Investigation of Pharmacognostical and Preliminary Phytochemical Characters of *Enicostemma Littorale*

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DOI: http://doi.org/10.5281/zenodo.2553733

Abstract

Objective: *To study detailed pharmacognostic profile and preliminary phytochemical investigation of whole plant of Enicostemma littorale (Gentianaceae) is a perennial herb found throughout India. It is traditionally used as anti-diabetic, urinary astringent, anti-periodic, anthelmintic, anti-inflammatory, and laxative and hepato protective.* ***Methods:*** *Leaf, stem and root samples of E.littorale were studied by Macroscopical, Microscopical, Physiochemical, Phytochemical analysis of powder of the plant and other methods for standardization recommended by WHO.* ***Results****: Macroscopically, the leaves are simple. lanceolate, obtuse or acute, fresh leaves are dark green in color; bitter taste and characteristic odor. Microscopically, the leaf showed the distinct epidermal layer of small squarish cells with prominent cuticle. Calcium oxalate crystals of druses or Sphaerocrystals are widespread in the leaf-mesophyll tissue. The anisocytic type stomata observed more prominently. Single basal cell, multi -cellular, Non-glandular trichomes are the diagnostic features noted from anatomical study of the leaf. The salient features of stem were epidermis with thick cuticle, unilayered small cells compactly arranged. The diagnostic characters observed in the transverse section of the roots are thin walled, compact parenchyma cells, narrow xylem fibres.Powder microscopy of the powder revealed the presence of parenchyma cells, xylem fibres and epidermis with anisocyticstomata. Preliminary phy to chemical screening revealed the presence oftannins, betacyanin, quinone, glycosides, phenols, flavanoids, and alkaloids.* ***Conclusions:*** *The above results given the valuable information of this plant it will helpful for the future studies of this plant.*

Keywords: *Enicostemmalittorale, Microscopy, Macroscopy, Phytochemical evaluation*

INTRODUCTION

Traditional systems using countries like developing countries used herbal drugs for the treatment of various diseases. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many parts of the world [1]. Due to less side effects and more compatible, more percentage of the people in the world particularly developing countries used the natural drugs. Most of the allopathic drugs prepared from isolated compounds from natural sources. The strategy of isolating the active principles from the medicinal plants and manufacturing a pharmaceutical preparation then became popular. Modern medicines and herbal medicines are complimentarily being used in areas for health care program in several developing countries including India. Of late, the interest in the plant products surfaces all over the world due to the belief that many herbal medicines are known to be free from side effects. It is the fact that the discovery of the new synthetic drug is time consuming & an expensive affair [2]. The utility of the synthetic drug is always accompanied with its single or multiple adverse effects and in some cases the curatives are not available. India is the richest source of herbal drugs [3]. In the

present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential [4]. In spite of the tremendous advances made in the modern medicine there are still a large number of ailments for which suitable drugs are yet to be found. Today, there is an urgent need to develop safer drugs for the treatment of dreadful diseases. Hence, there is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine [5].

The medicinal plant of *Enicostemmalittorale, belonging* to the family Gentianaceae is mostly used in southern parts of India. It is traditionally used as anti-diabetic, urinary astringent, anti-periodic, anthelmintic, anti-inflammatory, and laxative and carminative. It possesses antioxidant, hypolipidemic, anti-microbial, anti-nociceptive, anti-edematologinic and anti-tumor activities. It is small perennial plant. Stem is cylindric, glabrous with a decurrent ridge below each leaf [6]. Distribution is throughout India in areas up to 450m. Elevation, common coastal areas among grasses. Literature survey did not provide sufficient information about Pharmacognostical studies of leaf, stem and root of this plant. The current work aims to contribute in solving the problems of controversial drugs prevalent in ayurveda besides helping in laying down Pharmacopoeial standards. Therefore, keeping above view in mind various Macroscopic, Histological and physiochemical and quantitative microscopical studies and preliminary phytochemical investigation of leaves, stems and roots of *E.littorale*were carried out in the present study.

