# Phyto, Physicochemical Standardization of Medicinal Plant Enicostemma Littorale, Blume

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Abstract :Enicostemma littorale, Blume (Gentianaceae) is a glabrous perennial herb and it is found in open, sandy places among sparse grass close the beach throughout the dry zone in Sri Lanka. It is traditionally used to treat inflammatory and painful conditions like arthritis, back pain; diabetes mellitus and to regulate bowel functions. Phyto, physicochemical standardization of dried, matured whole plants of E. littorale has been carried out in the present study. The study includes organoleptic characters along with estimation of its physicochemical parameters such as loss on drying, pH, ash values, extractability in water and ethanol and preliminary phytochemical screening. The generated information of the present study will provide data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

Keywords - Enicostemma littorale, Indian gentian, Physicochemical, Phytochemical, Standardization

#### I. INTRODUCTION

Medicinal plants have been a major source of treatment for human diseases since time immemorial. One fourth of the world population i.e. 1.42 billion people are dependent on traditional medicines, particularly plant drug for curing ailments [1]. Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. The process of standardization can be achieved by stepwise pharmacognostic studies [2]. Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect [3]. Determination of extractive values, ash residues and active components (saponin, alkaloids & essential oil content) plays a significant role for standardization of the indigenous crude drugs [4]. *E. littorale*, Blume belongs to family Gentianaceae, commonly known as '*Vellarugu*' in Tamil, 'Indian gentian', in English, '*Mamajaka*' in Sanskrit. It is a flower producing glabrous perennial herb which grows up to a height of 1.5ft is found throughout India [5-7].

In Sri Lanka, it is found in open, sandy places among sparse grass close the beach throughout the dry zone particularly from northwestern to northeastern coastal belt [8]. It is traditionally used to treat (a) inflammatory and painful conditions like arthritis, back pain, (b) diabetes mellitus, and (c) to regulate bowel functions [6, 9]. This herb is also known for its anti-inflammatory effects [10, 11] anti-diabetic activity [12], antioxidant [13], hepatomodulatory [14]. hypolipidemic and anti-arthritic activity[15]. 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 [16], so that the medical benefits of the plant may be used properly and scientifically and reach to the larger populations of the world. There fore, in the present research work was to evaluate the physicochemical parameters and phytochemical constituents of the whole plant of *E. littorale*.

# II. MATERIALS AND METHODS

## **2.1 Collection of Plant materials**

Whole plants of *Enicostemma littorale* were collected during October to January (2011-2012) in and around Jaffna District. The plant was botanically authenticated and voucher specimen (Assess. No. 2454) was deposited in the Bandaranayaka Ayurveda Memorial Research Institute, Nawinna, Maharahama, SriLanka.

## 2.2 Preparation of plant material

The collected *E. littorale* whole plants were washed with tap water. The plants were cut in to small pieces and air-dried thoroughly under shade (at room temperature) for 2 months to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air-tight container for furthers analysis.

## 2.3 Preparation of water and ethanol extracts

The whole plant powder of the *E. littorale*, extracted with ethanol and water using hot extraction technique. A total of 10gm of individual whole plant powder of the *E. littorale* was taken and mixed with 50 ml distilled water (1:5) in a round bottom flask and gentle refluxed for 1½ hour separately. The residue was removed by filtration through Whatmann No. 1 filter paper and the aqueous extract was concentrated used on a Rotary evaporator (Buchi) to get 22% solid yield. Same procedure was followed using ethanol instead of distilled water to prepare the hot ethanol extract.

# 2.4 Organoleptic Evaluation

Organoleptic evaluation refers to evaluation of the whole plant powder of the E. littorale, by colour, odour, taste, texture, touch etc. The organoleptic characters of the sample were evaluated based on the method described by Siddiqui et al., 1995 [17].

## 2.5 Physicochemical Investigations

Six samples of whole plant powder of E. littorale were subjected for determination of physicochemical parameters such as loss on drying, ash values, pH value in 1% and 10% solution, aqueous, and alcoholic extractive values were carried out according to the methods recommended by the World Health Organization [18].

# 2.5.1 Determination of pH range

The pH of different formulations in 1% w/v (1g: 100ml) and 10% w/v (10g: 100ml) of water soluble portions of whole plant powder of E. littorale, were determined using standard simple glass electrode pH meter [19].

