

**QUANTITATIVE ESTIMATION OF PHYSICO-CHEMICAL AND  
PHYTOCHEMICAL CONSTITUENTS OF *ENICOSTEMMA*  
*LITTORALE* BLUME**

**Mayuri C. Rathod<sup>1</sup> and D. A. Dhale<sup>2\*</sup>**

<sup>1</sup>Department of Biotechnology, Veer Narmad South Gujarat University,  
Surat- 395007, Gujarat, India.

<sup>2</sup>PG-Department of Botany, SSVPS's, L.K. Dr.P.R. Ghogrey Science College,  
Dhule-424005, Maharashtra, India.

Article Received on  
22 Jan 2015,

Revised on 15 Feb 2015,  
Accepted on 09 March 2015,

**\*Correspondence for  
Author**

**Dr. Datta Dhale**

PG-Department of Botany,  
SSVPS's, L.K.Dr.P.R.  
Ghogrey Science College,  
Dhule-424005,  
Maharashtra, India.

**ABSTRACT**

*Enicostemma littorale* Blume is a well-known plant drug in Ayurved, Unani-Tibbi, Siddha, Allopathy, Homeopathy, Naturopathy and Home Remedies. The study includes seasonal variations (winter, summer and monsoon) and quantitative estimation of physicochemical parameters such as relative water content and phytochemical analysis includes total alkaloids, phenols, lipid, Chlorophyll-a, Chlorophyll-b, Carotenoid, Carbohydrates, amino acid, protein, etc. The higher carbohydrates content was noted in root and leaves. The higher phenol, protein and total amino acid content was noted in leaves, whereas, lipid content remained highest in stem of the studied plant. These observations will help in the Pharmacognostical identification, proper

collection timing and standardization of the drug in the crude form and also to distinguish the drug from its adulteration.

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

**INTRODUCTION**

Plants have been associated with the human health from time immemorial and they are the important source of medicines since human civilization (Kajaria *et al.*, 2011). The history of plants being used for medicinal purpose is probably as old as the history of mankind. The use of medicinal plants in the industrialized societies has been traced to the extraction and

development of several drugs from this plant as well as from traditionally used folk medicine (Shrikumar and Ravi, 2007).

*E. littorale* commonly known as, *Indian gentian*, in English, *Mamajaka* in Sanskrit. It is found in open, sandy places among sparse grasses. It is a flower producing glabrous perennial herb which grows up to a height of 1.5ft is found throughout India (Nadkarni, 2002; Kirtikar and Basu, 2003; Abirami and Gomathinayagam, 2011). It is pungent and very bitter, anthelmintic, cures 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 cures fever and also an anthelmintic. It is also used as stomachic and Vata. The plant is also acrid, thermogenic, digestive, carminative, stomachic, laxative, anti-inflammatory, liertonic, urinary astringent, depurative, revulsive and anti-periodic and useful in dyspepsia, colic, flatulence, helminthiasis, abdominal ulcers, hernia, constipation, dropsy, swellings, vitiated conditions of Kapha and Vata hepathopathy, 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 diabetes (Annonymous, 1986; Bhandari,1946; Chopra, 1956; Mattew, 1982; Cooke,1967; Vaidya, 1965; Vyas *et al.*, 1979). The plant possesses valuable medicinal properties but most of the advantages are still confined to tribal areas because of raw knowledge and absence of proper scientific standardization. For the useful application of the plant parts in modern medicine, physico-chemical and phytochemical standardization is very important (Saxena, *et al.*, 2012), so that the medical benefits of the plant may be used properly and scientifically and reach to the larger populations of the world. Some of the previous researchers work on preliminary finding but not go through the quantitative estimation (Vinotha Sanmugarajah, *et al.*, 2013; Rajamani Saranya *et al.*, 2013; Rathod and Dhale, 2014). Therefore, in the present research work was to evaluate the physicochemical parameters and phytochemical constituents of the whole plant of *E. littorale*.

## MATERIALS AND METHODS

The plant material of *Enicostemma littorale* Blume Syn. *E. axillare* (Lam.)Raynal. Family (Gentianaceae) are collected from the different part of Dhule Districts during different seasons *viz.* summer (March), Monsoon (July) and winter (November). The leaves, stem and roots were collected and kept separately. Further, the time of collection, source and the parts of the taxa under study are listed in Table 1.

