

**PHYTOCHEMICAL INVESTIGATION, DIURETIC AND ANTI  
INFLAMMATORY ACTIVITY OF ROOT AND STEM EXTRACTS OF  
*BOERHAAVIA ERECTA* LINN IN EXPERIMENTAL ANIMALS**

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**ABSTRACT:** The pharmacological and biological properties and chemical constituents of plants from the plant *Boerhaavia erecta* which is widely used in folk medicine. In the present study, the anti-inflammatory, diuretic activity of ethanolic, ethylacetate extracts and column isolated compound SU I of whole plant of *Boerhaavia erecta* were studied and the activity was compared with Diclofenac sodium, Furosemide as standard. The anti-inflammatory activity was found out by Carragenan induced paw edema method by using standard Diclofenac sodium. The diuretic activity was found out by Lipschitz *et al* method by using standard Furosemide. Preliminary phyto chemical screening showed the presence of Carbohydrates, proteins, amino acids, Saponin, flavanoids, Phyto sterols and Tannins are present in ethanol extract. The ethanolic extract exhibited significant in both activities. The anti-inflammatory activity showed \*\*\* P<0.001, \*\* P<0.01, \*P<0.02 . compared with standard.

**Keywords:** *Boerhaavia erecta*, Anti inflammatory activity, Diuretic activity.

## INTRODUCTION

*Boerhaavia erecta* Linn (Erect spiderling), Family: Nyctaginaceae, Indigeneous to tropical America and introduced to India, commonly found as a weed in many parts of India, especially in Gangetic plains, Maharashtra and Kerala. An erect, puberulous annual plant up to 8 inches high. Ribbed stems, ovate leaves, tiny flower clusters and inverted, cone shaped fruits on the ends of slender, base stalks. Plant parts used are Leaf, root and seeds. The leaf juices serves as lotion in ophthalmia. It is given internally as a blood purifier and to relieve muscular pain. The leaves are cooked and eaten as spinach. The roots and seeds are added to cereal, pancakes and other food. The oil made of the root is administered to people suffering from Jaundice [1,2,3,4]. It is also an antidote to snake venom. Topical use of roots has well anti inflammatory effect. Fresh juice of leaves is used in dropsy and chronic renal failure in a dose of 20ml two to three times a day. Roots boiled with milk are used in a single daily dose for maintaining health.

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## MATERIALS AND METHODS

### Plant material

The plant has been identified by Dr. Stephen, Dept. of Botany, American College, Madurai. The plant were collected from the watery areas of Ernakulam district, Kerala in the month of July. The plants were then washed with water to remove soil and other extraneous matter. The stem and roots of the plant was cut into small pieces and were dried under shade for 15 days. Then the dried material was homogenized to coarse powder and was stored in an airtight container.

### Extraction

650 grams of the dried coarse powder of *Boerhaavia erecta* Linn was extracted by continuous hot percolation method using different solvents namely pet ether (40-60°C), chloroform, ethyl acetate and ethanol. The extraction was carried out with 900 ml of each solvent for a period of 72 hours. At the end of the extraction the respective solvents were concentrated under pressure and the crude extracts were stored in a dessicator. The marc left was then dried and extracted were later subjected to qualitative tests for the identification of various constituents.

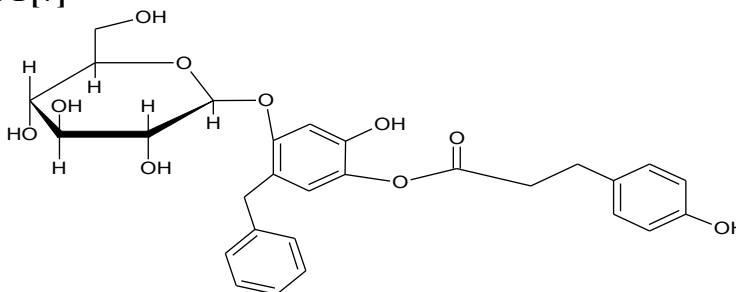
### Preparation of column chromatography

Ethyl acetate and alcoholic extracts were combined and chromatographed (15gms) over silica gel column. The solvents used were pet ether, chloroform, methanol and their mixtures in various proportions in the order of increasing polarity. Each 100ml of the elute was collected and concentrated. The obtained fractions were tested for the presence of various constituents and nature of the compounds. (S-II, S-III)

100gms of dried plant powder was extracted with chloroform and ethyl acetate to obtain a brown residue (3.5gms). The brown residue was boiled with 25ml of 2N acetic acid for 6 hours and left overnight at 4°C. The solution was distinctly alkaline (above pH-9) with ammonia and stirred for 2 hours. This solution was kept at 4°C for 18 hours. The extract was centrifuged in Remi analytical centrifuge at 8,000 r.p.m. for 15 min and pellet was recovered. The residue which is supposed to contain alkaloid was repeatedly extracted with chloroform. The chloroform layer was collected and shaken with anhydrous Na<sub>2</sub>SO<sub>4</sub> to remove any traces of water. The chloroform extract was evaporated and the crude alkaloid was fractionated over silica gel column. Each 50ml of the elute was collected. Two compounds were recovered and named as SA-I, S-I.

