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**COMPARATIVE PHYTO-PHYSCOCHEMICAL STUDIES ON  
SELECTED MEDICINAL PLANTS, ENICOSTEMMA LITTORALE  
BLUME AND WITHANIA SOMNIFERA DUNAL**

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**ABSTRACT**

Medicinal plants have been a major source of treatment for human diseases since time immemorial. The physicochemical evaluation of the plant material is an important parameter in detecting adulteration or improper handling of drugs. The present study was planned to compare the phyto-physicochemical analysis of *Enicostemma littorale* Blume (Whole plant) with *Withania somnifera* (L) Dunal (root). Both plants are medicinally important and used as anti-arthritic, anti-inflammatory, antioxidant and hepatoprotective. These plant materials were phyto, physico-chemically standardized according to the methods recommended by the World Health Organization and standard laboratory procedures. The highest percentage of hot water and ethanol extractable matter; total ash and acid insoluble ash ( $37.21 \pm 1.27\%$  &  $24.92 \pm 0.64\%$ ,  $8.16 \pm 0.1\%$  &  $1.89 \pm 0.1\%$ ) were found in *E. littorale* when compared with *W. somnifera* ( $30.82 \pm 1.09\%$  &  $10.0 \pm 0.21\%$ ,  $5.76 \pm 0.1\%$  &  $0.63 \pm 0.04\%$ ). Percentage of loss on drying was found in *W. somnifera* ( $9.12 \pm 0.10\%$ ) and it was slightly lower than *E. littorale* ( $10.25 \pm 0.33\%$ ). Preliminary phytochemical screening of these both plant materials revealed that presence of alkaloids, saponins, flavonoids, steroids, tannins, proteins, reducing sugar, fat and fixed oils and coumarins. This comparative information provides immense potential for studying their activities for arthritis and other disease conditions, both in pre-clinical and clinical stages, which lead to the preparation of useful pharmaceutical products. The present study has authenticated the usefulness of the identified plants for medicinal purposes. These species could also be seen as potential source of useful drugs due to their rich contents of phytochemical.

**Keywords:** Comparative study, Physicochemical, Phytochemical, *Enicostemma littorale*, *Withania somnifera*.

**INTRODUCTION**

Medicinal plants have been used for years in daily life to treat diseases all over the world. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare [1]. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [2]. Plants, besides providing nutrition, have always formed an important source of chemical compounds as secondary metabolites, which can be used for medicinal purposes [3]. The pharmacognostical parameters are major and reliable criteria for confirmation of the identity and determination

of quality and purity of the crude drugs [4]. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained [5]. The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of plant material. Correct identification and quality assurance of the starting materials are an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy [6]. *Enicostemma littorale* Blume (Gentianaceae) is a glabrous perennial herb. It grows throughout India up to 1.5 feet height and more frequently near the sea [7]. It is called as 'Mamajaka'

in Sanskrit and 'Indian gentian' in English and is being used as a folk medicine for various ailments like rheumatism, diabetic mellitus, skin diseases and constipation. It also reported to possess anti-inflammatory, antioxidant, anti-arthritis activity and anti-diabetic activity [8-14].

*Withania somnifera* (Linn) Dunal (Solanaceae) is commonly known as 'Ashwagandha', 'Indian ginseng' and 'Winter cherry'. It has been an important herb in the indigenous medicine system for over many years. The roots are the main portion of the plant used therapeutically [15] and prescribed for rheumatism, arthritis, cough tumours and low energy [16]. It is reported to have various biological activities including anti-inflammatory [17-19], anti-arthritis activity [20], analgesic effect [21], anti tumour activity [22] and hepato-protective effect [23].

Medicinal herbs have been known for centuries and are highly valued all over the world as a rich source of therapeutic agents for prevention of diseases and ailments [24]. Therefore, these plants can be exploited to find out effective alternative to synthetic drugs [25]. Hence the present work is aimed to describe the phyto, physicochemical variations between the two species.

## MATERIALS AND METHODS

### Collection of Plant materials

Whole plants of *Encostemma littorale* were collected during October to January (2011-2012) in and around Jaffna District. Roots of *Withania somnifera* were purchased from a reputed vendor of herbal material in Jaffna District, Sri Lanka.

These plants (*Withania somnifera* & *Encostemma littorale*) were botanically authenticated and voucher specimens (Assess. No. 2453 & 2454) were deposited in the Bandaranayaka Ayurveda Memorial Research Institute, Nawinna, Maharahama, Sri Lanka.

