International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Preliminary Phytochemical Screening of Different Extracts of Whole Plant of *Enicostemma littorale* Blume

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Abstract

Enicostemma *littorale* Blume (Gentianaceae family), which is commonly known as Mamajaka (Sanskrit), Vellarugu (Tamil) and Indian gentian (English). *E. littorale* is a perennial herb which grows in coastal areas of Northern and Eastern province of Sri Lanka. The whole plant is dried and powdered and used to treat rheumatism, swelling, back pain, diabetes mellitus, constipation, and skin diseases. The aim of this study is to evaluate the phytochemical constituents in different extracts of *E. littorale* according to the standard procedures. Quantitative estimation of some of the active constituents like alkaloids, flavonoids and saponins were also carried out. The preliminary phytochemical screening of hot and cold ethanol, methanol and aqueous extracts showed the presence of alkaloids, saponins, flavonoids, steroids, tannins, proteins, reducing sugar coumarins and quinones and absence of anthraquinones. Cold and hot water extracts showed the presence of fat and fixed oil. The total alkaloid and flavonoid contents were found to be 2.25 ± 0.01 % and 25.34 ± 0.24 % respectively and total saponin content was (Foaming Index) FI < 100. The phytochemicals identified in the present study may be used as tools for quality control of drugs prepared with *E. littorale* in the future, for the treatment of a variety of disease conditions.

Keywords: *Enicostemma littorale*; Different extracts; Phytochemical Screening

1. Introduction

Herbal medicine is widely practiced from ancient period throughout the world. These medicines are safe and environment friendly. According to World Health Organization 80% of the world's population relies on traditional medicine for their primary health care [1]. In the traditional system of medicine, which dates back many centuries, many herbal extracts are used to cure a variety of diseases [2].

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One such popularly used plant that is reported to have anti-diabetic, anti-inflammatory, anti-oxidant, hypolipidemic, and anti-arthritic effects is Enicostemma littorale Blume (Gentianaceae family), which is commonly known as Mamajaka (Sanskrit), Vellarugu (Tamil) and Indian gentian (English) [3]. E. littorale is a perennial herb with sessile lanceolate leaves which grows in coastal areas of Northern and Eastern province of Sri Lanka [4]. It is commonly available in and around the Jaffna District during rainy season. The whole plant is dried and powdered and used to treat rheumatism, swelling, back pain, diabetes mellitus, constipation, and skin diseases [5,6].The aim of the present study is to evaluate the phytochemical constituents in different extracts of *E. littorale* and quantitative estimation of quantification of some of the active constituents like alkaloids, flavonoids and saponins in whole plant of *E. littorale*.

2. Materials and Methods

2.1 Collection of Plant material

Whole plants of *Enicostemma littorale* were collected from the natural habitats during the month of October 2011 to January 2012 in and around Jaffna District.

2.2 Identification of Plant material

The botanical identity of the plant was authenticated (Accession No. 2554) and the voucher specimen of *Enicostemma littorale* has been deposited at Bandaranayaka Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka.

2.3 Preparation of Plant material

The collected *Enicostemma littorale* whole plants were washed thoroughly 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 pulveriser and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air-tight container for further analysis.

2.4 Preparation of the Plant extracts

2.4.1 Hot extraction: A total of 10 gm of powdered sample was taken and mixed with 50 ml distilled water in 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) for just as long as was required to remove the solvent, and re-dissolved the residue in a small volume (2 or 3 ml) of water.

2.4.2 Cold extraction: A total of 10 gm of powdered sample was successively extracted with 50 ml distilled water and stirred magnetically (Magnetic stirrer - Snijders) in a container for 1½ hour at room temperature. The extract was filtered through filter paper and concentrated by a Rotary evaporator for just as long as was required to remove the solvent, and re-dissolved the residue in a small volume (2 or 3 ml) of water [7].

Finally, the all extracts were collected in clean stoppered glass test tubes separately and used for phytochemical screening. Same procedures were followed using ethanol and methanol instead of distilled water to prepare the hot and cold ethanolic and methanolic extracts.