The different organs of the plant like leaves, Stems, roots and Fruits were collected and shade dried. The dried material was finely powdered sieved through muscline cloth and stored for chemical analysis.

**Table1: Details of Source, Time of Collection, Type of Sample and Plant Parts Obtained for the Study.**

S.N	Taxon	Type of sample	Source	Time of collection	Plant part used	Part medicinally used
1.	<i>Enicostemma littorale</i> Blume	Fresh Leaves stems and roots	Nimgul, Dahivel District Dhule	July, November, March	Leaves, stems, and roots	Whole plant leaves, stems, roots

#### i) Determination of Relative Water Content (RWC) of Leaf

Relative water content was estimated as per the method suggested by Jayaraman (1981). Leaf sample was weighed. This weight is an initial weight or fresh weight. The weighed leaf bits were kept in 20ml of distilled water in Petridis (9cm) and allowed to be soaked in water for three hours. After three hours they were removed from water, their surface blotted and weighed again. This was referred to as the turgid weight. After taking turgid weight, the leaf bits were dried in an oven at 80<sup>0</sup>C for 24 hours and then dry weight was taken.

Formula for calculation,

$$\text{RWC Percentage} = \frac{\text{Initial weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

$$\text{RWC} = \text{Relative water content.}$$

#### ii) Assay for total alkaloids

Quantitative estimations of alkaloids were carried out following the methods of Sairam and Khanna, (1971).

Each sample was ground to fine powder. To each one gram powder, 0.75 ml 25% ammonium hydroxide, 1 ml 95% ethyl alcohol and 2 ml ethyl ether were added. The material was allowed to stand for 12 hours and dried.

The dried material was extracted with chloroform for 24hours in a soxhlet apparatus, and the extract obtained was evaporated to dryness, and the residue was redissolved in chloroform. This solution was allowed to evaporate under infrared heat and the residue was mixed with 2.5ml 0.1N ethanolic (90%) HCl. The extract, thus obtained was centrifuged to take

supernatant and discard the pellet. The solution was evaporated and the total alkaloids were weighed after drying at 100<sup>0</sup>c.

### iii) Total phenols

Estimation of total extractable phenol compound was estimated by folin method of Swain and Hillis, (1959).

Reagent: Folin-Phenol

Standards: Glacial acetic acid, tannic acid

Procedures: Phenolic compounds were extracted by grinding 50mg (dry weight) sample of tissue using a chilled pestle and mortar with an aliquot of chilled 80% (v/v) ethanol. The homogenate was centrifuged at 13,000rpm for 15minutes. The supernatant was collected and residue pellet was extracted twice more as above. The supernatant of all three extractions was pooled and volume made up to 25ml with 80% ethanol.

A suitable aliquot of the ethanolic extract was diluted with distilled water to 8.5ml and after adding 0.5ml of folinphenol reagent. The content were mixed well, three minutes later, 1 ml of saturated sodium-carbonate solution (1 g/3ml) of distilled water was added and the mixture shaken thoroughly. Colour was allowed to develop for 60minutes and then read at 725nm in colorimeter standard curve was prepared using chlorogenic acid/ tannic/ gallic acid and all the concentrations were expressed in terms of mg/g of this compound.

### iv) Lipid (oil) in Leaf, Stem and Root

Agrawal, *et al.*, (1987) method was followed for the estimation of lipid. The material was dried for 12-17 hours at 60-70<sup>0</sup>C and ground to a coarse powder. 5g of weighed sample was taken in a cellulose thimble (the quantity of material would depend on its oil content.) the thimble was fixed in the soxhlet funnel and about 150-200ml. of petroleum ether was taken in the flat bottom flask (FBF). The funnel over the flask was fixed attached to the water switched off to let the apparatus cool (maintaining the water flow as such). Condenser and funnel were detached. Petroleum ether was evaporated in the FBF over hot plat at 80<sup>0</sup>C. when a small quantity (about 10ml) of ether was left in the flask, transferred it in weighed beaker (W<sub>1</sub>) of 50 or 100ml. rinsed the FBF twice small quantities of ether and transferred the washing in the beaker. The beaker was transferred in an oven at 70 ± 1<sup>0</sup>C till ether evaporated (presence of ether can be detected by its smell). The beaker was cooled in a desiccator and content. The oil percentage was calculated on the basis of the weight of plant material.

