

Research Article

Phytochemical and Pharmacognostic Value of *Enicostemma littorale*

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ABSTRACT

Enicostemma axillare (Lam.) Raynal, syn. *E.littorale* Blume family Gentianaceae, is a perennial herb found throughout the greater part of India. Locally it is known as chota chirayita and used in indigenous medicines in the treatment of fevers and as bitter tonic and forms hypoglycemic marketed formulations. Hence, this study was undertaken to determine the phytochemical studies and antioxidant activities by various methods. It was observed that the methanol, ethyl acetate and petroleum ether crude extracts of *E.littorale* possess a significant anti-microbial activity against a bunch of microorganisms responsible for causing many life threatening diseases like AIDS etc., Hence our results suggest that *Enicostemma littorale* may be a potential plant for developing better therapeutic drugs for treating various infectious diseases.

Key words: *Enicostema littorale*, Chota chirayita, phytochemical studies, phyto constituent; Anti-oxidant properties.

INTRODUCTION

Enicostemma littorale (Chota-Kirayat or Chota-Chirayata) is a glabrous perennial herb belongs to family Gentianaceae. It is traditionally used as antidiabetic, urinary astringent, antiperiodic, anthelmintic, anti-inflammatory, laxative and carminative. It possesses anti-oxidant, hypolipidemic, anti-microbial, anti-nociceptive, anti-edematogenic and anti-tumor activities.

Enicostemma littorale (Figure 3.1) is a glabrous, perennial herb, distributed throughout India including coastal region up to 450m in lower hills. Drug consists of dried whole plant (mostly root and vegetative parts) of *Enicostemma hyssopifolium* (Willd.) Verd. (syn. *Enicostemma littorale* Blume) belonging to family Gentianaceae. Hence, this study focused to determine the phytochemical studies and antioxidant activities by various methods.

MATERIALS AND METHODS

Plant material and preparation of extracts: The aerial plant parts of *E.littorale* at flowering stage were collected from the Tirunelveli District, Alangulam in March 2010. The plant species was identified (by Botanist Dr.P.Jayaraman at Plant Anatomy Research Centre (PARC)). The collected material was dried under the dry shade and powdered. The powdered plant material was extracted using solvents of increasing polarity such as chloroform, ethyl acetate, methanol and petroleum ether in a soxhlet extraction apparatus.

Phytochemical properties and phyto constituents of *Enicostema littorale*:

In Vitro Antioxidant Activity: In all these methods, a specific concentration of the extract or standard solution was used to give a final concentration of 1000 µg/ml. Absorbance was measured against a blank solution that

contained the extract without the reagent. A control experiment was performed without the reagent. A control experiment was performed without adding the extract or standard. The IC₅₀ value, which is the concentration of the sample required to scavenge 50% of free radicals, was calculated.

2, 2-Diphenyl-Picryl Hydrazyl (DPPH) Radical Scavenging Method: 50 µl of the sample was mixed with 1.35 ml of methanol and then 100 µl of 0.1 % methanolic DPPH was added. The control was prepared by adding 100µl of 0.1% methanolic extract to 1.4ml of methanol. The suspension was incubated for 30 minutes in dark condition. Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of the sample was compared with known standard (0.16%) of Butylated Hydroxy Toluene (BHT).

FRAP Method: 50µl of sample was mixed with 1.5 ml of working FRAP reagent and the absorbance at 593 nm was measured at 0 minute after vortexing. Thereafter, samples were placed at 37°C in water bath and absorption was measured after 4 minute. Ascorbic acid standard was also processed in the same way.

DPPH ASSAY

Reagents

DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) and TPTZ (3, 4, 6-tripyridyl-s-triazine) were purchased from Sigma, USA. All the other chemicals used were of analytical grade.

Radical scavenging activity - DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl assay)

Antioxidant activity or free radical scavenging activity of the methanolic extract against DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) was measured according to George et al.



Fig. 1: T.S of leaf through midrib.

