

DIURETIC POTENTIAL OF *AERVA LANATA* AND *ECBOLIUM LIGUSTRINUM* ROOT EXTRACTSNIMMAKAYALA SRIDHAR^{1*}, BONDADA V.V.S. SURYA KIRAN², MITHUN RUDRAPAL³

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This paper is available online at www.jprhc.in**ABSTRACT:**

Based on the ethnobotanical importance as diuretics *Aerva lanata* and *Ecbolium ligustrinum* were selected for present screening. Experiment was carried out on male wister strain albino rats using furosemide as standard drug. 20mg/kg and 200mg/kg oral doses are selected for standard and test respectively. For quantifying the diuretic activity the urine output, urine pH, sodium, potassium and chloride levels in the urine are measured. The diuretic index value of *Ecbolium ligustrinum* is 1.70 and nearer to the standard furosemide value 1.80. *Aerva lanata* group shows less diuretic index of 1.49 compared to standard. The ratio of the concentration of sodium ions to the potassium ions in control group was found to be 1.39 and the ratio for standard, *Aerva lanata* and *Ecbolium ligustrinum* are 1.78, 1.49 and 1.68 respectively. *Ecbolium ligustrinum* affected the amount of urine excreted and also the electrolyte concentration in urine. The present study concluded that roots of *Aerva lanata* and *Ecbolium ligustrinum* are having diuretic nature.

KEYWORDS: Diuretic activity, *Aerva lanata*, *Ecbolium ligustrinum*, Roots.**INTRODUCTION:**

Diuretics are the drugs which increase the process of urine formation. Some drugs like digitalis will also increases the urine out flow by mobilizing the oedema fluid when given to patients with congestive heart failure. But the term diuretic is generally restricted to the agent which acts directly on kidney¹. The diuretic effects of a molecule can be useful in variety of clinical compliances like congestive heart failure, pregnancy toxemia, cirrhosis, nephritic syndrome, renal failure and widely in hypertension patients². *Aerva lanata* (*L.*) *juss* is a small herb belongs to the family *Amaranthaceae*. It grows mainly in warmer parts of India. Traditionally it is useful as anticalculus, diuretic, demulcent, anthelmintic, ant diarrheal, anticholinergic, boric. Leaves of the plant are useful in hepatitis and roots in strangury. The flowers and roots are used in headache. The plant contains chemical constituents like beta-sitosterol, palmitic acid and alpha amyryl³. Due to its wide range of traditional applications different biological activities of the plant was extensively published.

Ecbolium ligustrinum (*Vahl.*) *vollesen* also known as *Ecbolium viride* is an erect glabrous herb belongs to the family *Acanthaceae*. The plant parts are mainly used for gout and dysuria in traditional medicine. Roots of the plants are used for jaundice, menorrhagia and rheumatism⁴. It was reported that root are having glycoflavones like orientin, isoorientin, vitexin, isovitexin⁵ and lignin like Ecbolin A⁶. The plant was reported to have radical scavenging⁷, hepatoprotective⁸ antidiabetic⁹ and antimicrobial¹⁰ properties. As per the ethnobotanical survey conducted in East Godavari district of Andhra Pradesh, we found that the roots of these two plants are used in folk medicine for their diuretic properties. The review of the scientific literature did not reveal any significant information on diuretic properties of these two plant roots. So it was consider worthwhile to elucidate the diuretic properties of roots of *Aerva lanata* and *Ecbolium ligustrinum*.

MATERIALS AND METHODS:**Animals:**

Wister strain male albino rats weighing 150-180g were selected for the experimentation. Rats were maintained in a husk lined polypropylene cages at standard environmental conditions of 25±2°C temperature and 55±10% relative humidity with 12:12 hr dark light cycle. The rats are fed with standard chow diet and water *ad libitum*. The study was started after the approval of Institutional Animal Ethical Committee (Reg no: 1633/PO/a/12/CPCSEA) by strictly following CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

Drugs and Chemical:

Standard drug furosemide was obtained from Aventis pharma, Mumbai (Lasix). The solvents of analytical grade used in the experimentation are obtained from EMerck Limited, Mumbai and SD fine chemical Limited, Mumbai.

Collection and Identification of Plant:

Plant material was collected from different areas of East Godavari district, Andhra Pradesh during the month of September 2013 on day time. The two plants were taxonomically identified by the experts of Botanical Survey of India, Hyderabad (BSI/DRC/2013-14/Tech./522 and BSI/DRC/2013-14/Tech./915).

Extraction of Plant Material:

Roots of both the plants were separated and made free from soil matter. They were dried and powdered by using hand pulveriser to a coarse powder. The roots of *Aerva lanata* and *Ecbolium ligustrinum* were extracted with Methanol by using Soxhlet apparatus at a temperature of 50-55°C for 8h. The extracts were distilled, concentrated and the semisolid mass was dried in vacuum desiccators.