**v) Chlorophyll Determination in the Leaf**

Chlorophyll-a, chlorophyll-b and carotenoids were extracted from the freshly plucked third leaf from the top using 80% acetone. Optical densities were recorded at 510, 645 and 663nm. The amount of Chl.-A, Chl.-B and Carotenoid were calculated in terms of mg pigment/g of fresh leaves by using the following formula (Duxbbury and Yentsch, 1956; Maclachalam and Zalik, 1963).

Formula for Caculation:

1. Chl. a (mg/g fresh weight)

$$\frac{12.3 D_{663} - 0.86 D_{645}}{d \times 1000 \times W} \times V$$

2. Chl. b (mg/g fresh weight)

$$\frac{719.3 D_{645} - 3.6 D_{663}}{d \times 1000 \times W} \times V$$

3. Carotenoids (mg/g fresh weight)

$$\frac{7.6 D_{480} - 1.49 D_{510}}{d \times 1000 \times W} \times V$$

Where : 'V' is the volume of the chlorophyll solution.

'd' is the length (cm) of light path, and

'W' is the fresh weight (g) of leaves.

**vi) Carbohydrates in Leaf, Stem and Root**

Carbohydrates were estimated by methods suggested by McGready (1950) and Nelson (1941).

**Reagents**

- 1) Somogy's reagent

4 g : CuSO<sub>4</sub>, 5 H<sub>2</sub>O.

24 g : Anhydrous Na<sub>2</sub>CO<sub>3</sub>.

16 g : Na – K tartarate (Rochette Salt)

180 g : Anhydrous Na<sub>2</sub>SO<sub>4</sub>

- 2) Nelson arsenomolybdate reagent

24 g : (NH<sub>4</sub>)<sub>6</sub> MO<sub>7</sub> O<sub>24</sub>, 4H<sub>2</sub>O (Ammonium molybdate).

3 g : Na<sub>2</sub>SO<sub>4</sub>, 7H<sub>2</sub>O.

Both solutions were mixed and incubated at 37°C for 24 hours before use and stored in brown bottle.

3) Standard sugar solution was prepared by dissolving 10 mg of glucose in 100 ml distilled water.

### Procedure

2g of samples were crushed with 80% ethanol in mortar by adding acid free sand then filtered through whatman filter paper. The filtrate and residue were collected separately.

The alcoholic residue was taken in 250 ml in conical flask, 150 ml distilled water and 5ml conc. HCl was added to it. Hydrolysed for 30 minutes and cooled to room temperature. Na<sub>2</sub>CO<sub>3</sub> was added bit-by-bit until the extract became neutral (pH- 7). The extract was filtered. Residue was discarded. Total volume of filtrate was noted and this was served as a sample for starch. First filtrate was taken in 100 ml conical flask and condensed on water bath up to 2-3 minutes and cooled to room temperature. Lead acetate and potassium oxalate 2g each (1:1), were added in 15 ml of distilled water, added to the filtrate and then filtered after mixing. Residue was discarded and the total volume of filtrate was served as a sample for reducing sugar.

20ml of this filtrate was taken in 150ml conical flask, 2ml of conc. HCl was added to it and corked. It was then hydrolysed for 30minutes and cooled at room temperature. Na<sub>2</sub>CO<sub>3</sub> was added bit-by-bit until the extract became neutral (pH, 7). Then this extract was filtered and the residue was discarded. The final volume of the filtrate was measured. It was served as a sample for total sugar.

0.5ml of aliquot sample was taken in each test-tube and 1ml of somogy's reagent was added to it. All tubes were placed in boiling water bath for 30minutes, cooled the tubes to room temperature and 1ml of Arsenomolybdate reagent was added to it. The contents were mixed thoroughly. Then the contents were diluted to a total volume of 10 ml and its absorbance measured at 560nm in spectrophotometer.

### vii) Total Amino Acid in Leaf, Stem and Root

The estimation of amino acid was carried out by Krishnamoorthy *et al.*, (1989) methods.

**Reagent**

1) Alcoholic ninhydrin

100ml alcohol + 400mg ninhydrine

2) Glycine (std.)

10ml glycine + 100ml distilled water.