# 2.5.2 Loss on drying / Moisture content (Gravimetric determination):

Separately place about 1.0g of whole plant powder of the E. littorale, in an accurately weighed moisture disc (Electronic measurement scale – Mettler Toledo). For estimation of loss on drying, it was dried at 105°C for 5 hours in an oven (Memmert), cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air-dried material.

# 2.5.3 Determination of hot water and ethanol-extractable matter

Separately place about 4.0g of whole plant powder of the E. littorale, in an accurately weighed, glassstoppered conical flask. For estimation of hot water-extractable matter, 100ml of distilled water was added to the flask and weighed to obtain the total weight including the flask (Electronic top loading balance - Citizen). The contents were shaken well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and boiled gently for 1 hour; cooled and weighed. The flask was readjusted to the original total weight with distilled water and it was shaken well and filtered rapidly through a dry filter. Then 25 ml of the filtrate was transferred to an accurately weighed, tarred flat-bottomed dish (Petri disc) and evaporated to dryness on a water-bath. Finally, it was dried at 105°C for 6 hours in an oven, cooled in a desiccator for 30 minutes, and weighed without delay. Same procedure was followed using ethanol instead of distilled water to determine extractable matter in ethanol. The extractable matter was calculated as the content of in mg per g of air-dried material.

#### 2.5.4 Determination of total ash

Two grams of the whole plant powder of the E. littorale, was placed in a previously ignited (350°C for 1 hour) and tarred crucible accurately weighed. Dried material was spread in an even layer in the crucible and the material ignited by gradually increasing the heat to 550°C for 5 hours in a muffle furnace (Nabertherm) until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material.

#### 2.5.5 Determination of acid-insoluble ash

Twenty- five (25) ml of hydrochloric acid (~70g/l) TS was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper (Whatmann 41) and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 550°C for 3 hours in a muffle furnace (Nabertherm) to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acid-insoluble ash content was calculated as mg per g of air-dried material.

## 2.5.6 Determination of water-soluble ash

Twenty- five (25) ml of water was added to the crucible containing the total ash, covered with a watchglass and boiled gently for 5 minutes. Insoluble matter was collected on an ash less filter-paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water - soluble ash content was calculated as mg per g of air-dried material.

## 2.5.7 Determination of sulfated ash

Ignited a suitable crucible (silica) at 550 °C to 650 °C for 30 minutes, cooled the crucible in a desiccator (silica gel) and weighed it accurately. One gram of the whole plant powder of the *E. littorale*, was placed in a previously ignited crucible, ignited gently at first, until the substance was thoroughly white. Cooled and moistened the sample with a small amount (usually 1 ml) of sulfuric acid (~1760 g/l) TS, heated gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moistened the residue with a small amount (usually 1 ml) of sulfuric acid (~1760 g/l) TS, heated gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moistened the residue with a small amount (usually 1 ml) of sulfuric acid (~1760 g/l) TS, heated gently until white fumes were no longer evolved, and ignited at 800 °C + 25°C until the residue is completely incinerated. Ensure that flames were not produced at any time during the procedure. Cooled the crucible in a desiccator (silica gel), weighed accurately. This was repeated until the sample reaches a constant weight and calculated the percentage of residue [20].

# 2.6 Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanol and water (hot) extracts of whole plant powder of *E. lttorale* were carried out using standard laboratory procedures, to detect the presence of different secondary metabolites (phytochemical constituents) such as alkaloids, flavonoids, saponins, tannins, steroid glycosides, phenols, coumarins, reducing sugars, protein, anthraquinones, quinines, Fixed oils and fats [16, 21-24].

# **2.6.1 Determination of Phenolic compounds**

Two to three drops of 1% ferric chloride (FeCl<sub>3</sub>) solution were added in to 2 ml portions (1%) of each extract. Phenolic compounds produce a deep violet colour with ferric ions.

# **2.6.2 Determination of Tannins**

Ferric chloride test- A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of a blue-black, or green blackish colour in the presence of ferric chloride precipitate was taken as evidence for the presence of tannins.

#### 2.6.3 Determination of Flavonoids

Shinoda test- The extract was dissolved in methanol (50%, 1-2 ml) by heating. To an alcoholic solution of each of the extract, three pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

#### **2.6.4 Determination Coumarins**

Coumarins form a yellow colour with 1% KOH in absolute ethanol. 1 ml of portions of 1% solutions of each in test tubes was treated with 3-4 drops of 1% KOH in absolute ethanol.