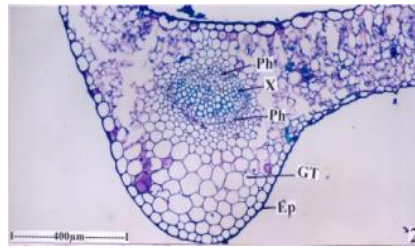


Fig. 2: Midrib portion enlarged

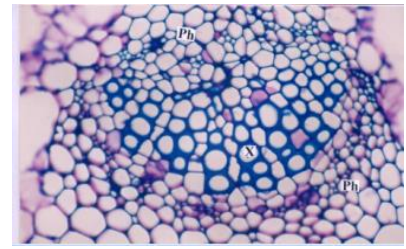


Fig.3: : Vascular tissues of the midrib

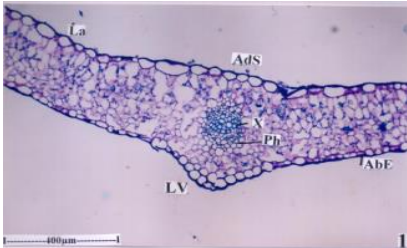


Fig.4 : T.S of leaf through lateral vein

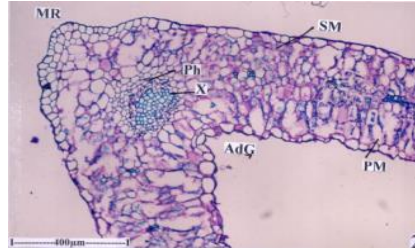


Fig.4: T.S of leaf with adaxially folded lamina.

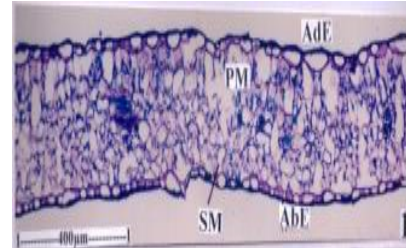


Fig.5: T.S of Lamina

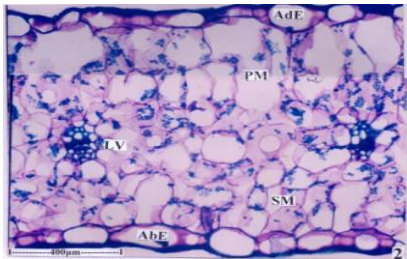


Fig.6: T.S of Lamina-enlarged

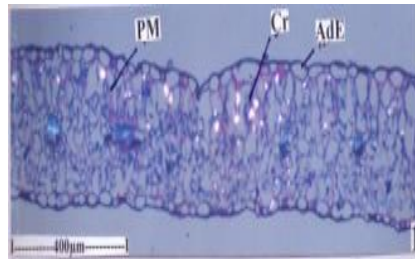


Fig.7: T.S. of Lamina showing crystal distillations

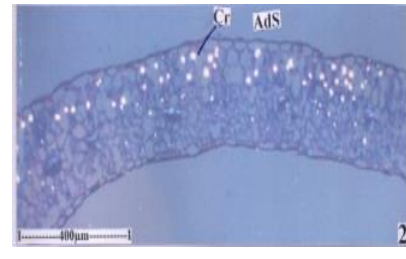


Fig.8: Crystal in the cells-enlarged

(A b E: Abaxial Epidermis; Ads.Adaxial side;

A d G: Abaxial Groove; La: Lamina; LV: Lateral vein; MR:Midrib; PM: Palisade Mesophyll; Ph: Phloem; SM: Spongy Mesophyll; X: Xylem).

(1996). The percentage inhibition of DPPH radical by the sample was calculated using the following formula

Inhibition % =

(absorbance of control (A517) – absorbance of sample (A517) * 100

Absorbance control (A517)

Principle

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radical with purple colour (absorbed at 517nm). If free

radicals have been scavenged, DPPH will degenerate to yellow colour. This assay uses this character to show free radical scavenging activity.

Procedure:

Preparation of sample: The extracts were dissolved in methanol in the concentration of 1mg/ml which was then used to determine its antioxidant activity.

Optical density of control – 0.801

IC₅₀ Calculation

Table 1: Preliminary phytochemical tests done on whole plant of *Enicostemma littorale* Using different solvent extracts

Test	Ethyl acetate	Methanol	Petroleum ether	Chloroform
Carbohydrate test	-	-	+	-
Tannins test	±	+	-	-
Saponin test	+	-	-	-
Flavonoid test	+	+	+	+
Alkaloid test	-	+	-	+
Anthocyanin and Betacyanin test	+	+	+	-
Quinones	+	+	+	-
Antraquinone	-	-	-	-
Glycosides test	-	+	-	-
Cardiac glycoside	+	-	+	-
Terpenoids test	+	-	+	-
Triterpenoids	-	-	-	+
Phenols	-	+	-	+
Coumarins	+	+	+	-
Acids	-	-	-	-
Protein and amino acid test	-	-	+	-

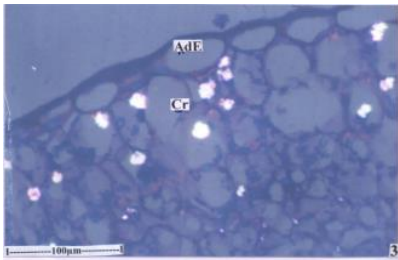


Fig.9: T.S. of Lamina showing crystal distriptions.