### Preparation of plant material

The collected *E. littorale* whole plants were cut in to small pieces and washed with tap water. The purchased *W. somnifera* roots were cut in to small pieces and boiled with cow's milk (1:1). These plant materials were air-dried thoroughly under shade (at room temperature) for 2-3 weeks to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and sieved up to 80 meshes separately. It was then homogenized to fine powder and stored in air-tight container for further analysis.

### Preparation of water and ethanol extracts

Shade dried and powdered plant materials were extracted with ethanol and water using hot extraction technique separately. A total of 10gm of individual powder was taken and mixed with 50 ml distilled water (1:5) in a round bottom flask and gentle refluxed for 1½ hour. 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 25% solid yield. Same procedure was followed using ethanol instead of distilled water to prepare the hot ethanol extract.

### Organoleptic Evaluation

Organoleptic evaluation refers to evaluation of the whole plant powder of the *E. littorale*, and root powder of *W. somnifera* by colour, odour, taste, texture, touch etc. The organoleptic characters of the sample were evaluated based on the method described by Siddiqui and Hakim 2005.

### Physicochemical Investigations

Six samples of whole plant powder of *E. littorale* and root of *W. somnifera* 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 (WHO, 1998).

### 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 both powdered plant materials was determined using standard simple glass electrode pH meter [26].

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

Separately place about 1.0g of both powdered plant materials in an accurately weighed moisture disc (Electronic measurement scale – Mettler Toledo) separately. For estimation of loss on drying, these were 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 / percentage of air-dried material.

### Determination of hot water and ethanol-extractable matter

Separately place about 4.0g of both powdered plant in an accurately weighed, glass-stoppered conical flask separately. 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 / percentage of air-dried material.

#### Determination of total ash

Two grams of the both powdered plant materials were placed in a previously ignited (350°C for 1 hour) and tarred separate crucibles 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/ percentage of air-dried material.

#### 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/ percentage of air-dried material.

#### Determination of water-soluble ash

Twenty- five (25) ml of water was added to the crucible containing the total ash, covered with a watch-glass 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 / percentage of air-dried material.

#### 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 both powdered plant materials were placed in a previously ignited separate crucibles, 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 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 (WHO, 2012).

#### Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanol and water (hot) extracts of whole plant powder of *E. litorale* and root powder of *W. somnifera* 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, quinones, Fixed oils and fats.

#### 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 [27].

#### 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.

#### 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.

#### 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 [28].

#### Determination of Steroid glycosides

Liebermann Burchard's test- Extract was dissolved in equal volumes of anhydrous acetic acid and chloroform (CHCl<sub>3</sub>) and cooled to 0°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.

#### 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 reagent (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 (Saxena et al., 2012)..

#### 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.

#### Detection of Quinones

To the test sample, sodium hydroxide is added. Formation of blue, green, or red colour indicates the presence of quinines [29].

#### Detection of Anthraquinones

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

#### 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.

#### 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.

#### 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.

#### 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.

### RESULTS AND DISCUSSION

The organoleptic characters of whole plant powder of *E. littorale* and root powder of *W. somnifera* were as shown in Table 1. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs (Nasreen and Radha 2011). Table 2 presents the result for the physicochemical analysis of whole plant powder of *E. littorale* and root powder of *W. somnifera*. It shows that the highest percentage of hot water and ethanol extractable matter; total ash and acid insoluble ash ( $37.21 \pm 1.27\%$  &  $24.92 \pm 0.64\%$ ,  $8.16 \pm 0.1\%$  &  $1.89 \pm 0.1\%$ ) were found in *E. littorale* when compared with *W. somnifera* ( $30.82 \pm 1.09\%$  &  $10.0 \pm 0.21\%$ ,  $5.76 \pm 0.1\%$  &  $0.63 \pm 0.04\%$ ). Presence of water soluble ash and sulfated ash ( $2.93 \pm 0.15\%$  &  $1.92 \pm 0.04\%$ ) in *W. somnifera* were slightly higher than *E. littorale* ( $2.75 \pm 0.1\%$  &  $1.30 \pm 0.04\%$ ) and percentage of loss on drying was found in *W. somnifera* ( $9.12 \pm 0.10\%$ ). It was slightly lower than *E. littorale* ( $10.25 \pm 0.33\%$ ).