Acute Toxicity Study:

The toxicity of plant extracts on experimental animal was tested according to the OECD-423 (Organisation of Economic Co-operation and Development) guideline. After 24h of the oral administration of both extracts (2000mg/kg) none of the rats showed significant signs of toxicity. The observation was continued for 14 days twice daily during which we does not found any drug related behavioural changes¹¹.

Diuretic Activity:

For the elucidation of diuretic activity we adopted the method of Lipschitz *et al*^{12, 13}. The experimental rats were divided into four groups of six animals each, which were deprived of food and water 18h before the experiment. Prior to the administration of drugs the bladder was emptied by pulling the tail at its base and then 25ml/kg dose of normal saline was given to each animal. Group 1 serves as control and received deionised water, Group 2 received standard drug furosemide 20mg/kg, Group 3 and Group 4 received 200mg/kg doses of *Aerva lanata* and *Ecbolium ligustrinum* methanol extracts respectively. After the administration of the drugs the animals were transferred to metabolic cages which are designed to separate the faeces and urine collection. The urine was collected into a graduated cylinder from the funnel outlet and the volume was measured up to a period of 5hours. Prior to the urine collection the cylinders were rinsed with mineral oil for prevention of urine evaporation. For quantifying the diuretic activity the urine output, urine pH, sodium, potassium and chloride levels in the urine are measured. The concentration of sodium and potassium were measured by flame photometry¹⁴ and chloride concentration by gravimetric titration against 0.02N silver nitrate solution using 5% potassium chromate as indicator¹⁵.

Statistical Analysis:

Data was represented as mean \pm SEM and analysed by one- way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION:

The results of the evaluations carried for diuretic assay on the test extracts were shown in the Table 1. The results indicating that the methanol extract of the roots of *Ecbolium ligustrinum* is having more activity than that of methanol extract of *Aerva lanata* roots. The urine volumes of two test groups (*A. lanata*: 6.15 \pm 0.24ml/100g/5h; *E. ligustrinum*: 7.00 \pm 0.85 ml/100g/5h) showing significant variation compared to that of control group (4.12 \pm 0.99 ml/100g/5h) and also significant compared to that of standard (7.43 \pm 0.25 ml/100g/5h). These values reviling the diuretic nature of test plant extracts.

Table 1: Diuretic activity of *Aerva lanata* and *Ecbolium ligustrinum* root extracts

Drug	Dose (mg/kg)	Urine Volume (ml/100g/5h)	Urine pH	Diuretic index	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	Na ⁺ /K ⁺
Control	-	4.12 \pm 0.99	7.00 \pm 0.88	-	101.00 \pm 4.01	72.54 \pm 1.32	60.88 \pm 5.39	1.39
Furosemide	20	7.43 \pm 0.25	6.71 \pm 0.59	1.80	159.30 \pm 8.26	89.34 \pm 2.95	92.67 \pm 1.79	1.78
<i>A. lanata</i>	200	6.15 \pm 0.24	6.85 \pm 0.38	1.49	111.80 \pm 3.42	74.83 \pm 1.85	61.92 \pm 2.10	1.49
<i>E. ligustrinum</i>	200	7.00 \pm 0.85	6.76 \pm 0.43	1.70	145.08 \pm 2.38	86.30 \pm 6.23	87.39 \pm 1.77	1.68

All the values are expressed as mean \pm SEM (n=6); $p < 0.05$ compared with control.

The diuretic index value of *E. ligustrinum* is 1.70 and nearer to the standard furosemide value 1.80. *A. lanata* group shows less diuretic index of 1.49 compared to standard. The pH values of test and standard groups dose not showing significant difference compared to that of control group. When we estimated the excretion of sodium for 5h, the value of standard drug was high (159.30 \pm 8.26 mmol/L) followed by the value of *E. ligustrinum* (145.08 \pm 2.38 mmol/L). Though *A. lanata* shows good urine output it does not having significant effect on sodium excretion (111.80 \pm 3.42 mmol/L). The potassium and chlorine ion excretion values are also similar to that of sodium. The concentration of potassium and chlorine dose not showed much variation on administration of *A. lanata* extracts. The ratio of the concentration of sodium ions to the

potassium ions in control group was found to be 1.39 and the ratio for standard, *A. lanata* and *E. ligustrinum* are 1.78, 1.49 and 1.68 respectively. From the urinary electrolyte concentrations we can state that methanol extract of *Ecbolium ligustrinum* roots was most effective in increasing urinary electrolyte concentration. The methanol extract of *Aerva lanata* roots was showing its effect only on urinary volume but not on electrolyte concentration.