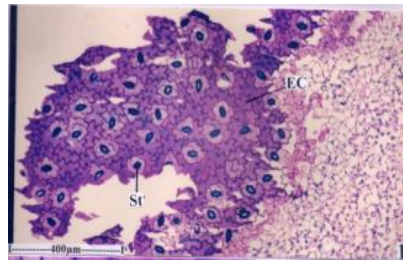


Fig.10: Para dermal sections of the Epidermis

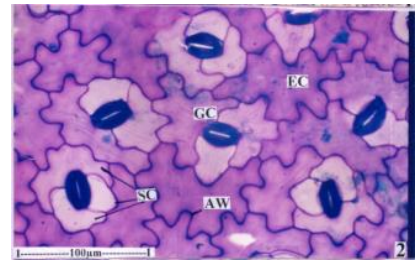


Fig.11a: stomata in surface view

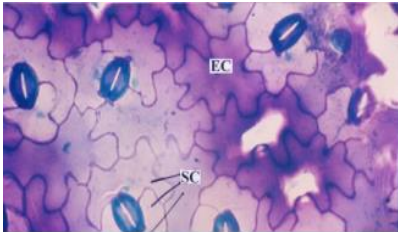


Fig.11b: Enlarged stomata in surface view

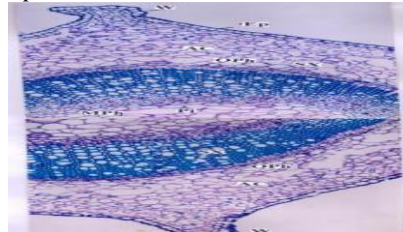


Fig.12: T.S of young stem

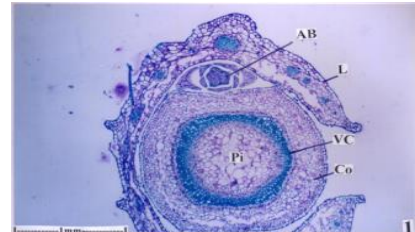


Fig.13: Two wings enlarged

All sections as seen under polarized light. (AdE.Adaxial Epidermis; Ads: Adaxial side; Cr.crystal; PM:Palisade Mesophyll) AW: Antielinal walls; EC.Epidermal cells; GC: Guard cells; SC: subsidiary cells; st: stomata, AC.Aerenchymatous cortex; Ep: Epidermis; Mph: Medullary phloem; Pi.Pith; Oph: Outer phloem; Sx: Secondary xylem; W: Wings

$$IC_{50} = \frac{\text{concentration of sample} \times 50}{\text{Final inhibition \%}} \mu\text{g/mg}$$

IC₅₀ value:

Table 2: Phytochemical Constituents of *E.littorale*:(Titrometric Method):

Phytochemicals	Percentage (%)
Alkaloids	0.02%
Flavones.	0.35%
Terpenoids	0.04%
Triterpenoids.	Trace
Total Phenolic acids	5.83%
Lignins	0.28%
Saponins	Nil
Macromolecules	
Protein	10.62%
Carbohydrates	5.83%
Lipids	0.16%
Fibre Content	0.12%
Ash value (Dry weight basis)	1.92%

Methanol = 92.71 µg/mg

Ethyl acetate=251.88 µg/mg

Petroleum ether=100.64 µg/mg

RESULTS AND DISCUSSION

Results of phytochemical investigation of *E.axillare* showed the presence of tannins, flavanoid, alkaloid, betacyanin, quinone, glycosides, and phenol (T.Clarina et al., 2012). According to ethanobotanical claim, this plant is used in typhoid fever, dropsy, malaria and skin diseases(Sharada L Deore 2008). As it is already reported that this plant contains phenolic and terpenoid compounds, our study focused to evaluate its anti-microbial activity possessed by this plant. The crude extracts of *E.littorale* was used to assess its anti-microbial activity against Gram

positive ,Gram negative bacteria and some fungal strains through the above mentioned assays. All the phytochemical tests showed the presence of tannins, flavanoids, alkaloids, betacyanin, quinone, glycosides, and phenols in methanolic crude extract.