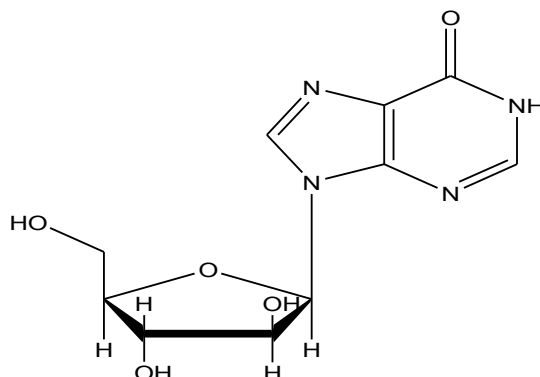
### Phytochemical investigation [5,6]

#### Compound SA-I [7]



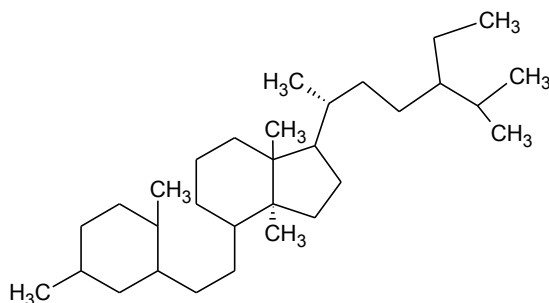
A pale white solid obtained from the chloroform extract of alkaloid, has the melting point of 280°C and Rf value 0.75 (solvent system Chloroform: methanol 9:1). IR spectra shows the bands at 3561, 1610 and 740cm<sup>-1</sup> N-H stretching. The peak at 1712cm<sup>-1</sup> C-H stretching. NMR spectra SA-I shows <sup>1</sup>HNMR singlet at 1.4δ ppm CH<sub>2</sub> proton, singlets 2.2δ ppm aromatic methyl proton, 7.2-8.4δ aromatic hydrogen and CH-coupling.

### Compound S-I



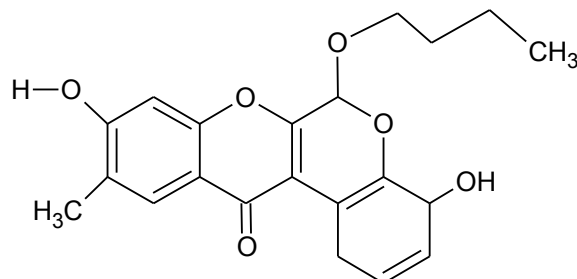
A dark brown solid obtained from the chloroform extract of alkaloid, has the melting point 275°C and Rf value 0.618 (solvent system Chloroform: ethyl acetate: methanol 7:2:1). IR spectra shows the bands at 3650-3521cm<sup>-1</sup> N-H stretching, a strong band at 2846cm<sup>-1</sup> C-H stretching, 1710.5cm<sup>-1</sup> C=O group, 1118.5cm<sup>-1</sup> C-O group, 971.9-898cm<sup>-1</sup> C-H bending. NMR spectra of compound S-I shows singlets at 1.2-1.4δ ppm CH<sub>2</sub> protons, singlets at 1.9δ C=C-CH<sub>3</sub>, singlet at 2.05δ C=O-(CH<sub>3</sub>), triplet at 4.4δ O-H or C-H, multiplet at 7.1-7.4δ ppm aromatic hydrogen, doublet at 7.8δ hetero aromatic ring.

### Compound S-II [8]



A yellow solid obtained from the ethyl acetate and ethanolic extract, has the melting point 130°C and Rf value 0.80 (solvent system Chloroform: pet ether 9:1). IR spectra the bands at 3525-3247cm<sup>-1</sup> O-H group, a strong band at 2854.1cm<sup>-1</sup> C-H stretching, the peak at 1722.1cm<sup>-1</sup> C=O group, 1461cm<sup>-1</sup> C-H bending. NMR spectra of compound S-II shows doublet at 1.25δ ppm CH<sub>2</sub> protons, doublet at 0.9δ may be due to methyl and methylene protons.

