Research Article

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PHARMACOGNOSTIC CHARACTERIZATION OF *ENICOSTEMMA LITTORALE* BLUME

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ABSTRACT

Enicostemma littorale Blume, Syn. *Enicostemma axillare* (Lam.) Raynal. is a well-known plant used as drug in Ayurved, Unani-Tibbi, Siddha, Allopathy, Homeopathy, Naturopathy and Home Remedies. The present communication deals with the pharmacognostic evaluation on the different parts of *Enicostemma littorale* Blume (*Enicostemma littorale*) Family-Gentianaceae. Macroscopic and microscopic examinations of the different organ, observations and differential microchemical test have been carried out for the authentication of the samples. Physiochemical values such as the moisture contents, percentage of total ash, acid insoluble ash, acid soluble ash, water soluble ash, extractive values as petroleum ether-soluble extractives, ethanol-soluble extractives, methanol-soluble extractives and water-soluble extractives were calculated as well as color reactions of powder and extract with different chemicals were performed to observe fluorescence analysis. The extracts were subjected to qualitative screening test for various constituents. This revealed the presence protein, glycosides, alkaloids, tannins and phenolic compound, steroid reducing sugars and saponin glycosides. These observations will help in the Pharmacognostical identification and standardization of the drug in the crude form and also to differentiate the drug from its adulteration.

Keywords: *Enicostemma littorale*, Gentianaceae, pharmacognosy, Ayurveda, Unani-Tibbi, Homeopathy, Naturopathy.

INTRODUCTION

Botanical Name : *Enicostemma littorale* Blume
Family : Gentianaceae
Sanskrit Name : Nagajihva, Mamajjakah, Nahi
Marathi Name : Nay, Kadvi-nayi, Mamijwa
Hindi Name : Kadvinayi, Chotachirayita
English Name : White head

Figure1: Habit of *Enicostemma littorale* Blume

Enicostemma littorale is an erect, perennial herb, 5–30 cm tall, simple or branched at the base. Stem is cylindric, glabrous with a decurrent ridge below each leaf. Leaves are sessile sometimes narrowed into a petiole-like base, longer than the internodes; lamina (5.0-8.0×0.3-1.0)cm, linear to lanceolate or narrowly oblong, entire, obtuse and mucronate at the apex, somewhat narrowing towards the base, 3-nerved from the base, glabrous. Inflorescence in many flowered auxiliary clusters, numerous in the axils of each pair of leaves. Flowers are white with green lines, drying yellowish, sessile or subsessile; 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 subcircular, 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 point¹.

Distribution

Distribution is throughout India in areas up to 450m. elevation, common coastal areas among grasses. When growing in good soil, away from the sea this plant attains larger size with large broad leaves than the one near the sea.

Medicinal Properties and Uses

Plant is pungent and very bitter, anthelmintic and reduces fever. Plant is very bitter and is used in Madras as stomachic. It is also a tonic and laxative. The plant is crushed and applied locally in snack-bite. It is also used as stomachic and Vata². The plant is also acrid, thermogenic, digestive, carminative, laxative, anti-inflammatory, liertonic, urinary astringent, depurative, repulsive and anti-periodic and useful in dyspepsia, colic, flatulence, abdominal ulcers, hernia, constipation, dropsy, swellings, vitiated conditions of Kapha and Vata, hepatothy, glycosuria, leprosy, skin diseases, prutitus, intermittent, fever and malaise. Powder is given with honey as a blood purifier and in dropsy. The leaves are used in diabetes³⁻⁹.

According to the World Health Organization (WHO), herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries¹⁰. The work cited up to the articles can be focused on phytochemicals and many pharmacological activities. Whereas animal research has thrown light on anti-inflammatory, analgesic,

anti-malarial, hepatoprotective, hepatomodulatory, anti-hyperglycemic, hypoglycemic, antioxidant, antitumor, hypolipidemic and antihelminthic activities of phytochemicals. Swertiamarin should particularly be screened for large scale clinical trials¹¹⁻¹⁵. The pharmacognostical characters reported in this work may serve as a valuable source of information and provide suitable diagnostic tool for the standardization as well as identification of adulterants in future investigation or application. It will also be immense useful to carry out further research and re-validation of its use.