MATERIALS AND METHODS

Collection and Authentication

Enicostemma littorale leaf, stem and root were collected, in and around Palakkad, Kerala, India and authenticated by taxonomist and the plant authenticated specimen is deposited in the Department of Pharmacognosy, Sanjo College of pharmaceutical studies, Palakkad, the authentication specimen number is SCPS/P.COG/002/2019. The fresh leaves, stems and roots were kept for shade drying. The whole plant was dried and powdered by using hammer mill to get a desired size. The powdered drug was stored in an air tight vessel for the purpose of further process.

Pharmacognostic Standardization

Macroscopical characters of leaves stem and roots are observed. Histological characters of the leaves, stem and roots were studied by using very thin transverse section taken by hand and stain with using phloroglucinol and Con HCl and mounted in glycerin [7]. The powder drug were stained and mounted, the diagnostic characters are observed [8].

Quantitative Microscopy

The quantitative microscopy such as vein-islet number, vein- terminal number, stomatal number and stomatal index were determined as per standard procedure [9].

Physio-chemical Evaluations

The physiochemical chemical parameters of Total ash, water soluble ash, acid insoluble ash, acid soluble ash, extractive values of different solvents and loss on drying were evaluated by using leaf, stem and root powder. Fluorescence analysis was studied by using different reagents with leaf, stem and root powder [10].

Preliminary Phytochemical Screening

The ethanol, petroleum ether, benzene, chloroform and aqueous extract of *E. littorale* Linn. was subjected to tests for

the presence or absence of the major class of compounds by standard methods [11].

EXTRACTION OF PLANT MATERIAL

Powdered plant material to be extracted by Soxhletextractionapparatus. In this method 1Kg of powdered drug to be taken in the thimble part of the apparatus, extract with different solvent according to their increasing polarity, like Pet ether, benzene, chloroform, ethanol and water. The extracts were filtered and concentrated.These extracts were used for the preliminary phytochemical studies for find out the presence and absence of the various phytoconstituents [12].

RESULTS

Macroscopical characters

It is an erect, perennial herb, 5–30 cm tall, simple or branched at the base. Cylindrical shape stem, it has decurrent ridge below each leaf. Macroscopically, the leaves are simple. lanceolate, obtuse or acute, fresh leaves are dark green in color; bitter taste and characteristic odour. Flowers are white with green lines, drying yellowish, sessile or sub-sessile; bracts long, shorter than the calyx, lanceolate-acuminate, crenate. Calyx tube 1–2 mm long; lobes are usually unequal, (0.7-1.5×0.4-0.7)mm, triangular to lanceolate, acute at the apex and narrowly scarious at the margin, or obovate to sub-circular, obtuse and mucronate at the apex, with wide scarious margin. Corolla tube is 3.5–6.0 mm long; lobes (1.5-2.0×0.7-1.0) mm, ovate and abruptly narrowing to an acute or mucronate apex. Stamens inserted below the sinuses, just above the middle of the tube; filaments 1.5– 2.3 mm long, with a double hood at the insertion point.

Figure 1: *Macroscopy of E.littorale.*

HISTOLOGICAL CHARACTERS

The detail and systemic Pharmacognostical evaluation would give valuable information for the future studies.

LEAF

The leaf is dorsiventral, mesopmorphic and amphistomatic. The adaxial surfaces are smooth and even. The midrib is planoconvex in sectional view with flat adaxial side and hemispherical abaxial side. The adaxial epidermis of the midrib is thin with small squarish or rectangular cells, the abaxial epidermis fairly wide with larger squarish cells and prominent cuticle.The vascular bundle is single, are shaped having several parallel radial multiples of xylem elements, phloem occurs both on the outer and inner sides of the xylem (bicollateral vascular bundles). The ground tissue of the midrib comprises of dilated, thin walled, circular, compact parenchyma cells. The midrib is 600µm vertically and 500µm horizontally. Abaxial side veins are conical shape, butadaxial side it should be flat. The size of vein is 300-380µm thickness (Figure 2, 3, 4, 5).