A brown solid obtained from the ethanolic extract, has the melting point 240°C and Rf value 0.81 (solvent system Chloroform: methanol 9:1). IR spectra shows the bands at 2927-2854 cm<sup>-1</sup> C-H stretching, 1718.2cm<sup>-1</sup> C=O group, 1461cm<sup>-1</sup> C-H bending, 971.9cm<sup>-1</sup> C-H (out of plane). The NMR spectra of compounds S-III singlet at 1.3 and 1.2δ CH<sub>2</sub> protons.

**Compound S-III****Preliminary phytochemical investigation [9,10,11,12]**

The qualitative chemical test of various extracts of *Boerhaavia erecta* was carried out using standard procedure. Carbohydrate, Phytosterols, Tannins, Saponins, Terpenoids, Flavonoids, Protein and Aminoacids and Alkaloids are present in the extracts.

**Animals used**

Albino wistar rats 120-150 gms and Swiss albino mice 25-30 gms of either sex were obtained from the standard animal house, Madurai. The animals were maintained in a well ventilated room with 12:12 hour light / dark cycle in polypropylene cages [13]. The animals were fed with standard pellet feed and water was given *adlibitum*.

**Screening of Anti-Inflammatory activity**

Anti-inflammatory Activity of was studied in adult albino rats of either sex weighing between 120-150gms by Carrageenan induced hind paw oedema method [14,15]. Animal were divided into four groups containing 6 animals per each. Group I (control) was given a 1% Sodium Carboxy methyl Cellulose solution. Group II of animals received 6.75mg/kg of Diclofenac sodium which was considered as standard. Group III and Group IV were treated with 112.5 mg/kg SA I and ethylacetate extract respectively dissolved in Sodium Carboxy methyl Cellulose solution. Doses were given orally with the help of an oral catheter. 0.1 ml of 1% solution of carrageenan was administered to the rats into the plantar surface of the right hind limb to induce paw oedema. Paw volume was measured plethysmographically after 1h, 2h and 3h, 4h of carrageenan injection and paw swelling in groups of drug treated were compared with control. Percentage inhibition of edema was calculated by using the following formula (Table 1)

$$\% \text{inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

where

**V<sub>t</sub>**- means increase in paw volume in rats treated with test compounds

**V<sub>c</sub>**-means increase in paw volume in control group of rats.

**Table 1: ANTI INFLAMMATORY ACTIVITY OF EXTRACT AND COMPOUND OF *Boerhaavia erecta* Linn. AGAINST CARRAGEENAN INDUCED PAW****EDEMA IN ALBINO RATS**

S.No	Drug	Mean increase in edema $\pm$ SEM (X $\pm$ SEM)% inhibition of Edema				
		0 Hour	1 hour	2 hours	3 hours	4 hours
1	control	0.34 $\pm$ 0.013	0.47 $\pm$ 0.073	0.56 $\pm$ 0.082	0.75 $\pm$ 0.097	0.80 $\pm$ 0.012
2	Diclofenac sodium	0.28 $\pm$ 0.011 17.6%	0.38 $\pm$ 0.060 19.1%	** 0.35 $\pm$ 0.015 37.5%	*** 0.34 $\pm$ 0.079 54.6%	*** 0.32 $\pm$ 0.012 60.0%
3	Ethanollic extract	0.31 $\pm$ 0.047 8.8%	0.37 $\pm$ 0.010 21.2%	* 0.40 $\pm$ 0.079 28.5%	** 0.49 $\pm$ 0.059 34.6%	** 0.51 $\pm$ 0.079 36.2%
4	Compound SA-I	0.30 $\pm$ 0.048 11.7%	0.40 $\pm$ 0.039 14.8%	* 0.44 $\pm$ 0.098 21.4%	** 0.51 $\pm$ 0.014 32.0%	** 0.53 $\pm$ 0.036 33.7%

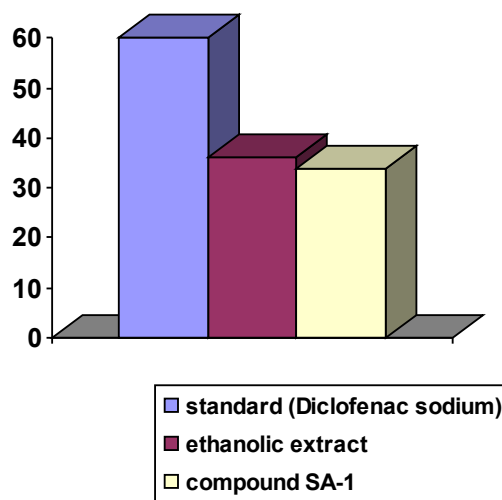
SEM : Standard Error Mean

\*\*\* : P&lt;0.001

\*\* : P&lt;0.01

• : P&lt;0.02

Percentage inhibition =  $\frac{\text{Volume of control} - \text{Volume of test}}{\text{Volume of control}} \times 100$