## 2.6.5 Determination of Steroid glycosides

Libermann Burchard's test- Extract was dissolved in equal volumes of anhydrous acetic acid and chloroform (CHCl<sub>3</sub>) and cooled to  $0^{\circ}$ C. The mixture was transferred to a dry test tube and concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was introduced to the bottom of the tube. Formation of a reddish brown or violet- brown ring at the interface of the two liquids indicates the presence of steroids.

## **2.6.6 Determination of Alkaloids**

Mayer's Test- One ml portions of each extract was acidified with 2-3 drops of 1M Hydrochloric acid and treated with 4-5 drops of Mayer's regent (Potassium Mercuric Iodide) Formation of a yellow or white coloured precipitate or turbidity indicates the presence of alkaloids.

Dragendroff's Test- Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

## **2.6.7 Detection of Proteins**

Xanthoproteic Test- The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

#### 2.6.8 Detection of Quinones

To the test sample, sodium hydroxide is added. Formation of blue, green, or red colour indicates the presence of quinones.

#### 2.6.9 Detection of Anthraquinones

For examining the anthraquinone derivatives prepare a specimen in potassium hydroxide solution, anthraquinones give blood red colour.

# 2.6.10 Determination Saponins

Foam Test- 0.5 g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

#### 2.6.11 Detection of reducing sugar

Fehling's test- To a test tube 1 ml each a Fehling's A and B solutions were added and mixed. To this ~2 ml of plant extract was added and heated on a boiling water bath for ~10 minutes. Formation of brick red or orange precipitate indicates the presence of reducing sugar/ carbohydrates.

# 2.6.12 Detection of Fixed oils and fats

Spot test- A drop of concentrated extract was pressed in between two filter papers and kept undisturbed. Oil stain on the paper indicates the presence of oils and fats.

#### 2.7 Statistical analysis

Statistical analysis of the results obtained in each experiment was carried out by use of the Ms Excel 2007 statistical software and mean values along with standard deviation were recorded.

# III. RESULTS

The Organoleptic characters of the Whole plant of E. *littorale* course powder; aqueous and alcoholic extracts are tabulated as Table No. 1. The Average physicochemical parameters of the Whole plant of E. *littorale* course powder are tabulated as Table No. 2. The Preliminary phytochemical screening for various functional groups is tabulated as Table No. 3.

Parameters	Raw	Aqueous extract	Ethanolic extract
Appearance	Powder	Liquid	Liquid
Touch	Coarse	Smooth	Smooth
Colour	Greenish brown	Light brown	Dark green
Taste	Bitter	High bitter	High bitter
Odour	Characteristic	Characteristic	Characteristic

TABLE 1: Organoleptic Properties of Whole Plant of E. littorale

TABLE 2: Physicochemical Parameters of Whole Plant of E. littorale

Parameters	Whole Plant of E. littorale		
Loss on drying	$10.25\pm0.33$		
Total ash value	$08.16\pm0.09$		
Water soluble ash	$02.75\pm0.08$		
Acid insoluble ash	$01.89\pm0.07$		
Sulfated ash value	$01.30\pm0.10$		
pH of 1% w/v formulation solution	$05.12\pm0.02$		
pH of 10% w/v formulation solution	$04.87\pm0.04$		
Water soluble (hot) extractive value	37.21 ± 1.27		
Ethanol soluble (hot) extractive value	$24.92\pm0.64$		

Values are expressed as mean $\% \pm$  S.D., except pH values are expressed as mean  $\pm$  S.D., n = 06.

TABLE 3: Phytocochemical Screening for Aqueous and ethanolic extracts of Whole Plant of E. littorale

No	Components	Aqueous extract	Ethanolic extract
01	Phenolic compound	+++	+++
02	Tannins- Ferric chloride test	+++	+++
03	Flavonoids- Shinoda test	++	+++
04	Coumarins	++	+++
05	Steroid-glycosides- L. Burchard's test	+++	+++
06	Alkaloids		
	Mayer's Test	+++	+++
	Dragendroff's Test	+++	+++
07	Protein- Xanthoproteic Test	++	+
08	Quinone	++	+++
09	Anthraquinones	0	0
10	Saponins- Foam test	++	++
11	Reducing sugars- Fehling's test	+++	+++
12	Fixed oil and Fats-Spot test	++	0

+++ = appreciable amount, ++ = average amount, + = trace amount, 0 = absent

#### **IV. DISCUSSIONS**

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal, whole plant of *E. littorale* was subjected to systematic organoleptic evaluation, physicochemical and phytochemical screening by aqueous & ethanolic extracts to determine the amount of soluble constituents in a given amount of medicinal plant material and are helpful in determining the quality and purity of a crude drug, especially in the powdered form.