MATERIALS AND METHODS

The fresh, healthy, mature plants and its parts were collected from farm of Dhule which was away from pollution. The plant materials were identified using the Flora of Dhule and Nandurbar District¹⁶, India at Post-graduate Department of Botany, SSVS Sansthas, L.K. Dr.P.R.Ghogrey Science College, Deopur, Dhule-(M.S) India and herbarium was also preserved. The voucher specimen number is SCD/H.No.-145. The leaves stem and roots were washed and used for the present study. The macroscopic observations of the mature plants, leaves, inflorescences and other parts were noted. For microscopic studies, some plant material preserved in 70% alcohol. Leaf epidermal studies were carried out on fresh specimens. Peels were removed mechanically using some chemicals. They were stained in 1% safranin mounted in glycerin and made semi-permanent by ringing with DPX solution. Stomatal index (SI), stomatal frequency, vein-islet, vein termination number and palisade ratio were calculated as defined by Salisbury^{17,18}, viz.,

$$SI = \frac{S}{E + S} \times 100$$

Where 'S' = number of stomata per unit area and 'E' = number of epidermal cells in the same area. Stomatal index (SI), stomatal frequency, vein-islet, vein termination number and palisade ratio have been calculated of an average of 10 readings.

Transections of leaf, petiole, stem and root were taken by free hand. Fresh and preserved materials were used. Sections were stained in safranin (1 %), light green (1 %) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine. Microphotographs of leaf, petiole, stem and root sections were taken by using DIGI-EYE High resolution Cameras affixed to microscope Olympos CH 201. For study of vessels, fragments of plant organs, especially stem at nodal region and root, were macerated using nitric acid (10%) and potassium dichromate (10%) solution in equal proportions. The vessel elements were stained with aqueous safranin (1%), dehydrated and mounted in DPX. Some vessel members were also examined in glycerine. The line and cellular sketches of the figures were drawn using a Camera Lucida. The range of length and diameter of vessel elements was determined by the measurement of 20 – 25 vessel elements. The fresh, healthy plants were rooted out and washed with water. They were separated as root, stem and leaves and shade dried. The dried plant materials were pulverized into fine powder using a grinder (mixer). About 1 kg of powdered material was

prepared. After that powder were kept into air tight bags. Physiochemical values such as the percentage of total ash, acid insoluble ash, acid soluble ash, extractive values as petroleum ether-soluble extractives, ethanol-soluble extractives, methanol-soluble extractive, and water-soluble extractives were calculated according to the methods described in the Indian pharmacopoeia^{19,20}. Phytochemical studies such as qualitative examination were done on the dried powdered material for this about 5 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; ethanol, methanol, diethyl ether, and chloroform²¹. The respected solvents were evaporated (at 40°C) with the help of heating mantle. Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods²²⁻²⁵. The positive tests were noted as present (+++) appreciable amount, (++) moderate amount, (+) trace amount and (-) completely absent.

RESULTS AND DISCUSSIONS

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance^{26,27}. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. The result of this study as follows:

Microscopic Characters

Dermal Characters

Epidermal cells of adaxial epidermis are mostly with anisocytic stomata. Occasionally anomocytic stomata are also present; Epidermal cell with wavy margins. Cells are elongated at the coastal region. Epidermal cells of the abaxial epidermis are mostly elongated and straight walled at the coastal region. Most dominant type of stomata on abaxial surface is anisocytic (Figure 2: A). Occasionally diacytic and anomocytic stomatas are also seen. Epidermal cell with spongy wavy walls (Figure 2: A, B). Epidermis is also shows non-glandular, multicellular trichomes having a single basal cell.

T. S. of leaf of *E. littorale* (Figure 2: C)

The epidermis unilayered on upper and lower epidermis with thick cuticle, cuticle striated hence appearing lobed in cut section at the midrib, there is a single layered hypodermis at an abaxial side. Mesophyll is made up of compact chlorenchyma. A vascular bundle is present in the cortex of midrib region. Stele is haplostele. Xylem element is facing upwards. The mesophyll of lamina is differentiated into two layered palisade towards the abaxial epidermis and 3-5 layered spongy chlorenchyma. Epidermis is studded with nonglandular epidermal

trichomes. The trichomes are multicellular having a single basal cell.

