haplostele; chlorenchymatous single-layered epidermis and hypodermis is made up of Mesophyll tissues is compact chlorenchymatus with mucilage cells. Lamina is differentiated into two layers such as palisade tissue towards adaxial epidermis i.e. upper surface of the leaf and spongy chlorenchyma is just at the abaxial side means lower surface of the leaf. Vascular bundle is present in the cortex region of midrib. It is of conjoint type i.e. xylem and phloem are arranged together in the same radius (collateral) and open i.e. with cambium. Bundle sheath extension is of chlorenchymatous. Xylem elements are facing upwards. Lateral leaf is having lamina indentations on both sides. Leaf surface showing stomata on abaxial epidermis (lower surface) is of anisocytic and anomocytic types, two guard cells are surrounded by 3 subsidiary cells from which 2 are larger and one is distinctly smaller (Figure 5).
Table 1. Macroscopic characteristics of plant material

<table>
<thead>
<tr>
<th>Characters</th>
<th>Fresh</th>
<th></th>
<th>Dry</th>
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<tbody>
<tr>
<td><strong>Color</strong></td>
<td>Leaves</td>
<td>Stems</td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>Whitish green</td>
<td>Green</td>
<td>Yellowish white</td>
<td>Dark green</td>
<td>No change</td>
</tr>
<tr>
<td><strong>Odor</strong></td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td><strong>Taste</strong></td>
<td>Bitter</td>
<td>Bitter</td>
<td>Bitter</td>
<td>Bitter</td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td>Lamina narrowly linear-oblong</td>
<td>Terete or 4-angled</td>
<td>Cylindrical, branched-profusely branched</td>
<td>Crooked</td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td>(5.0-8.0 x 0.3-1.0) cm</td>
<td>15-45 cm or 1.5 feet</td>
<td>L=15-20 cm, D=2.5 cm</td>
<td>(4.0-7.0 x 0.2-1.0) cm</td>
</tr>
<tr>
<td><strong>Touch</strong></td>
<td>Smooth</td>
<td>Soft</td>
<td>Hard</td>
<td>Course</td>
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</table>

Abbreviation: L: Length, D: Diameter, CR: Characteristics

Figure 2. Organoleptic characters of *Enicostemma axillare*; A. Fresh whole plant, whitish-green leaves, and stems, whitish-yellow roots, B-C. Dried dark green leaves and powder, D. Dried yellowish-green stems, E. Dried yellowish green stem powder, F-H. Fresh roots, dried crooked roots, and light brown powder

Figure 3. Root T.S. of *Enicostemma axillare*; (A-B): Fresh Roots: Epidermis (EP), 1-layered, cells irregular, (Outer cortex-OCX) broadly Parenchymatous (PAR). Fibers many-layered (F), arranged between the cortical parenchyma and the secondary phloem (SX); Proto xylem (PX), Metaxylem (MX): inside Primary phloem (P.Phl) presence of Xylem (X). Pith and Root hairs absent. (C-D): Dried Roots; epidermis 1-layered, cells irregularly shaped. Outer cortex is broadly parenchymatous. Fibers many-layered, arranged between the cortical parenchyma and the secondary phloem (SPhl). Root hairs absent.
Figure 4. Stems transversal section of *Enicostemma axillare*; A-B. Fresh Stems: epidermis 1-layered (EP), cells round, parenchymatous (PAR) without intercellular spaces, covered with cuticle (Ct), Conjoint vascular bundle with presence of outer cambium just beneath the secondary phloem and inner cambium below to the secondary xylem, pith is into the center; C. Showing hexagonal outline, 2-layered. Cortex (Cx), parenchymatous (PAR), chlorophyll (Chl); D. Hypodermis (H) 2 layered collenchymatous (CO), Protoxylem (PX), and Metaxylem (MX). E. Fresh Stems: 1-layered Epidermis (EP), cells round, Parenchymatous (PAR) without intercellular spaces, covered with thick cuticle (Ct). F. vascular cambium, phloem is present in the continuous band on both side of xylem band, sphaeraphides are occasionally seen. G-I. Dried Stems: 1-layered Epidermis (EP), compactly arranged unicellular small round cells are Parenchymatous (PAR), Hypodermis (H), Collenchymatous (CO), 2-layered. Thick Cortex (Cx), parenchymatous chlorenchyma (PAR, Chl), Subepidermal Sclereides (Sep, Scl), Phloem (Phl) both External (Ex) and Internal (In), secondary included (Sec, in) [included phloem (phi): phloem tissue lying within the Secondary xylem SX]. There is an initial sec. growth with the addition of Secondary xylem (SX) towards the inner side (SX, in). Vascular cylinder consists of Secondary Xylem (SX), Secondary Phloem (SP), and Vascular Cambium (VC) path phloem (P), Angles are having Multicellular wing like structures (MWLS).

**Fluorescence characteristics and powdered analysis**

For the powder analysis we have taken 14 reagents and tested against crude powder of *Enicostemma axillare* of different plant parts like roots, stems and leaves and visualized against different types of light sources such as visible, UV 254 nm and UV 365 nm. In these investigations, each source of light has shown different sort of color variation in reagents tested on plant samples. Crude powder extract as such, as well as in distilled water; it has not shown any color variation in any light source. For leaves, stem and roots it has shown henna green, yellowish-green and straw yellow colors respectively in all the three types of light sources i.e. visible, UV 254 nm and UV 365 nm. Similar color variations are observed in with reagents such as picric acid, Conc. H$_2$SO$_4$, Conc. HNO$_3$, HCl, acetic acid, lactic acid, Iodine, Sudan III, Ammonia, NaOH in methanol and NaOH (details are given in Table 2).