Interesting we observed that all the four extracts exhibited significant antimicrobial activity against all microorganisms used in the study. The percentage of inhibition of these phytochemical extracts is shown in table 1. The IC₅₀ value for Methanol, Ethyl acetate and Petroleum ether was evaluated to be 92.71 µg/m, 251.88 µg/mg and 100.64 µg/ml respectively. Similarly, the antioxidant properties determined by FRAP, DPPH method showed that the IC₅₀ value for Methanol, Ethyl acetate and Petroleum ether to be 92.71 µg/m, 251.88 µg/mg and 00.64 µg/mg respectively. From the results obtained, we found that the methanol and ethyl acetate extracts exhibited prominent antimicrobial activity against all the microorganisms as compare to petroleum ether and chloroform extracts.

CONCLUSION:

This is the first report of *Enicostemma littorale* showing a remarkable anti-microbial activity against a wide spectrum of microorganisms responsible for causing serious pathological conditions. This prominent antimicrobial activity of *E.littorale* may be due to presence of large amounts of tannins, phenolic acid, flavanoid, terpenoids and glycoside groups. Therefore, we suggest that *Enicostemma littorale* possessing significant anti-microbial activity could be a potential plant for treating various dreadful diseases like AIDS, chlorera, malaria,encephalitis and other viral fevers. Further research needs to be done in order to isolate and identify a wide range of individual constituents that are present in this potential plant.

Anatomical Features

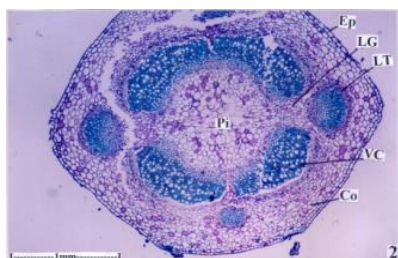


Fig.14: T.S of supra-nodal region of the stem.

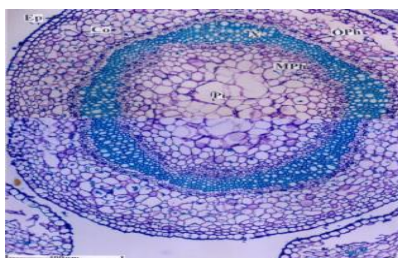


Fig.15: T.S of Nodal region showing unilacunar-single traces system

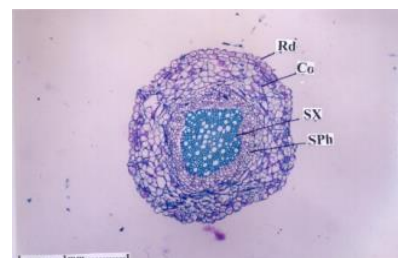


Fig. 16: T.S of internodal region of old stem

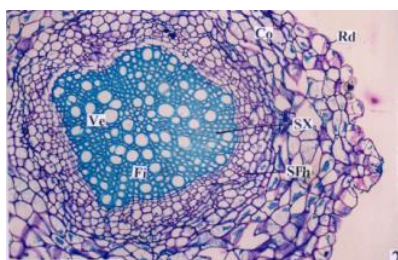


Fig.17: T.S of thin Root

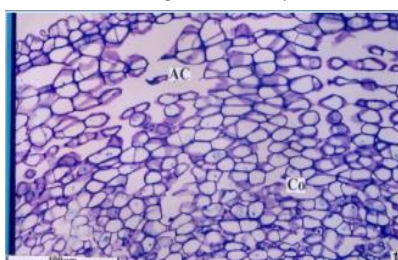


Fig.18: T.S of thin Root enlarged

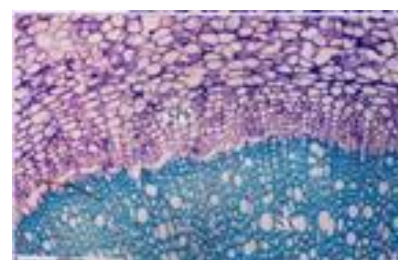


Fig.19: Thick-Root: cortical zone

(Co: Cortex; Ep:Epidermis; Mph. Medullary phloem; Oph: Outer phloem; pi.pith X: Xylem).(AB:Axillary bud; Co:Cortex; Ep:Epidermis; L:Leaf sheath; LG:Leaf-gap; LT:Leaf-trace; Pi.Pith; VC:Vascular cylinder). AC: Aerenchymatous cortex; Co: Cortex-inner zone; Ep; Epidermis; Sph: secondary phloem; SX: Secondary xylem

Leaf: The mature leaves have prominent mid rib with a flat lamina. The young leaves are folded along the midrib into a V-shaped outline. The mature leaf has a plano-convex midrib with a flat adaxial side and a broadly conical abaxial part. It is 600 µm wide. The midrib consists of a distinct epidermal layer of small squarish cells with a prominent cuticle. The ground tissue of the midrib is homogenous and parenchymatous. The cells are thin-walled, angular, and compact.