T. S. of Petiole of *E. littorale* (Figure 2: D)

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 parenchymatous 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 stem of *E. littorale* (Figure 2: E)

The transverse section of the stem shows hexangular in outline, angles extending into a multicellular wing like structure. Epidermis with thick cuticle, unilayered small cells compactly arranged; following the epidermis is a thick cortex 6-15 layered which is parenchymatous with scattered chlorenchymatus cell. Cortex is in wing 2-5 layers of compact Parenchyma. 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. Sphaeraphides are occasionally seen in pith cells. Central pith is present.

T. S. of root of *E. littorale* Blume (Figure 2: F)

The transverse section shows circular in outline. Epidermis single layered, cortex 7-10 layered parenchymatous, stele consisting of star shaped and xylem is surrounded by phloem zone. Vessels are embedded with thick-walled xylem sclerenchyma. Pith obscure.

Quantitative microscopy

The leaf microscopic characters as stomatal frequency, stomatal index and palisade ratio were determined (Table 1).

Vessel Elements from macerated stem

A survey of about 40-50 vessel elements were carried out to observe various characters of vessels elements viz., size, wall thickening, shape, tail, and characters of perforation plate like number, orientation and shape. The ranges of the length and width of vessel element was determined by the measurement of 20-25 vessel elements and were classified as per Radford²⁸, which is reproduced here for perusal.

S. No.	Class	Category	Size range
1	A	Extremely short	less than 175μ
2	B	Very short	175 to 250μ
3	C	Moderately short	251 to 350μ
4	D	Medium size	351 to 800μ
5	E	Moderately long	801 to 1100μ
6	F	Very long	1101 to 1900μ
7	G	Extremely long	Over 1901μ

Results of ranges of the length and width of vessel element have been presented in Table 2. The observations show that class-D type (Range-381-763) found large quantity i.e. 59.37% while Class-A,B and E found less amount (Figure 3).

Qualitative Phytochemical screening

The extracts and powder drug were subjected to phytochemical screening for the presences of type of phyto-constituents. The extracts and powder were found to contain protein, glycosides, alkaloids, tannins and phenolic compound, steroid reducing sugars and saponin glycosides (Table 3).

Fluorescence analysis

The fluorescence analysis is sensitive and enables the precise and accurate determination over a satisfactory concentration. The fluorescence color is specific for each compound. A non fluorescent compound may fluorescence if mixed with impurities that are fluorescent. The color of the extract from organic and inorganic solvents was observed under ordinary light. (Table 4)

Physical constants

Results of moisture contents, ash analysis and extractive values of the dried leaves, stem and root have been presented in Table 5.

CONCLUSION

The morphological, microscopic and physicochemical characters reported in this work can serve as a valuable source of information for botanical study, quality control and provide suitable diagnostic tool for the standardization as well as identification of adulterants from the drug. It will also be immensely useful to carry out further research and revalidation of its use. The microscopic features may help in laying down micro-morphological standards for authentication of the original drug. In the present investigation researcher observed the high extractive values in ethanol compared to other solvents used. The fluorescence color was specific for each compound. The high ash contents indicate the large amount inorganic salts or extraneous matter. It may be used as enhancement of information for Ayurvedic pharmacopoeia.

Figure 2: Anatomical features of *Enicostemma littorale* Blume

A: adaxial surface of leaf showing diacytic stomata and epidermal cells; B: abaxial surface of leaf showing diacytic stomata; C: T.S. of leaf; D: T.S. of petiole; E: T.S. of stem; F: T.S. of root

Figure 3: Vessel elements (VE) of *Enicostemma littorale* Blume

A: Cylindrical VE with spiral thickening; B, H: Linear VE with irregular pitted thickening; C: Spiral VE with irregular pitted thickening; D: Wedge shaped VE with irregular pitted thickening; E: Top Shaped VE with irregular pitted thickening; F: Fusiform VE with irregular pitted thickening; G: Oblong VE with irregular pitted thickening; I: VE with long pointed tail; J: VE with long blunt tail; K: VE with Short blunt tail; L: VE with transverse end wall; M: VE with oblique end wall