**Procedure**

500mg plant material was grounded in mortar and pastel with few drops of cold 80% ethanol. Then 2.5ml of distilled water and 25ml. of boiling 80% ethanol were added to it. The extract was centrifuged for 15minutes at 10,000 rpm. Supernatant was made to 30ml with distilled water.

1ml of sample was taken in a test-tube and alcoholic ninhydrin was added to it. Test-tube were kept at 60<sup>0</sup>C for 20minutes. The test-tube were cooled and read at 420nm in spectrophotometer. Glycine was used as standard.

**viii) Protein in Leaf, Stem and Root:**

The protein was quantitatively estimated by the method of Lowry *et al.*, (1951).

Reagents / Chemicals :

0.1 N NaOH, 2% Na<sub>2</sub>CO<sub>3</sub>, 0.5% CuSO<sub>4</sub>, 1% Na-K-tartrate, 5% Trichloro acetic acid/perchloric acid.

Lowry A - 2% Na<sub>2</sub>CO<sub>3</sub>, in 0.1 N NaOH.

Lowry B - 0.5% CuSO<sub>4</sub> in 1% Na-K-tartrate.

Lowry C - 98ml A and 2ml B.

Lowry D - Folin – phenol reagent

BSA - (Bovine Serum albumin) std.

10 mg/100 ml distilled water.

**Procedure**

1 to 2g plant material was homogenized with 80% ethanol. The extract was centrifuged and the supernatant was discarded. 5% Trichloroacetic acid (TCA) or perchloric acid (PCA) was added to residue and incubated at 80<sup>0</sup>C for 20minutes. The pallette was recentrifuged and the supernatant was discarded. 2% Na<sub>2</sub>CO<sub>3</sub> in 0.1N NaOH was added to the residue and incubated for an hour at 300C. again centrifuged and the residue was discarded. The final volume of supernatant was measured and it was used as a sample for protein.

1ml of aliquot of sample was taken and 5ml reagent C was added to it and mixed thoroughly. The sample was incubated for 10minutes and 1ml of reagent D was added to it. The colour intensity was read at 660nm using spectrophotometer. The protein concentration of an unknown sample was calculated using standard graph.

## 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 (Thomas, *et al.*, 2007; Usha *et al.*, 2009). 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.

### Relative Water Content of Leaves

The percentage of relative water content of leaves of *E. littorale* was higher in monsoon (88.03%) over that of winter (70.20%) and summer (70.47%). The percentage of relative water content of leaves were found to be in increasing order of winter < summer < monsoon (Table 2).

**Table 2: Relative Water Content of leaves of *E. littorale***

Season	Initial weight (g)	Turgid weight (g)	Dry weight (g)	Relative water Content (%)
Winter	0.250	0.330	0.0615	70.20
Summer	0.300	0.385	0.0494	70.47
Monsoon	0.350	0.390	0.0558	88.03

### Total Alkaloids

The estimation of total alkaloid content was carried out in different parts like leaves, stem and root during winter, summer and monsoon.

The total alkaloid content of leaves was ranging from 2.1% to 5.5%, to 11.81% in stem and 1.76% to 7.81% in root (Table 3). The percentage of total alkaloid content were in increasing order from leaves < root < stem.



**Table 3: Total Alkaloid contents of *E. littorale***

Plant part	(mg.g <sup>-1</sup> dry weight)	
	Season	Total Alkaloids in Chloroform (%)
Leaves	Winter	5.50
	Summer	6.16
	Monsoon	5.59
Stem	Winter	3.40
	Summer	9.70
	Monsoon	1.76
Root	Winter	2.10
	Summer	11.81
	Monsoon	7.81

**Total Carbohydrates**

Carbohydrates are of special importance because they are direct products of photosynthesis and are; therefore, the primary energy storage compound and the basic organic substances from which most other organic compounds found in plants are synthesized.

Leaves and stem harvested during the summer, monsoon and winter showed almost identical range of total sugar content (1.31-1.37mg/g dry wt.). Root was observed to be rich with total sugar content (1.59 mg/g dry wt.). The leaves were the poorest source of starch (0.223mg/g dry wt.) as compared to stem (0.270mg/g dry wt.) and root (0.290mg/g dry wt.).

The total carbohydrate content in leaves ranged from 0.127% to 0.175% highest being observed during monsoon (0.175%). Leaves harvested during the summer showed low level of total carbohydrates) 1.27mg/g dry wt.). The total carbohydrate concentration of stem was higher in monsoon (0.193%) over that of winter (0.154%) and summer (0.146%). The total carbohydrate concentration of root showed comparatively higher level (i.e. 0.144% to 0.214%).