T.S OF LAMINA

Even and smooth surface lamina with uniform thickness. Spongy mesophyl tissue and palisade cells are not easily diffentiated with the mesophyll tissue. Adaxial side epidermis is similar size and shape of abaxial side. Epidermal cells have prominent cuticle and cylindrical shape. Both surfaces of the lamina have more prominently anisocyticstomata and occasionally diacytic stomata are occurs. Single basal cell, multi -cellular, Non-glandular trichomes are also observed both surfaces. The size of epidermal cells is 30 to 40µm thickness. Middle zone of the mesophyll tissues are large but adaxial side is short and cylindrical shape. Spongy parenchyma cells to form wide air chambers. Towards the lamina part, the cells assume vertically oblong, cylindrical

shape forming loosely arranged vertical filaments with intervening air-chambers. The vascular strands of the lateral vein-lets are small, centrally located, circular and collateral.(Figure 6,7,8) Sphaerocrystals of calcium oxalate spread throughout the mesophy The druses are solitary in each cell and are mostly restricted in the palisade zone The druses are up to 15µm in diameter (Figure 9,10).

EPIDERMAL CELLS ANDSTOMATA MORPHOLOGY

The epidermal cells, as seen in surface view of the para dermal sections, are wide with their highly waxy anticlinial walls. Due to waxy walls, the cells appear much lobed and amoeboid in outline. Fine, parallel lines of cuticular striations are visible on the surface of the epidermal cells. The stomata are anisocytic type. Each stoma has three, unequal subsidiary cells, un- sheathing the guard cells. The guard cells are elliptical measuring 30 µm long and 20µm wide. The stomatal pores are narrow and slit-like. Single basal cell, multi -cellular, Non-glandular trichomes (Figure 11, 12, 13).

T. S. of Petiole

The transverse section of the petiole shows circular in outline. Epidermis with thick cuticle, unilayered small cells compactly arranged; following the epidermis is a thick cortex 5-12 layered which is parencymatous with scattered chlorenchymatus cell. Stele consisting of band of xylem disrupted at poles. Polar xylem is lax, phloem is present in the form of continues band on both side of xylem band. Central pith is present.

T. S of the Stem

Young inter node, the young stem along the inter-node is four-angled with short stumpy wings along the four corners. The stem is 2.5mm thick. It consists of a thin and distinct epidermal layer of thin walled squarish cells, with thin smooth cuticle (Figure 14, 15). Stomata are seen at certain places of the epidermis. Inner of the epidermis is fairly wide. Parenchymatous cortex includes small, thin walled, loosely arranged cells. The cells of the wings also have similar type of cells as in the cortex. The vascular cylinder is slightly four angled, the four angles being thicker than the intervening portions. The thicker portions have dense xylem tissue; the primary xylem elements occur in close parallel radial rows along the inner portion; the outer portion consists of randomly distincted narrow vessels and dense thick walled fibres. Phloem occurs in thin continuous layers along the inner as well as outer portions of the xylem cylinder. The thinner portions of the xylem cylinder have thin segments of xylem with a few vessels and thick walled fibres (Figure 18).

Supramodel Region of the Stem

The region just above the node exhibits petiolar leaf-sheath, axillary bud and stem portion. The leaf-sheath has thick and conical and lateral veins. The stem is 1.35 mm thick. It has prominent epidermal layer of squarish cells and thin cuticle. The cortical zone consists of one or two layers of small, fairly thick walled compact hypodermal cells; the remaining cortical portion includes thin walled, less compact angular parenchyma cells. The xylem cylinder is closed without gaps; it is uneven thickness and comprises diffusely distributed, narrow, thick walled vessels and fibers. The xylem is amphi phloicsiphons cylinder, that is, phloem occurs both along the inner and outer portions of the xylem cylinder phloem elements are thin continuous lines. The pith is wide and parenchymatous. The central core of the pith cells are dilated tending to distinguishing rate. The Node exhibits unilaculear –single trace structure. These are a single leaf-gap with single trace (Figure 16, 17).