Table 1: Quantitative microscopy of the leaves of *E. littorale*

S. No.	Parameter	Range	Mean
1	Stomatal frequency (upper surface)	145-185	165
2	Stomatal frequency (lower surface)	200-250	225
3	Stomatal index (upper surface)	14.5-18.5	16.5
4	Stomatal index (lower surface)	20.0-25.0	22.5
5	Veinlet number	9.1-13.1	11.1
6	Vein termination	8.5-13.1	10.8
7	Palisade ratio	1.13-1.18	1.16

Table 2: Classification and Relative Frequency of Vessels Found In *E. littorale*

S.No.	Class	Range (µm)	Percentage (%)
1	Class -A	116-166	9.00
2	Class -B	182-249	9.00
3	Class -C	256-336	13.63
4	Class -D	381-763	59.37
5	Class -E	830-962	9.00

Table 3: Qualitative Phytochemical of *E. littorale*

S.No.	Test	Leaves Ex.in		Stem Ex. in		Root Ex. in	
		Enol	Mnol	Enol	Mnol	Enol	Mnol
1	Protein	++	++	+++	+++	++	++
2	Glycosides	-	-	+	+	-	-
3	Alkaloids	++	++	++	++	++	++
4	Tannins and Phenolic compound	++	++	++	++	+	+
5	Steroid	+	+	++	++	++	++
6	Saponin glycosides	++	+	+	+	+	+
7	Reducing sugar	+++	+++	++	+++	++	+++
8	Fat	++	++	++	++	++	++

(+++ appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent; Enol= Ethanol; Mnol=Methanol; Ext=Extract

Table 4: Fluorescence Analysis of *E. littorale*

S. No.	Test	Leaf	Stem	Root
1	Powder	Dark green	Light green	Light yellow
2	Pd+Iodine	Red	Reddish brown	Red
3	Pd+5%FeCl ₃	Orange greenish	Dark orange	Light orange
4	Pd+1N NaOH	Dark green	Light green	Light green
5	Pd+Acetic acid	Greenish yellow	Greenish yellow	Light yellow
6	Ext+A.A.+50%H ₂ SO ₄	Bluish green	Light green	Yellowish green
7	Pd+50%H ₂ SO ₄	Light green	Light yellow	Yellowish brown
8	Pd+50%Conc. HCl	Dark yellow	Light yellow	Light yellow
9	Pd+Ammonia	Dark green	Light green	Light green
10	Ext+4%NaOH+1%CuSO ₄	Dark green	Green	Green
11	Ext+40%NaOH+1%Lead acetate	Dark green	Dark green	Greenish yellow
12	Pd+50%HNO ₃ +Picric acid	Dark green	Light green	Light green
13	Pd+Satu.Picric acid	Greenish yellow	Light green	Light green

Abbreviations: Pd= Powder A.A=Acetic Acid, Ext=Extract

Table 5: Physical Evaluation (% w/w) of *E. littorale*

Parameter	Value (% w/w)			
	Leaves		Stem	Root
Moisture content	6.10		6.50	6.30
Extractive values				
a) Petroleum Ether	5.50		4.50	3.20
b) Ethanol	7.85		8.50	7.70
c) Methanol	6.55		6.70	6.20
d) Water	8.65		9.98	8.85
Ash values				
a) Total ash	11.50		10.45	10.10
b) Acid insoluble Ash	6.40		6.20	6.10
c) Acid soluble Ash	5.10		4.25	4.00
d) Water soluble Ash	4.30		3.25	5.52