2.5 Organoleptic Evaluation

2.6

Organoleptic evaluation refers to evaluation of the whole plant of *E. littorale* crude powder, and it's aqueous and alcoholic extracts by colour, odour, taste, texture, etc. The organoleptic characters of the sample were evaluated based on the method described by [8].

2.6 Preliminary phytochemical screening

The preliminary phytochemical screening of the hot and cold ethanol; methanol and aqueous extracts of the whole plant of *E. littorale* were carried out using standard laboratory procedures, to detect the presence of different secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroid glycosides, phenols, coumarins, reducing sugars, protein, anthraquinones, quinones, fat and fixed oil [9 - 13].

2.7 Quantitative estimations

2.7.1 *Estimation of Total Alkaloid*: Quantitatively, alkaloid was determined using the procedure forward by Harborne, 1973; as described by [14].

Briefly, five grams (5 g) of whole plant powder was weighed into 250 ml beaker and 200 ml of 20% acetic acid was added and covered to stand for 4hr. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration through the accurately weighed filter paper. The residue is the alkaloid, which was dried at oven for 4 hours and weighed. Total alkaloid content was calculated as mg per g of air-dried material [14].

2.7.2 Estimation of Total Flavonoids: Flavonoids were determined using the procedure forward by Boham and Kocipaiabyazan (1994) as described by [14].

Briefly, 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman No. 42 (125 mm) filter paper. The filtrate was later transferred into accurately weighed crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weight is flavonoids. Total flavonoid content was calculated as mg per g of air-dried material [14].

2.7.2 Estimation of Total saponin (Determination of foaming index):

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index. Saponins were determined according to the method described by World Health Organization [15].

Reduce about 1 g of the whole plant powder weighed accurately and transferred to a 500-ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cooled and filtered into a 100 ml volumetric flask and added sufficient water through the filter to dilute to volume. Poured the decoction into 10 stopper test-tubes (height 15cm, diameter 15mm) in series of successive portions of 1 ml, 2 ml, 3 ml, up to 10 ml and the volumes in each tube adjusted with water to 10ml. The tubes were stopper and then shaken them in a lengthwise motion for 15 seconds, two shakes per second. After allowed the tubes to stand for 15 minutes and the height of the foam was measured by means of a graduated tape with millimetre scale.

2.7.3 Determination of volatile oil: Fresh whole plants of *E. littorale* were washed to remove dirt, chopped into small pieces and ground in a blender. The material was subjected to hydro distillation using Clavenger-type glass apparatus for 4 hours separately. Then, observation done whether the volatile oil present or absent [15, 16].

2.8 Statistical analysis

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

3. Results and Observations

The Organoleptic characters of aqueous and alcoholic extracts of the *whole plant of* E. littorale, are tabulated as Table no.1. The phytochemical screening for secondary metabolites is tabulated as Table no. 2. The quantitative test for some of the active constituents is tabulated as Table no. 3.

Table 1. Organoleptic properties of aqueous and alcoholic extracts of whole plant of *E. littorale*

Name of the crude powder/ extracts	Appearanc	Colour	Taste	Odour
Crude powder:	Powder	Greenish brown	High bitter	Characteristic
Aqueous extracts:				
Hot water extract	Liquid	Brown	High bitter	Characteristic
Cold water extract	Liquid	Golden brown	High bitter	Characteristic
Alcoholic extracts:				
Hot ethanol extract	Liquid	Dark green	High bitter	Characteristic
Cold Ethanol extract	Liquid	Dark green	High bitter	Characteristic
Hot methanol extract	Liquid	Dark green	High bitter	Characteristic
Cold methanol extract	Liquid	Dark green	High bitter	Characteristic

Table 2. Phytocochemical Screening of cold and hot aqueous and alcoholic extracts of whole plant of *E. littorale*