T. S. of the root

Both thin and thick roots were studied. The thin root is 1.3 mm in diameter. It

consists of fairly distinct rhizodermal layer of small papillae cells. The cortex is homocellular and consists of thin walled, compact parenchyma cells. The vascular cylinder is circular, solid and dense. It includes central xylem which consists of wide, circular, thick walled, solitary and diffusely distributed vessels and thick walled narrow xylem fibers. The vessels are up to 40µm wide. Unsheathing the xylem cylinder is fairly a wide phloem zone which includes short, radial lines of cells and small groups of sieve-elements (Figure 19,20) The thick root has wider cortex and well developed secondary xylem and secondary phloem. The epidermal layer in discontinuous with cells broken at several places. The cortical cells are larger, and thin walled; they are less compact and wide air-spaces seen in the cortex .Secondary phloem is nearly 200µm wide. The cells in the outer part of the phloem are crushed in to their dark lines; those in the inner part are intact. They occur in fairly regular radial files. The phloem rays are short, narrow and distinct. The sieve elements are in several seathered clusters. Secondary xylem is nearly 2mm in diameter. It consists of narrow, thick walled solitary vessels which are diffuse and seathered. Xylem fibers are thick walled with liquefied walls (Figure -21, 22).

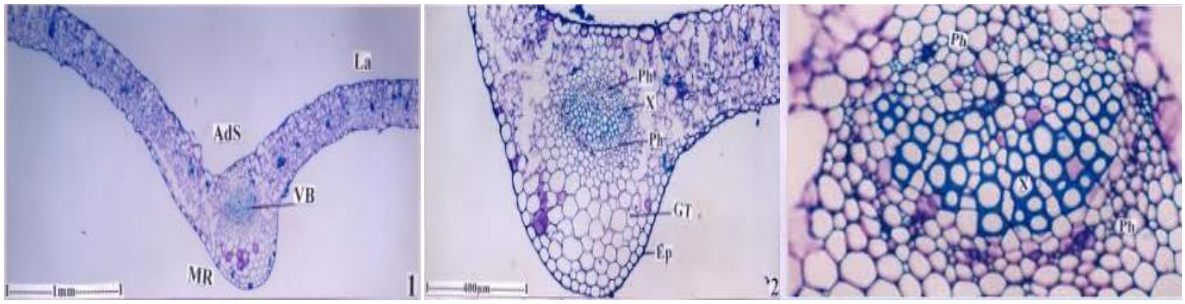

Figure 2, 3, 4: *T.S of leaf through midrib, Mid rib portion enlarged, Vascular tissues of the mid rib.*

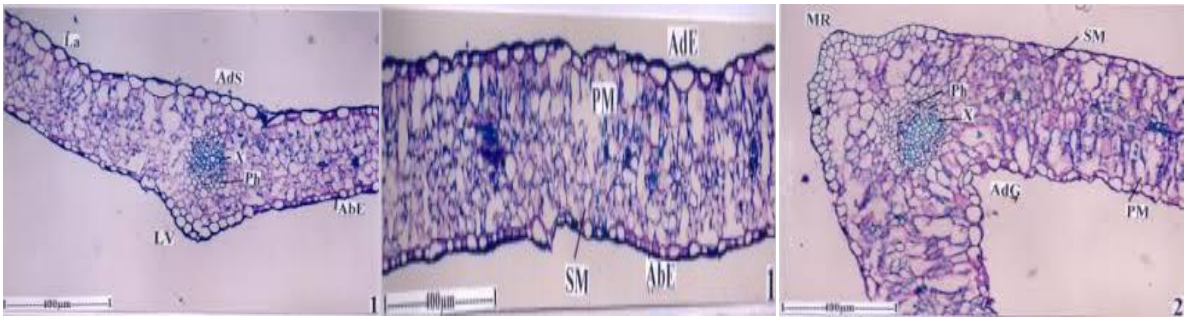

Figure 5, 6, 7: *T. S of leaf through lateral vein, T.S of lamina, T. S of leaf with folded lamina.*