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REFERENCES

1. Gupta RS, Singh D. Hepatomodulatory role of *Enicostemma littorale* Blume against oxidative stress induced liver injury in rats. *Afr J Agri Res* 2007;2:131-138.
2. Agharkar SP. Medicinal Plants of Bombay Presidency, Scientific Publication, Jodhpur, India; 1991.
3. Anonymous, Indian Pharmacopoeia. vol. 2.3rd Ed. Govt. of India, Ministry of Health, Controller of Publications, New Delhi, India; 1986.
4. Bhandari CR. Vanaushadhi Chandrodya (Hindi), Bhanpura, Indore, Vol. II; 1970. p.549.
5. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants, PID, CSIR, New Delhi; 1956.
6. Matthew KM. The flora of Tamil Nadu Carnatic, The Rapinet Herbarium St. Joseph's College, Tiruchirapalli, India Vol. II; 1982. p.973.
7. Theodore Cooke, Flora of Presidency of Bombay, Botanical Survey of India, Calcutta; 1967.
8. Vaidya BG. Some Controversial drugs of Indian Medicine, V.J. Res. Indian Med 1965; 7(2):45-48.
9. Vyas DS, Sharma VN and Khanna NK. Preliminary studies on anti-diabetic properties of *E. littorale*, *Jour. RS. Ind. Med. Yoga and Homeo* 1979; 14(3):63-65.
10. Sharma KA, Kumar R, Mishra A, Gupta R. Problems associated with clinical trials of Ayurvedic medicines. *Rev. Bras. Farmacogn. Braz. J. Pharmacogn* 2010;20(2):276-281. <http://dx.doi.org/10.1590/S0102-695X2010000200023>
11. Rajamani S, Thirunavukkarasu T, Munisami H, Ranganathan B, Ernest D. Pharmacognosy of *Enicostemma littorale*: A review, *Asian Pac J Trop Biomed* 2013; 3(1): 79-84. [http://dx.doi.org/10.1016/S2221-1691\(13\)60028-3](http://dx.doi.org/10.1016/S2221-1691(13)60028-3)
12. Abirami P and Gomathinayagam M. A Review on *Enicostemma littorale*, *Pharmacologyonline Newsletter* 2011; 1: 75-83.
13. Sekar BH, Deepika B, Manjubharathi N, Alamelu M, Chandran M. Phytochemical screening of different parts of plant *Enicostemma axillare* (Family – Gentianaceae), *RJPBCS* 2011; 2(3):971-974.
14. Sharada LD, Khadabadi SS, Bhangure L and Ghorpade DS. *In-vitro* antimicrobial studies on *Enicostemma axillare* (Lam.) Leaves, *Natural Product Radiance* 2008; 7(5): 409-412.
15. Sanmugarajah V, Thabrew I, Sivapalan SR. Phyto, Physicochemical Standardization of Medicinal Plant *Enicostemma littorale*, Blume, *IOSR Journal of Pharmacy* 2013;3(2): 52-58. <http://dx.doi.org/10.9790/3013-32205258>
16. Patil DA. Flora of Dhule and Nandurbar District, Bishen Singh Mahendrapal Singh, Dehra Dun (India); 2003.
17. Salisbury EJ. On the cases and ecological significance of stomatal frequency with special references to the wood land flora *Phil Trans. Roy, Soc. London* 1927; 216B: 1-65.
18. Salisbury EJ. The interpretation of soil climate and the use of stomatal frequency index of water reduction to plant, *Beih. Bot. Zeni-ralb* 1932; 49: 408-420.
19. Anonymous, Indian Pharmacopoeia. vol. 2.3rd Ed. Govt. of India, Ministry of Health, Controller of Publications, New Delhi, India; 1966.
20. Anonymous, Indian Pharmacopoeia. vol. 2.3rd Ed. Govt. of India, Ministry of Health, Controller of Publications, New Delhi, India; 1985. p. A74 - A75.
21. Vogel AI. Elementary practical organic chemistry (second edition), Orient Longman Limited; 1988. p.45-168.
22. Harborne JB. Phytochemical Methods, Chapman Hall, London; 1988. p. 100-101.
23. Trease E and Evans WC. Pharmacognosy, Billiare Tindall, London; 1987.
24. Ajaiyeoba EO. Phytochemical and antimicrobial studies of *G gynandra* and *B coriacea* extracts, *Afr. J. Biomed. Res* 2000;3(3):161-165.
25. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal Plant. *African J. Biotechnology* 2005; 4(7): 685-688.
26. Thomas S, Patil DA, Patil AG, Chandra Naresh. Pharmacognostic evaluation and physicochemical Analysis of *Averrhoa carambola* L. *Fruit Journal of Herbal Medicine and Toxicology* 2009; 2(2): 51-54.
27. Kuamari Usha J, Navas M, Mathew D, Rajasekharan S. Pharmacognostic studies on *Acrotrema noctianum* Wight – A promising ethnomedicinal plant. *Indian Journal of Traditional Knowledge* 2009; 8(3): 334-337.
28. Radford AE, Dickson WC, Massey JR and Bell CR. Vascular Plant systematic, Harpere and Row, New York; 1974.

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