As seen in Table1, both the aqueous and ethanolic extracts of whole plant of *E. littorale* had similar organoleptic properties except for the colour of the both extracts. Physicochemical parameters of whole plant powder of *E. littorale* were estimated based on the methods recommended by World Health Organization (WHO). As apparent from Table 2, Percent weight loss on drying or moisture content value was found to be  $10.25 \pm 0.33$ . The less value of moisture content of drugs could prevent content bacterial, fungal or yeast growth through storage [25]. The ash values total ash; water soluble ash, acid insoluble ash and sulfated ash value were found to be  $8.16 \pm 0.1\%$ ,  $2.75 \pm 0.1\%$ ,  $1.89 \pm 0.1\%$  and  $1.30 \pm 0.1\%$  respectively. Ash values used to find out quality, authenticity and purity of unsophisticated drug and also these values are important quantitative standards [26]. The pH of 1% w/ v and 10% w/ v solutions were found to be  $05.12 \pm 0.02$  and  $04.87 \pm 0.04$  respectively. These values were showed not much difference in the pH of water soluble portions of whole plant of *E. littorale*. The solubility percentage of *E. littorale* in aqueous hot extraction is higher (37.21±1.27%), when compared with ethanolic hot extraction (24.92±0.64%). The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent [27].

As seen in Table 3, the preliminary phytochemical screening of hot aqueous and ethanolic extracts indicated the presence of alkaloids, saponins, flavonoids, steroids, tannins, proteins, quinines, reducing sugar, phenols and coumarins; and does not indicate the presence of anthraquinones. These constituents may be possibly responsible for the biological activities of *Enicostemma littorale*.

#### V. CONCLUSION

As there is not enough evidence for detailed physicochemical and phytochemical evalution on whole plant of *Enicostemma littorale* Blume is reported. Therefore present work is taken up in the view to completely standardize the herb in accordance to parameters of World Health Organization (WHO) Guidelines and standard laboratory procedures. In the present study whole plant of *E. littorale* was thoroughly investigated for their organoleptic characters; physicochemical characters and major active constituents to analyze their quality, safety and standardization for their safe use. The generated information of the present study will provide data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

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#### REFERENCES

- [5] K. Nadharni, Indian Materia Medica, Vegetable kingdom, (Bombay Popular Prakashan, Vol. I, 2002, 485).
- [6] K. R. Kirtikar and B. D. Basu, Indian Medicinal Plants, (Bishen Singh Mahendra Pal Singh, Vol. III, 2003, 1655-1656.

S. Kala, M. Johnson, N. Janakiraman, A. Anto Arockiaraj, S. Iyan Raj and Dorin Bosco, Pharmacognostic and phytochemical studies on some selected ethnomedicinal plants of Tamilnadu, South India, Int. J. Med. Arom. Plants, Vol. 1, No. 2, 2011, 89-94.

<sup>[2]</sup> D. Nivedithadevi and R. Somasundaram, Pharmacognostical and Qualitative Phytochemical Studies on the aerial parts of tephrosla purpurea (L), International Journal of Research in Biological Sciences, Vol. 2, No. 2, 2012, 48-53.

<sup>[3]</sup> S. Nasreen and R. Radha, Assessment of Quality of Withania somnifera Dunal (Solanaceae) Pharmacognostical and physicochemical profile, International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 3, Issue 2, 2011, 152-155.

<sup>[4]</sup> Hina Fazal, Nisar Ahmad and Mir Ajab khan, Physico-chemical, Phytochemical Evaluation and DPPH- Scavenging Antioxidant potential in Medicinal Plants used for herbal formulation in Pakistan, Pak. J. Bot., Special Issue 43, 2011, 63-67.