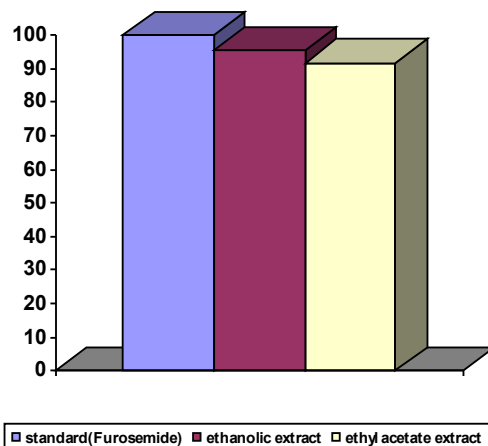


### Diuretic activity

The method of Lipschitz et al [16,17] was employed for the assessment of diuretic activity. The animals were fasted and deprived of water for 18 hrs prior to the experiment, were divided into 4 groups having 6 rats each. The first group of animals serving as control received normal saline, the second group received 30 mg/kg furosemide as standard drug, and the remaining group received the ethanol and ethylacetate extract at a dose of 100mg/kg. Immediately after dosing, the animals were separately placed in metabolic cages (3 in each cage) suitable for collection of urine in graduated measuring cylinders at  $25\pm 0.5^{\circ}$  through out the experiment. Urine samples were collected for 3 hours, while animals were deprived of food and water. During this period, no water or food was made available to animals.  $\text{Na}^+$ ,  $\text{K}^+$  concentrations were measured by Flame photometry [18] and  $\text{Cl}^-$  concentration was estimated by titration [19] with silver nitrate solution (N/50) using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20 mg/kg of furosemide per kg body weight in series of supportive experiments. (Table 2).

**Table 2: DIURETIC ACTIVITY OF ROOT AND STEM EXTRACT OF *Boerhaavia erecta* Linn**

S.No	Drug/extract	Volume of urine Mean $\pm$ SEM	Urine volume deviated from control	%diuretic activity
1	Control	1.96 $\pm$ 0.06	-	-
2	Standard(Furosemide)	3.96 $\pm$ 0.033	2	100
3	Ethanollic extract	3.83 $\pm$ 1.0.033	1.87	95.40
4	Ethyl acetate extract	3.76	1.8	91.83



### Statistical analysis

All the results are expressed as mean  $\pm$  standard error. The data was analyzed statistically using ANOVA followed by student 't' test [20] at a probability level of \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.02$ .

## RESULTS AND DISCUSSION

The diuretic activity on the ethanolic and ethyl acetate extracts of the root and stem of *Boerhaavia erecta* Linn were screened. The ethanolic extract exhibit significant diuretic activity when compared with fruosomide as standard. Diuretics relive pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure [21]. Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ethanol, aqueous and chloroform extract may produce diuretic effect by increasing the excretion of Sodium, Potassium and Chloride.

The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles [22]. The regulation of Sodium, Potassium balance is also intimately related to renal control of acid-base balance. The Potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended [23]. In present study chloroform, aqueous and ethanol extracts showed elevated levels of Potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavonoids, saponins and terpenoids are known to be responsible for diuretic activity [24,25]. These active principles in aqueous and chloroform extracts may be responsible for diuretic activity. Results of present investigation showed that ethanol is most effective in increasing urinary electrolyte concentration of all the ions i.e Sodium, Potassium and Chloride followed by chloroform and aqueous extracts while petroleum ether extract did not show significant increase in urinary electrolyte concentration.

About the anti-inflammatory activity on the root and stem extracts of *Boerhaavia erecta* Linn. the ethanolic extract exhibited significant anti inflammatory activity when compared to compound SA-I by keeping diclofenac sodium as standard. Carrageenan induced paw edema was taken as a proto type of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator leaves through a common trigger mechanism. The development of carrageenan induced edema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin [26,27,28] and the delayed phase is sustained by the leucotrienes and prostoglandins [29]. Flavonoids and tannins are reported to inhibit PG Synthetase synthesis [30]. Most of the NSAIDS have well balanced anti inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity. The plant extract possess a marked anti-inflammatory activity and hence may pose itself as very good anti-inflammatory drug. Still further investigation with respect to pharmacological and phytochemical profile of the drug needs to be carried out. Three distinct phases are observed during inflammation which are the histamine and serotonin released in the first phase, Kinin and Prostoglandin are released in the second and third phases respectively [31]. Carrageenan induced hind paw oedema in the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. The extract *Boerhaavia erecta* produced significant inhibition of Carrageenan induced paw oedema. The inhibition was however less than that of the standard drug.

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