The concentration of total carbohydrate were found to be in increasing order of leaves < stem < root (Table-4).

**Table 4: Seasonal variations of total carbohydrates levels of different plant parts of *E. littorale*.**

Plant Part	Season	(mg.g <sup>-1</sup> dry weight)		
		Total sugar	Total starch	Total carbohydrates
Leaves	Winter	1.380	0.220	1.600
	Summer	1.140	0.130	1.270
	Monsoon	1.430	0.320	1.750
Stem	Winter	1.300	0.240	1.540
	Summer	1.340	0.120	1.460
	Monsoon	1.480	0.450	1.930
Root	Winter	1.140	0.300	1.440
	Summer	1.720	0.100	1.820
	Monsoon	1.660	0.480	2.140

### Protein

The protein content of leaves was highest (0.466%) in Winter over that of monsoon (0.060%) and summer (0.247%). The range of protein content of stem was from 0.074% to 0.422%. The protein content root was very low in monsoon. The range in root was from 0.30% to 0.385% and showed higher in winter (0.320%). The percentage of protein content showed increasing order of stem < root < leaves (Table 5).

### Amino Acids

The amino acid content of leaves was 0.861% in summer, 0.448% in winter and (0.062%). The ranges of amino acid content of stem were from 0.065% to 0.775%. Maximum concentration of amino acid was noted during summer (0.775%). The ranges of amino acid content of root were from 0.175% to 0.749%. The concentrations of amino acids were found to be in increasing order of stem < root < leaves (Table 5).

**Table 5: Seasonal variations of total Proteins and Amino acids levels of different plant parts of *E. littorale*.**

Plant part	Season	(mg.g <sup>-1</sup> dry weight)	
		Total proteins (%)	Total Amino Acids(%)
Leaves	Winter	4.66	4.48
	Summer	2.47	8.61
	Monsoon	0.60	0.62
Stem	Winter	1.83	2.75
	Summer	4.22	7.75
	Monsoon	0.74	0.65
Root	Winter	3.20	2.55
	Summer	3.85	7.49
	Monsoon	0.30	1.76

### Lipid

The lipid concentration of leaves was higher in winter (0.059%) over that of summer (0.014%) and monsoon (0.0144%). In stem, lipid concentration was ranging from 0.0030% to 0.1% and it was significantly higher in winter (0.1%, Table 19). The lipid content of root was higher in winter (0.02%) over that of summer (0.019%) and monsoon (0.0035%). The concentrations of lipid were found to be in increasing order of root < leaves > stem (Table 6).

### Total Phenols

The concentrations of total phenols were ranging from 0.339% to 0.495% in leaves. Highest content of phenol was observed during summer (0.495%) in leaf. The total phenol content of stem exhibited maximum levels in summer (0.472%) over that of winter (0.292%) and monsoon (0.284%) (Table 6). The total phenol content of root was not significantly different as it ranges between 0.0236% to 0.466%. It was higher in winter (0.466%) (Table 19). The concentration of total phenols were found to be in increasing order of root < stem < leave (Table 6).

**Table 6: Seasonal variations of some Lipids and Phenols in different plant parts of *E. littorale*.**

Plant part	Season	(mg.g <sup>-1</sup> dry weight)	
		Total Lipid (%)	Total Phenol(%)
Leaves	Winter	0.59	3.39
	Summer	0.14	4.95
	Monsoon	0.14	4.03
Stem	Winter	1.00	2.92
	Summer	0.15	4.72
	Monsoon	0.03	2.84
Root	Winter	0.20	4.66
	Summer	0.19	2.63
	Monsoon	0.03	2.36

### Chlorophyll

The chlorophyll a, content of leaves was raised in monsoon (0.476 mg/g fresh wt.) over that of winter (0.449 mg/g fresh wt.) and summer (0.471 mg/g fresh wt.). The chlorophyll b, content of leaves was higher in monsoon (0.1825 mg/g fresh wt.) over that of winter (0.1432 mg/g fresh wt.) and summer (0.0676 mg/g fresh wt.). The carotenoid, content of leaves was accumulated more in monsoon (0.1897 mg/g fresh wt.) over that of winter (0.1801 mg/g fresh wt.) and summer (0.1403 mg/g fresh wt.). The range of chlorophyll a was found to be in

increasing order of winter < summer < monsoon, but the range of chlorophyll and carotenoid were found to be in increasing order of summer < winter < monsoon (Table 7).