The vascular strand of the midrib is single, triangular in outline, and bicollateral. The vascular strand comprises several, parallel, compact files of 3-5 xylem elements in each file; the xylem cells are thick-walled, angular, and wide. The xylem elements are up to 20 µm wide. Phloem occurs in small, discrete units both outer and inner portions of the xylem (Fig.6). Lateral vein is fairly prominent with a broad abaxial part and a flat adaxial part. It is 380 µm thick. The vascular strand of the lateral vein is single, small, and bicollateral.

Lamina: The lamina is uniform in thickness and the surfaces are smooth and even. The mesophyll tissue is less distinct in the differentiation of the palisade and spongy mesophyll. The adaxial and abaxial epidermal layers are similar in shape and size. The cells are wide and cylindrical with a prominent cuticle. Stomata occur on both surfaces of the lamina. The epidermal cells are up to 30 µm thick. The mesophyll tissue consists of an adaxial zone of two layers

wide, short, cylindrical cells and a middle zone of large, much-lobed spongy parenchymatous cells which are interlinked with each other, forming wide air-chambers. Towards the lamina part, the cells assume a vertically oblong, cylindrical shape, forming loosely arranged vertical filaments with intervening air-chambers. The vascular strands of the lateral veinlets are small, centrally located, circular, and collateral.

Crystals: Calcium oxalate crystals of druses or sphaerocrystals are widespread in the leaf-mesophyll tissue. The druses are solitary in each cell and are mostly restricted to the palisade zone. The druses are up to 15 µm in diameter.

Epidermal Cells And Stomatal Morphology: The epidermal cells, as seen in surface view of the paradermal sections, are wide with highly waxy anticlinal 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, sheathing the guard cells. The guard cells are elliptical, measuring 30 µm long and 20 µm wide. The stomatal pores are narrow and slit-like.

Stem: Young internode, the young stem along the internode is four-angled with short stumpy wings along the four corners. The stem is 2.5 mm thick. It consists of a thin and distinct epidermal layer of thin-walled squarish cells

Table 3: Anti-Oxidant Activity

Sample	Optical density						% of Inhibition					
	5min	10'	15'	20'	25'	30'	5min	10'	15'	20'	25'	30'
Methanol	0.388	0.381	0.378	0.376	0.375	0.373	51.56	52.43	52.80	53.05	53.189	53.93
Ethyl-acetate	0.98	0.98	0.97	0.97	0.96	0.96	-22.34	-22.34	-21.09	-21.09	-19.85	-19.85
Petroleum Ether	0.407	0.406	0.406	0.405	0.404	0.403	49.18	49.31	49.31	49.43	49.56	49.68

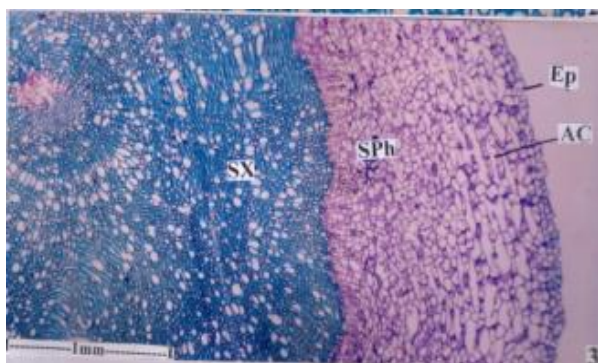


Fig.20. Thick-Root Secondary phloem, Secondary xylem.

(AC: Aerenchymatous cortex; Co: Cortex-inner zone; Ep; Epidermis; Sph: secondary phloem; SX: Secondary xylem).

with thin smooth cuticle. 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.

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 fibres. The xylem is amphiphloic siphous 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 distinctegushing rate. The Node exhibits unilaculear –single trace structure. These are a single leaf-gap with single trace.

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 fibres. The vessels are up to 40µm wide. Ensheathing the xylem

cylinder is fairly a wide phloem zone which includes short, radial lines of cells and small groups of sieve-elements.

Thick –Root: 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 fiberes are thick walled with liquefied walls.

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