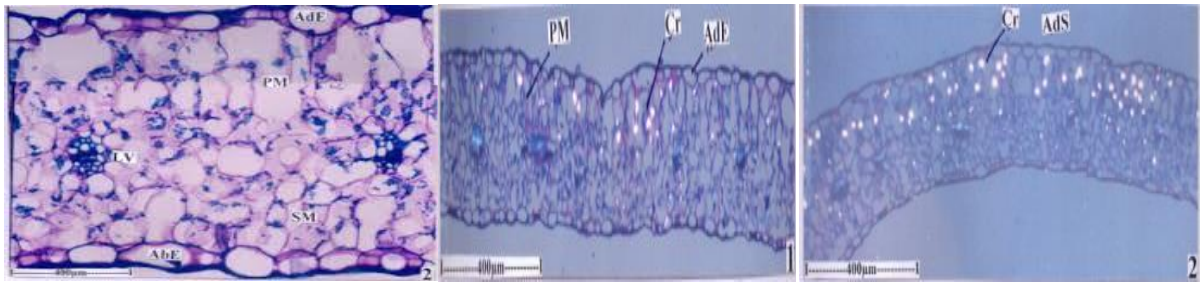

Figure 8, 9, 10: *T.S of Lamina-enlarged, T.S. of Lamina showing crystals, Crystal in the cells-enlarged.*

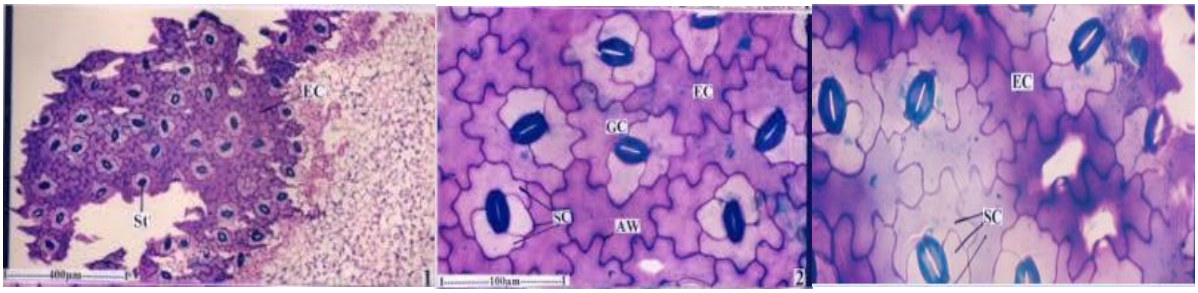

Figure 11, 12, 13: *Para dermal sections of the Epidermis, Stomata in surface view, Enlarged Stomata*

Figure 14, 15, 16: *T.S of young stem, two wings enlarged, T.S of supra-model region- stem.*

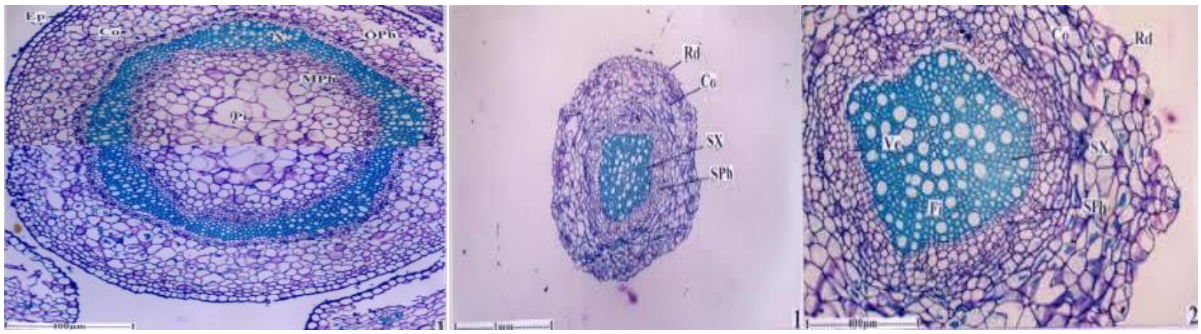

Figure 17, 18, 19: *T.S of Nodal region, Internodal region of old stem, T.S of thin root.*