Constant value: Total ash value of the crude extract of the *Enicostemma axillare* were calculated and it was found that among total ash content; (12.5%) of leaves, (11.45%) of stems and (11.10%) of roots; water-soluble were (5.30%) of leaves, (4.25%) of stems and (6.52%) of roots; acid insoluble ash were (7.40%) of leaves, (7.20%) of stems and (7.10%) of roots and acid-soluble ash were calculated as (6.10%) of leaves, (5.25%) of stems and (5.00%) of roots (see Table 3).
Figure 5. Anatomical details of leaves of *Enicostemma axillare*; A–C. T.S.; minor leaf veins (MLV) with Phloem transverse cells (PTC); Midrib region (M), D–H. Midrib (M): 1-layered epidermis chlorenchymatous epidermis (EP, Chl). Hypodermis (H): Mesophyll without Mucilage (Mu). Lamina is differentiated into 2-layered i.e. palisade towards the adaxial epidermis (upper) and spongy chlorenchyma just at the abaxial epidermis (lower), mesophyll is compact chlorenchyma. Vascular bundle (VB): present in cortex of midrib region, conjoint (Cj) i.e. [Xylem(X) and Phloem (P)] arranged together in same radius (collateral) and open with cambium. Bundle sheath extension chlorenchymatous, Stele is haplostele, Xylem elements are facing upwards, Minor leaf veins (MLV) with Phloem transverse cells (PTC) are present, stomata are of anisocytic and anomocytic). I–K. (L) lateral leaf showing lamina indentations, mesophyllous chlorophyll, presence of palisade tissue on upper surface and spongy tissue at the basal epidermis. L–O. leaf surface showing stomata (ST) on abaxial surface (AB) i.e. lower surface, anisocytic (AN), guard cells (GS), 3 subsidiary cells (SC) i.e. 2 larger and 1 distinctly small.
In this study, we observed prominent features of *Enicostemma axillare* for identification and authentication of true crude drugs and established physicochemical parameters for screening of commercial samples. The distinguishing characters of stem were the presence of anisocytic stomata, minor leaf veins, phloem transfer cells, and numerous sclereids. Macroscopic study shows leaves with glabrous-surface, roots showed glabrous root surface and stem was tall simple to branch and maybe prostrate or ascending. This sort of analysis by the micro and macroscopic parameters is easy to handle and establishes authenticity of the plant (WHO, 1998) and it’s very necessary towards the quality assessment of crude drugs (Luz et al. 2019). Color variation indicates presence of different types of components and essential oils present in it. The ash value includes percentage of water-soluble, acid-soluble and acid-insoluble ash values for leaf stem and root. These parameters are constant for the plant and can be used to set a standard for screening (WHO 1996). This parameter establishes the inorganic content and adulterant present in crude drugs. It is also important for the estimation and detection of salts, metals, and silica (Musa et al. 2006). The identification marks of crude drugs are very essential for efficacy and safety and also reproducibility of herbal drugs (Nayak et al. 2010). The crude drug material sold in the commercial market is present in very deformed state and with the naked eyes; it is hard to assure good quality and in such cases, there are more chances of adulterations.

In summary, in the present study, we have found resourceful information in relation to pharmacognostic characteristics and anatomical peculiarities of the *Enicostemma axillare* drugs to identify plants and their products to avoid that from adulterations with sub-standard products. Authentication is the first step towards ensuring quality of starting material. Due to diminishing supply as well as overuse of the availability of raw drugs has become a dangerous problem in the Ayurvedic scenario. It would also help scientists to utilize such needful information regarding the plant material identity. After thoroughly investigating, we concluded that the *Enicostemma axillare* is a commonly used medicinal plant. In macroscopic evaluation we found that there is difference between fresh plant parts and dry plant parts like leaves, stems and roots on the basis of color, shape and size. In Physical constant value determination, the ash values used to evaluate quality, authenticity, as well as purity of unsophisticated drugs and also values obtained, are necessary in quantitative standards. Efforts will be undertaken to continue Pharmacognostic studies and Anatomical peculiarities to avoid adulteration. There is not sufficient evidence or detailed anatomical and physicochemical evaluation on *Enicostemma axillare*. Due to which present work is taken in the manner to completely standardize the herbs according to the standard laboratory procedure.

### Table 2. Powder analysis of crude extract of *Enicostemma axillare*

<table>
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<th>Test reagent</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Leaves</th>
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<td>SY</td>
<td>NC</td>
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<td>NC</td>
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<td>CG</td>
<td>PY</td>
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<td>LB</td>
<td>DB</td>
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<td>Y</td>
<td>OC</td>
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### Table 3. Physical constant value of crude drugs

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<th>Roots</th>
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</thead>
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<tr>
<td>Total ash</td>
<td>12.50</td>
<td>11.45</td>
<td>11.10</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>5.30</td>
<td>4.25</td>
<td>6.52</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>7.40</td>
<td>7.20</td>
<td>7.10</td>
</tr>
<tr>
<td>Acid soluble ash</td>
<td>6.10</td>
<td>5.25</td>
<td>5.00</td>
</tr>
</tbody>
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REFERENCES