The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth (Nasreen and Radha 2011). Total ash value of plant material indicated the amount of minerals and earthy material attached to the plant material. The water soluble extractive value was indicating the presence of sugar, acids and inorganic compounds and alcohol soluble extractive values indicated the presence of polar constituents.

**Table 1. Organoleptic Properties of Whole Plant of *E. littorale* and root of *W. somifera***

Parameters	Whole Plant of <i>E. littorale</i>			Root of <i>W. somifera</i>		
	Raw	Aqueous extract	Ethanol extract	Raw	Aqueous extract	Ethanol extract
Appearance	Powder	Liquid	Liquid	Powder	Liquid	Liquid
Touch	Coarse	Smooth	Smooth	Coarse	Smooth	Smooth
Colour	Greenish brown	Light brown	Dark green	Whitish brown	Light brown	Dark yellow
Taste	Bitter	High bitter	High bitter	Bitter	Bitter	Bitter
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic

**Table 2. Physicochemical Parameters of Whole Plant of *E. littorale* and root of *W. somifera***

Parameters	Whole Plant of <i>E. littorale</i>	Root of <i>W. somifera</i>
Loss on drying	10.25 ± 0.33	9.12 ± 0.10
Total ash value	08.16 ± 0.09	5.76 ± 0.09
Water soluble ash	02.75 ± 0.08	2.93 ± 0.15
Acid insoluble ash	01.89 ± 0.07	0.63 ± 0.04
Sulfated ash value	01.30 ± 0.10	1.92 ± 0.04
pH of 1% w/v formulation solution	05.12 ± 0.02	5.73 ± 0.02
pH of 10% w/v formulation solution	04.87 ± 0.04	5.64 ± 0.03
Water soluble (hot) extractive value	37.21 ± 1.27	30.82 ± 1.09
Ethanol soluble (hot) extractive value	24.92 ± 0.64	10.00 ± 0.22

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

**Table 3. Phytochemical Screening for Aqueous and ethanolic extracts of Whole Plant of *E. littorale* and root of *W. somifera***

No	Components	Whole Plant of <i>E. littorale</i>		Root of <i>W. somifera</i>	
		Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract
01	Phenolic compound	+++	+++	+++	+++
02	Tannins- <i>Ferric chloride test</i>	+++	+++	+++	+++
03	Flavonoids- <i>Shinoda test</i>	++	+++	+	+++
04	Coumarins	++	+++	++	+++
05	Steroid-glycosides- <i>L. Burchard's test</i>	+++	+++	++	+++
06	Alkaloids <i>Mayer's Test</i> <i>Dragendroff's Test</i>	+++	+++	++	+++
		+++	+++	++	+++
07	Protein- <i>Xanthoproteic Test</i>	++	+	++	++
08	Quinone	++	+++	0	0
09	Anthraquinones	0	0	0	0
10	Saponins- <i>Foam test</i>	++	++	+	++
11	Reducing sugars- <i>Fehling's test</i>	+++	+++	+++	+++
12	Fixed oil and Fats- <i>Spot test</i>	++	0	++	0

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

Secondary metabolites were found in good proportion in alcoholic and aqueous extracts when compared. These secondary metabolites may be responsible for various pharmacological effects of ethanolic, methanolic and aqueous extracts of preparations. The phytochemical active compounds of hot water and ethanol extract of *E. littorale* and *W. somnifera* were qualitatively analyzed and the results are presented in Table 3. In analysis of Tannin compounds brownish green colour developed to indicate the presence of Tannin. Similarly based on the presence or absence of colour change indicate positive and negative results are indicate. In these screening process alkaloids, saponins, flavonoids, steroids, tannins, coumarins, phenols, proteins, reducing sugars, quinones, fixed oil and fats gave positive results for both plant materials exception quinones, gave negative results for *W. somnifera*. Anthraquinones gave negative results for both plant materials. The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity [30].

## CONCLUSION

Herbal drug which are used in various traditional medicine needs detailed investigation with ethno-pharmacological approach. In the present study Phyto-Physicochemical analysis of Whole Plant of *E. littorale* and root of *W. somnifera* has been carried out according to World Health Organization Guidelines and standard laboratory procedures. This comparative information provides immense potential for studying their activities for arthritis and other disease conditions, both in pre-clinical and clinical stages, which lead to the preparation of useful pharmaceutical products. The present study has authenticated the usefulness of the identified plants for medicinal purposes. These species could also be seen as potential source of useful drugs due to their rich contents of phytochemical.

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