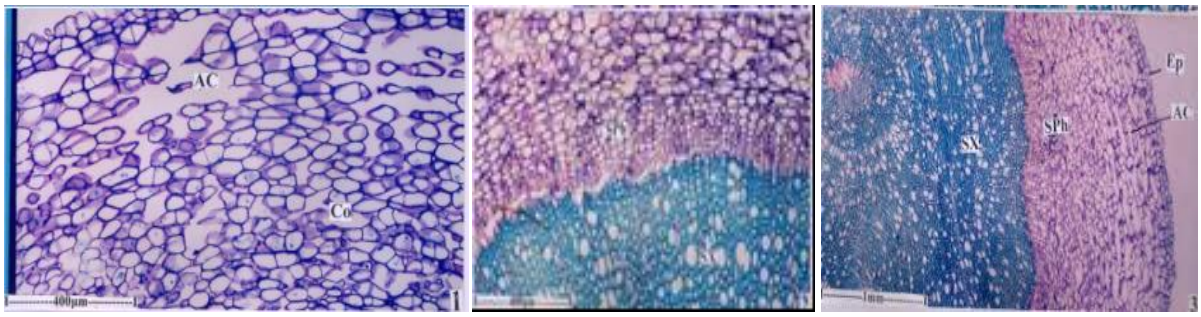

Figure 20, 21, 22: *Thin root enlarged, T.S of thick root, Thick-Root Secondary phloem, xylem.*

POWDER MICROSCOPY

Powder characteristics revealed the presence of glandular trichomes, covering trichomes, multiple trichomes, aerenchyma cells, collenchymas cells, vascular bundles, spiral vessels, fibres, anisocytic stomata, squarish cells, Sphaerocrystals of calcium oxa; late, xylem, phloem, compact parenchyma and xylem fibres.

QUANTITATIVE MICROSCOPY

The quantitative microscopy such as vein-islet number, vein- terminal number, stomatal number and stomatal index were

determined and the results were tabulated (Table 1).

Table 1: *Quantitative Evaluation of the Crude Drug of Leaf of E.littorale.*

Standardization parameters	
Vein islet no	11.3/sqmm
Vein termination no	10.4/sqmm
Stomatal number (upper)	16.66
(Lower)	22.66
Stomatal index (upper)	166
(Lower) 225	
Palisade ratio	1.16

PHYSIOCHEMICAL PARAMETERS

The powdered drug was evaluated for its physico-chemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated (Table 2). The ash contents showed the amount of inorganic matter present in the sample and the acid insoluble ash almost within 2.2 % which expresses low siliceous matter present in the sample.

Table 2: *Physico chemical evaluation of E.littorale*

Standardisation parameters	**Leaves(%w/w)**	**Stem(%w/w)**	**Root(%w/w)**
Total ash	9.5±0.03	8.9±0.024	8.7±0.028
Acid insoluble ash	6. 40±0.02	6.20±0.02	6. 10±0.02
Acid soluble ash	5.10±0.019	4.25±0.023	4.00±0.024
Water soluble ash	4.30±0.023	3.25±0.021	5.52±0.024
Loss on drying	6.10	6.50	6.30

EXTRACTIVE VALUES

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug and the results were tabulated (Table 3).

Table 3: *Extractive Values of Aerial Parts of Extracts of E.littorale with Different Solvents.*

Extracts	**Leaves**	**Stems**	**Roots**
Petroleum Ether Extract	5.5	4.5	3.2
Methanol Extract6.55	6.70		6.20
Ethanol Extract	7.85	8.50	7.70
Aqueous Extract	8.659.98		8.85

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The powdered drug and various extracts such as petroleum ether extract, benzene extract, chloroform extract, ethanol extract and aqueous extract were subjected to preliminary phytochemical screening of their presence or absence of the constituents and the results were tabulated (Table 4).

Table 4: *Preliminary Phytochemical Tests for Drug Powder and Various Extracts of E.littorale.*

Test	**Petroleum ether**	**Benzene**	**Chloroform**	**Ethanol**	**Aqueous**
Sterols	+	+	+	+	+
Terpenoids	-	-	-	-	-
Carbohytrates	+	-	+	+	+
Flavanoids	-	-	+	+	-
Proteins	-	-	-	-	+
Alkaloids	-	-	+	+	+
Glycosides	-	-	-	-	-
Saponins	-	-	-	-	-
Tannins	-	-	-	+	+
Mucliage	-	-	-	+	+
Resins	-	-	-	+	-
Oil/Fats	-	-	-	-	-

FLUORESCENCE ANALYSIS

The powdered drug and they were treated with solvents and the color changes were observed under Ultra violet light and the results were tabulated (Table 5).