- [7] P. Abirami and M. Gomathinayagam, A Review on Enicostemma littorale, Pharmacologyonline 1, 2011, 75-83.
- [8] M.D. Dassanayake and F. R. Fosberg, A Revised Handbook to the Flora of Ceylon, Oxford and IBH Publishing Co., New Delhi, 1981.
- [9] P.T.V. Lakshmi, A. Annamalai and C. Ramya, A study on the Genetic Diversity Analysis of a Medicinally potential herb-Enicostemma littorale Biume (Gentianaceae), International Journal of Pharma and Bio Sciences, Vol. 2, No. 4, 2011, pp. 238-445.
- [10] Amritpal Singh, Samir Malhotra and Ravi Subban, Anti-inflammatory and Analgesic Agents fro Indian Medicinal Plants, International Journal of Integrative Biology, Vol. 3, No. 1, 2008, 57-72.
   [11] S.J. Shivakumar, B. Shivakumar, F. Jayachandran, & H. M. Surash, Evaluation of anti-inflammatory activity of Enjocetamma.
- [11] S.I. Shivakumar, B. Shivakumar, E. Jayachandran, & H. M. Suresh, Evaluation of anti-inflammatory activity of Enicostemma littorale Linn, Journal Biomed, Vol. 4, No. 4, 2009, 402-404.
- [12] S.L. Vishwakarma, D. Sonawane Rakesh, M. Rajani, & Ramesh K Goyal, "Evaluation of effect of aqueous extract of Enicostemma littorale blume in streptocin- induced type I diabetic rats", Indian Journal of Experimental Biology, Vol. 48, 2010, pp. 26-30.
- [13] R. Sathishkumar, P.T.V. Lakshmi & A. Annamalai, Effect of drying treatment on the content of antioxidants in Enicostemma littorale Blume, Research Journal of Medicinal plant, Vol. 3, 2009, 93-101.
- [14] R.S. Gupta, & Dharmendra Singh, Hepatomodulatory role of Enicostemma littorale Blume against oxidative stress induced liver injury in rats, African Journal of Agricultural Research, Vol. 2, 2007, 131-138.
- [15] R. Gopal, A. Gnanamani, R. Udayakumar, & S. Sadulla, Enicostemma littorale Blume A potential hypolipidemic plant, Natural Product Radiance, Vol. 3, No. 6, 2004, 401-405.
- [16] P.N.N. Saxena, Shrivastava & R.C. Saxena, Preliminary Physico-Phytochemical Study of stembark of Alstonia scholaris (L) R. BR. - A Medicinal Plant, IJPSR, Vol. 3, No. 4, 2012, 1071-1075.
- [17] Siddiqui & M.A. Hakim, Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, anuary, Central Council for Research in Unani Medicine (CCRUM), New Delhi, (appendix), 1995, 24-25.
- [18] World Health Organization, (WHO), Organization Mondiale De La Sante, Quality control methods for plant materials, 559, Rev. 1, Original English, 1998, 08-67.
- [19] Neeraj Choudhary & Bhupinder Singh Sekhon, An overview of advances in the standardization of herbal drugs, J. Pharm Educ. Res., Vol. 2, Issue 2, 2011, 55-70.
- [20] World Health Organization, (WHO), Final text for revision of The International Pharmacopoeia 2.3. SULFATED ASH, Document QAS/11.401 FINAL, 2012, 1-2.
- [21] C.K. Kokate, K.R. Khandelwal, A.P. Pawer & S. B. Gokhale, Practical Pharmacognosy, 3<sup>rd</sup> edition, Nirali Prakashan, Pune, 1995, 137.
- [22] N.R. Farnsworth, Biological and Phytochemical screening of Plants, Journal of pharmaceutical Science, Vol. 55, 1996, 225-276.
   [23] Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur & Harleen Kaur, Phytochemical screening and Extraction: A
- Review, Internationale Pharmaceutica Sciencia, Vol. 1, Issue 1, 2011, 98-106.
  [24] A.K. Gupta, Neeraj Tandon, Madhub Sharma, Quality Standards of Indian Medicinal Plants, Indian Council of Medical Research,
- New Delhi, Vol. 7, 2008, 331-339.
  [25] M.K. Pandey, G.N. Singh, Rajeev Kr Sharma & Sneh Lata, Standardization of Yakrit Plihantak Churna: An Ayurvedic Poly herbal formulation, IJPSR, Vol. 3, No. 1, 2012, 171-176.
- [26] Paramjyothi Swamy & Syed Kamil Mulla, Preliminary Pharmacognostical and Phytochemical Evaluation of Portulaca quadrifida Linn", International Journal of Pharm. Tech. Research, Vol. 2, No. 3, 2010, 1699-1702.
- [27] Shwetajain, Chanderachud Sharma, Pankaj Khatri, Atul Jain & Ankur Vaidya, Pharmacognostic and Phyto chemical Investigations of the Leaves of Zizyphus xylopyrus (Retz) Willd, International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 3, Issue 2, 2011, 122-125.



Photograph of Medicinal Plant Enicostemma littorale Blume