**Table 7: Determination of Chlorophyll of Leaves of *E. littorale*.**

Plant Part	Season	(mg.g <sup>-1</sup> dry weight)		
		Chlorophyll-a	Chlorophyll-b	Carotenoid
<i>E. littorale</i> Leaves	Winter	0.449	0.143	0.180
	Summer	0.471	0.067	0.140
	Monsoon	0.476	0.182	0.189

## DISCUSSION

The present work deals with the pharmacogostic studies of Nai (*E. littorale*) The two taxa are selected, because Nai is very popular in classics, in ethnic use as well as modern galenicals as anti-malarial and anti-diabetic drug. The plants are of common occurrence along with grasses and other small herbs, cosmopolitan in distribution and locally abundant in high rain fed areas.

It is evident from Table 2 that leaves show higher relative water content in all the three seasons. The water content was highest about 88% in Mansoon. Alkaloids constitute one of the major chemical groups to which plants owe their medicinal properties. It is evident from the Table 3 that the alkaloid content of root is higher as compared to leaves and stem.

Phytochemical evaluation data is presented in Table 4 the highest carbohydrate content was noted higher in root while, the highest Amino Acid, protein content was noted in the leaves (Table 5). Lipid content of stem remained highest amongst all the organs. Whereas, Phenol content of leaves shows higher values (Table 6). Comparative pigment estimation for three pigment showed that the chlorophyll-a normally remains higher than that of chlorophyll-b and carotenoid (Table 7).

This parameter is of particular importance because it helps to envisage the dry product from the fresh collection. This knowledge also helps in fixing up the prices of the products. These observations will help in the Pharmacognostical identification, proper collection timing and standardization of the drug in the crude form and also to distinguish the drug from its adulteration.

## CONCLUSION

The medicinally important plant *E. littorale* is studied for their Phytochemical contents. The plant is very popular in classics, in ethnic use as well as modern galenicals as anti-malarial and anti-diabetic drug. Fresh plant samples were collected from the different part of Dhule Districts during different seasons (winter, summer and monsoon).

The chemical investigations carried out on field grown samples are summarized below. The alkaloids were found in higher magnitude in the roots, moreover they showed higher values during monsoon. Therefore, the monsoon collection is recommended if the alkaloids are to be used as medicine. Quantitative estimation of carbohydrates, protein, amino acid, phenol and lipid in various plant parts during different seasons were carried out. The higher carbohydrates content was noted higher in root. The higher Amino Acid, Phenol protein content was noted in leaves. Lipid content of stem remained highest among all the organs. Chlorophyll-a normally remains higher than that of chlorophyll-b and carotenoid. The water content was highest about 88% in Monsoon.

## REFERENCES

1. Abirami P. and Gomathinayagam M., 2011. A Review on *Enicostemma littorale*, Pharmacologyonline, 2011; 1: 75-83.
2. Agrawal A. K., Singhal A. and Gupta C. M., 1987. Functional drug targeting to erythrocytes in vivo using antibody bearing liposomes as drug vehicles; Biochem. Biophys. Res. Commun. 1987; 148: 357–361.
3. Anonymous, 1986. Indian Pharmacopoeia, vol. 2, 3rd Ed. Govt. of India, Ministry of Health, Controller of Publications, New Delhi, India.
4. Bhandari C.R., 1946. Vanaushadhi Chandrodaya (Hindi), Bhanpura, Indore, 1946; 2: 549.
5. Chopra R.N., Nayer S.L., Chopra I.C., 1956, Glossary of Indian Medicinal Plants, PID, CSIR, New Delhi.
6. Cooke T., 1967, Flora of the Presidency of Bombay. Botanically Survey of India, Calcutta.
7. Duxbury, A.C. and Yentsch, C.S., 1956, Plankton Pigment monographs, J. Marine Res., 1956; 15: 190-191.
8. Jayaraman, J., 1981. Laboratory manual in Bio-Chemistry. Wiley Eastern Limited, New Delhi, 114-117.