Table 5: *Fluorescence Analysis of E.littorale.*

Solvents used	Leaf	Stem	Root
Powder as such	Dark green	Light green	Light yellow
Powder +Iodine	Red	Reddish brown	Red
Powder + 5% $Fecl_3$	Orange greenish	Dark orange	Light orange
Powder + 1N NaOH	Dark green	Light green	Light green
Powder + Acetic acid	Greenish yellow	Greenish yellow	Light yellow
Powder+Acetic acid+50%H_2SO_4	Bluish green	Light green	Yellowish green
Powder + 50% Con HCl	Dark yellow	Light yellow	Light yellow
Powder +Ammonia	Dark green	Light green	Light green
Extract+1% $cuso_{4+}$4%NaOH	Dark green	Green	Green
Extract+40%NaOH+1% Lead acetate	Dark green	Dark green	Greenish yellow
Powder+ 50% HNO_3 + Picric acid	Dark green	Light green	Light green
Powder + Saturated picric acid	Greenish yellow	Light green	Light green

DISCUSSION

My study has focused on examining Pharmacognostic and Preliminary phytochemical study of *Enicostemma littorale.* Leaves stem and root. Normalization of the macroscopic and microscopic characteristics of the *Enicostemma littorale*. Drug remains essential in other to identify and avoid falsification. Thus comparing the cross section of the leaf, stem and root anatomy showed structural similarities, the leaf showed the distinct epidermal layer of small squarish cells with prominent cuticle, the vascular strand comprises several, parallel compact files of 3-5 xylem elements in each file; Calcium oxalate crystals of druses or sphaerocrystals are widespread in the leaf-mesophyll tissue. The anisocytic type stomata observed more prominently and occasionally diacytic stomata are also observed. Single basal cell, multi -cellular, Non-glandular trichomes are the diagnostic features noted from anatomical study of the leaf. The salient features of stem were epidermis with thick cuticle, unilayered small cells compactly arranged. Sphaeraphides are occasionally seen in pith cells. Parenchymatous and the cells are compact and scattered chlorenchymatous cells are observed. Phloem occurs in thin continuous layers along the inner as well as outer portions of the xylem cylinder. Central pith is present. The diagnostic characters observed in the transverse section of the roots are thin walled, compact parenchyma cells, thick walled, narrow xylem fibres. Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs [13]. The leaves are simple. lanceolate or linear or linear oblong or elliptic-oblong, obtuse or acute, fresh leaves are dark green in colour; dried leaves are greenish-brown in colour, bitter taste and characteristic odour. Powder characteristics revealed the presence of glandular trichomes, covering trichomes, multi pletrichomes, aerenchyma cells, collenchymas cells, vascular bundles, spiral vessels, fibres, anisocytic stomata, squarish cells, Sphaerocrystals of calcium oxa; late, xylem, phloem, compact parenchyma, xylem fibres. These diagnostic elements are consistent with

botanical standards and WHO guidelines [14, 15]. The study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and non-physiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 6.5 ± 0.1, which is below 10%. This result comply with the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reactions , fermentation and give less chance to microbial growth and contamination in drugs [16]. Therefore, for proper conservation of drugs made from the leaves of *Enicostemma littorale*. It would be desirable to use those whose water content is less than or equal to 10%. The determination of total ash gave us a rate of 9.5 ± 0,03. This value indicates the level of minerals in drugs. Insoluble ash in hydrochloric acid gave a rate of 6.40 ± 0,02. Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements [17]. This result is in agreement with Srikanthet al. who found rate of 0.97% and 0.5% respectively [18]. The maximum extractive value was found in distilled water (9.98%) followed by Ethanol (8.50%) Methanol (6.70%), Petroleum ether (5.5%). All the extracts of the drug was subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed presence of tannins, betacyanin, quinone, glycosides, phenols, flavanoids, and alkaloids. These parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material. Though *Enicostemma littorale* is a weed, it is a highly reputed drug used in Ayurveda. Histological evaluation and preliminary phytochemical studies give us the details of this plant; it will help further investigation of this plant in future.