Components	Different Extracts					
	Cold Ethanol	Hot Ethanol	Cold Methanol	Hot Methanol	Cold aqueous	Hot aqueous
Phenolic compound	+++	+++	+++	+++	+++	+++
Flavonoids-*Shinoda test*	+++	+++	+++	+++	+++	+++
Coumarins	+++	+++	+++	+++	++	++
Quinones	+++	+++	+++	+++	++	++
Anthraquinones	0	0	0	0	0	0
Tannins- *Ferric chloride test*	+++	+++	+++	+++	+++	+++
Saponins- *Foam test*	++	++	++	++	++	+
Protein- *Xanthoproteic Test*	+	+	+	+	++	++
Steroid-glycosides-*Libermann Burchard's test*	+++	+++	+++	+++	+++	+++
Alkaloids						
Mayer's Test	+++	+++	+++	+++	+++	+++
Dragendroff's Test	+++	+++	+++	+++	+++	+++
Reducing sugars- *Fehling's test*	+++	+++	+++	+++	+++	+++
Fixed oil and Fats	0	0	0	0	++	++

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

Table 3. Total alkaloids, flavonoid and saponin contents in powder of *Enicostemma littorale*

Name of the plant material	Total alkaloids	Total flavonoids	Total saponins (Foaming Index)
Whole plant powder of *E. littorale*	2.25±0.01	25.34 ±0.24	FI < 100

Values are expressed as mean% ± S.D., n=3

4. Discussion

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, phytochemical standardization is very important so that the medical benefits of the plant may be used properly and scientifically and reach to the larger populations of the world [13].

The secondary metabolites such as alkaloids, flavonoids, lignins, terpenoids, steroids, glycosides, coumarins and phenols in plant materials produce the curative effect when they are used in the traditional medical practice [17].

As seen in Table 1, both the aqueous and alcoholic extracts of whole plant of *E. littorale* had similar organoleptic properties except for the colour of the both extracts.

As apparent from Table 2, the preliminary phytochemical screening of cold and hot ethanol, methanol and aqueous extracts showed the presence of alkaloids, saponins, flavonoids, steroids, tannins, proteins, reducing sugar coumarins and quinones and absence of anthraquinones. Cold and hot aqueous extracts showed the presence of fat and fixed oil. Higher flavonoids, coumarins and quinones contents were found in the cold and hot ethanol and methanol extracts than in the cold and hot aqueous extracts of whole plant of E. littorale.

Previous preliminary phytochemical screening studies have shown that presence of triterpenoids, flavonoids, alkaloids and coumarins in aqueous extract of *E. littorale* [18]; presence of flavonoids, polyphenols, phytosterol, carbohydrate, amino acid and protein in 85% methanol extract of *E. littorale* [19]; presence of terpenoides, tannins, phenols, coumarin, flavonoids, protein and sugar in methanol extract extract of *E. littorale* [20] and presence of flavonoids, polyphenols, phytosterol, alkaloids, terpenoids, tannins, saponins, carbohydrates, glycosides & protein in methanol extract of *E. littorale* [21].

As seen in Table 3, the total alkaloid (20% acetic acid extract) and flavonoid (80% of aqueous methanol extract) contents were found to be 2.25 ± 0.01 % and 25.34 ± 0.24 % respectively and total saponin (hot water extract) content was (Foaming Index) FI < 100. The considerable amount of volatile oil was not determined in fresh whole plant of *E. littorale*.

The phytoconstituents quantified in the present study exhibit great deal of medicinal importance like alkaloids can be act antioxidant and immunomodulatory agent and generally flavonoids can be act antioxidant and anti-inflammatory property. The quantified values of the above phytoconstituents can be used as a major tool for obtaining a quality control profile for a drug.

5. Conclusion

Phytochemical screening of different extracts and phytochemical estimation of some active constituents of whole plant of *E. Ittorale* has been carried out according to standard laboratory procedures. The phytochemicals identified in the present study may be used as tools for quality control of drugs prepared with *E. littorale* in the future, for the treatment of a variety of disease conditions. Further the mechanism of actions of this plant and identify the constituent compounds responsible for the pharmacological activities should be carried out in future.

Acknowledgment

The financial support provided Higher Education Twenty first Century (HETC) Project, Ministry of Higher Education, Sri Lanka, and the assistance given by Dr. (Mrs.) Menuka Arawwawala, Industrial Technology Institute (ITI), Colombo, to conduct the qualitative and quantitative analysis are gratefully acknowledged.

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