9. Kajaria D.K., M. Gangwar, A.K. Sharma, G. Nath, .B. Tripathi, J.S. Tripathi and S.K. Tiwari., 2011. Comparative evaluation of phenol and flavonoid content of polyherbal drugs. Pharmacology online, 2011; 3: 1365-1373.
10. Kirtikar K. R. and Basu B. D., 2003, Indian Medicinal Plants, (Bishen Singh Mahendra Pal Singh, 2003; 3: 1655-1656.
11. Krishnamoorthy R., Ambazhagan M. and hagwat K.A., 1989. Testing Salt tolerant variability in the nutritional quality of seeds produced by rice cultivars subjected to salinity. Seed Science and Technology, 1989; 17 : 269-275.
12. Lowry O.H., Rose brough N.J., Farr A.L. and Randall R.L.,1951. Protein measurements with the Folin-Phenol reagent. J. Biol. Chem., 1951; 193: 265-275
13. MacLachlan, S. and Zalik S., 1963. Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. Can. J. Bot., 1963; 41: 1053-1062.
14. Matthew K. M., 1982. The flora of Tamilnadu Carnatic, The Rapinet Herbarium St. Joseph's College, Tiruchirapalli-620002, India Vol. II; 973.
15. McCready R.; Guggolz J.; Silviera V.; Owens H., 1950. Determination of starch and amylose in vegetables, application to peas. Anal. Chem, 1950; 22: 1156-1158.
16. Nadkarni K., 2002, Indian Materia Medica, Vegetable kingdom, (Bombay Popular Prakashan, Vol. I: 485).
17. Nelson N., 1941. A Photometric adoption of the somogyi method for the determination of glucose, J. Biol. Chem., 1941; 153: 375-380
18. Rajamani Saranya, Thirunavukkarasu Thirumalai, Munisami Hemalatha, Ranganathan Balaji, Ernest David, 2013 Pharmacognosy of *Enicostemma littorale*: A review Asian Pac J Trop Biomed, 2013; 3(1): 79-84
19. Rathod Mayuri C., and Dhale D. A., 2013. Pharmacognostic Characterization Of *Enicostemma Littorale* Blume, International Journal of Research in Ayurveda and Pharmacy (IJRAP); 2013; 04(6): 893-898
20. Sairam T.V. and Khanna P., 1971. Effect of Tyrosine and Phenyl alanine on growth and production of alkaloid in *Datura tubula*, tissue culture, Lloydia, 1971; 34: 170-171.
21. Saxena P.N.N., Shrivastava and Saxena R.C., 2012. Preliminary Physico-Phytochemical Study of stembark of *Alstonia scholaris* (L) R. BR. - A Medicinal Plant, IJPSR, 2012; 3 (4): 1071-1075.
22. Shrikumar S. and Ravi T.K., 2007, Approaches towards development and promotion of herbal drugs. Phcog. Rev, 2007; 1:180-184

23. Swain, T.; Hillis, W. E., 1959, The phenolic constituents of *Purmus domestica*. I. The quantitative analysis of phenolic constituents. *J. Sci. Food. Agric*, 1959; 10: 63-68.
24. Thomas S., Patil D.A., Patil A.G., Naresh Chandra, 2009. Pharmacognostic evaluation and physicochemical Analysis of *Averrhoa carambola* L. *Fruit Journal of Herbal Medicine and Toxicology*, 2009; 2(2): 51-54.
25. Usha Kuamari J., Navas M., Mathew D., Rajasekharan S., 2009. Pharmacognostic studies on *Acrotrema arnottianum* Wight – A promising ethnomedicinal plant. *Indian Journal of Traditional Knowledge*, 2009; 8(3): 334-337.
26. Vaidya B.G., 1965. Some Controversial drugs of Indian Medicine, *V.J.Res.Indian Med*, 1965; 7(2):45-48.
27. Vinotha Sanmugarajah, Ira Thabrew, Sri Ranjani Sivapalan, 2013 Phyto, Physicochemical Standardization of Medicinal Plant *Enicostemma Littorale*, Blume; *IOSR Journal of Pharmacy*, 2013; 3 (2): PP 52-58
28. Vyas D.S., Sharma V.N. and Khanna N.K., 1979, Preliminary studies on anti-diabetic properties of. *E. littorale*, *Jour.RS.Ind. Med.Yoga and Homeo*. 1979; 14(3):63-65.