CONCLUSION

World health organization has emphasized the need to ensure quality control of the raw materials used for ayurvedic medicines by using modern techniques and by applying suitable parameters and standards. In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation , which are beingreported for the first time in this plant and could be helpful in authentication and preparation of asuitable monograph for the proper identification of *Enicostemma littorale* for the future.

Acknowledgement

The author is thankful to the Director and Principal of Sanjo College of Pharmaceutical studies, vellapara, Palakkad, Kerala for providing facilities to carry out the present research.

Competing Interests

Authors have declared that no competing interests exist.

REFERENCES

1. Thendral Hepsibha B et.al. In vitro studies on antioxidant and free radical scavenging activities of *Azimatetracantha lam* leaf extracts. *Indian Journal of Science and Technology*. 2010; 5(0974-6846): pp. 571-577.
2. Rao VE & Prasada Rao PRS. Occurrence of triterpenoids in *Azimatetracantha*. *Curr Sci*. 1978; 47: pp. 857.
3. Ismail TS, Gopalakrishnan S & Begum VH et al. Anti-inflammatory activity of Salaciaoblonga Wall. And *Azimatetracantha Lam*. *J Ethno pharmacol*. 1997; 56: pp. 145-152.
4. Duraipandiyan.V & Gnanasekar. M, et.al, antifungal activity of triterpenoid

isolated from *Azimatetracantha* leaves. *Foliahis to chemicaetcy to biologica*. 2010; 48(2): pp. 311-313.
5. Pandya DJ, Desai TR, Nadpara NP & Mehta HA. pharmacognostic study and establishment of quality parameters of leaves of bombax insigne linn. *Int j pharmacognphytochem res*. 2010; 2(3): pp. 1-5.
6. Gupta RS & Singh D. Hepatomodulatory role of *Enicostemma littorale* Blume against oxidative stress induced liver injury in rats. *Afr J Agri Res*. 2007; 2: pp. 131–138.
7. Evans WC, Trease and Evans'Pharmaconosy, 14th ed., W.B.Sounders Company Ltd., London; 1996. pp. 545-546.
8. Johansen DA. Plant Microtechnique. McGraw- *Hill, New York, USA*. 1940.
9. Indian Pharmacopoeia. *Controller of Publication, Delhi, India*. 1995; 2: pp. A-54.
10. Horbone, JB. Phytochemical methods- A guide to modern techniques of plant analysis. *Chapman and Hall, London*. 1998; 129, 203: pp. 42.
11. Trease GE & Evans WC. Pharmacognosy. *Williams Charles Evans as edited in 15th edition. Saunders publisher London*; 2004: pp. 137-44.
12. Khandelwal KR. Practical Pharmacognosy- Techniques and Experiments. *Pune: Nirali Prakashan*. 2002.
13. Fouraste I. Le contrôle des plantes médicinales. *Actualités Pharmaceutiques*. 1990; (278): pp. 55-58.
14. Kumar S, Kumar V & Prakash O. Microscopic evaluation and physicochemical analysis of Dilleniaindica leaf. *Asian Pac. J Trop Biomed*. 2011; 1:337-340.
15. Nasreen S, Radha R. Assessment of quality of WithaniasomniferaDunal (Solanaceae): Pharmacognostical and physicochemical profile. *Int J Pharm Sci*. 2011; 3(2): pp. 152.
16. Organisation de l'unitéafricaine/commission scientifique technique et de la recherche (OUA/CSTR). *Pharmacopéeafricaine, méthodesgénéralesd'analyses. Edn 1, Publisher, Lagos (Nigéria)*. 1998, 254.
17. Sambo MH. Etude du traitementtraditionnel du diabète par unerecetteet les écorces de tronc de Manilkaramultinervis Dub (Sapotaceae). *Th Pharm., Univ.de Bamako, Mali*. 2005: pp. 125.
18. Srikanth K, Vikram G, Archana P, Rajinikanth M & Ram SN. Pharmacognostic and hytochemical investigations in Strychnospotatorum Linn. *F. J of Pharm and Phyt*. 2013; 2(4): pp. 46-51.