Furosemide is a loop diuretic act on the thick ascending limb and inhibits the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ carrier. It is used in sports for achieving rapid weight loss during screening and also to mask the other agents present in the urine¹⁶. The effect of *Ecbolium ligustrinum* root extract was comparable to that of furosemide. So we can estimate that the mode of action is same. The studies on phytochemicals present in the *Aerva lanata* reveals the presence of alkaloids, carbohydrates, flavanoids, glycosides, terpenoids, proteins and steroids. It was also found that the whole plant consists of 27 different types of terpenoids in which 5 are only present in roots¹⁷. The presence of 6 types of saponins also evidentiary¹⁸. The phytochemical studies on roots of *Ecbolium ligustrinum* reported to contain alkaloids, phenolic compounds, flavanoids and tannins¹⁹. The flavanoids present in the two plants may be responsible for the diuretic activity as reported earlier that flavanoid glycosides cause diuresis²⁰. The chemical molecules present in the extracts may individually or synergistically responsible for the results obtained. The *Ecbolium ligustrinum* is having more activity but not comparable in terms of quantitative activity elicited by the standard due to the use of crude extracts. By the isolation of individual active principles from these roots will be more helpful in elucidating and quantifying the activity more clearly. Searching new molecules in plants and developing the competitive leads for fulfilling safe pharmacological therapeutic needs is the major strategy following by pharmacologists now a days. In this prospect we have selected two plants for screening the diuretic potential and we got the expected positive results. The present study thus concluded that roots of *Aerva lanata* and *Ecbolium ligustrinum* are having diuretic properties and justifies the folk use as diuretic.

REFERENCES:

- 1) Peter A. Friedman, William O. Berndt. Diuretics. In: Charles R. Craig, Robert E. Stitzel, authors. Modern pharmacology with clinical applications. 6th ed. United States: Lippincott Williams & Wilkins; 2004. p. 244.
- 2) B. C. Koti, A. Purnima. Diuretic activity of extracts of *Centrathem anthelminticum*. International Journal of Green Pharmacy 2008; 2: 228-31.
- 3) C.P. Khare. Indian Medicinal Plants: An Illustrated Dictionary. New York: Springer; 2007.
- 4) A. Elumalai, M. Chinna eswaraiyah, M.Lakshmi. A short review on un-explored medicinal plant: *Ecbolium viride*. International journal of research in ayurveda and pharmacy 2011; 2: 1539-40.
- 5) Nair A.G.R, Ramesh P, Sankarasubramanian S. Occurrence of glycoflavones in Acanthaceae. Phytochemistry 1975; 14: 1644.
- 6) Venkataraman R, Gopalakrishnan S. A lignan from root of *Ecbolium linneanum* Kurz. Phytochemistry 2002; 61:963–66.
- 7) Ashoka Babu V. L, Arunachalam G, Jayaveera K. N, Madhavan. V, Shanaz Banu. Free radical scavenging activity of methanolic extract of *Ecbolium viride* (Forssk). Alston roots. Der Pharmacia Lettre 2011; 3: 285-88.
- 8) Preethi Priyadharshni S.P, T.Satyanarayana, B.Ganga Rao, Rajesh.K. Hepatoprotective activity of *Ecbolium viride* Forssk. Alston. Acanthaceae on experimental liver damage in rats. International Research Journal of Pharmaceutical and Applied Sciences 2011; 1:27-33.
- 9) Ranjitsingh B Rathor, Rama Rao D, Prasad Rao. Phytochemical screening and antidiabetic, antioxidant effect of *Ecbolium ligustrinum* flower extracts. Indian Journal of Research in Pharmacy and Biotechnology 2013; 1: 575-80.
- 10) K Francina Cecilia, R Ravindhran, V Duraipandiyar. Evaluation of Antimicrobial efficacy of *Ecbolium viride* Forssk. Alston root extracts. Asian Journal of Pharmaceutical and Clinical Research 2012; 5: 239-41.
- 11) Ghosh MN. Fundamentals of experimental pharmacology. 3rd ed. Kolkata: Hilton and Company; 2005. 190-7.
- 12) Lipschitz WL, Hadidian, Kerpcer A. Bioassay of Diuretics. Journal of Pharmacology and Experimental Therapeutics 1943; 79: 97-110.
- 13) Basavaraj C. Koti, Purnima Ashok. Diuretic activity of extracts of *Mimusops elengi* Linn. Bark. International Journal of Green Pharmacy 2010; 4: 90-92.
- 14) Jeffery GH, Bassett J, Mendham J, Denny RC. Vogel's text book of quantitative chemical analysis. 5th ed. England: Addison Wesley Longman Ltd; 1989. p. 801.
- 15) Beckette AH, Stenlake JB. Practical pharmaceutical chemistry. Part I. 1st ed. New Delhi: CBS Publishers; 1997. p. 197.
- 16) HP Rang, MM Dale, JM Ritter, RJ Flower, G Henderson. Rang and Dale's Pharmacology. 7th ed. New York: Elsevier publishers; 2012. p. 353.
- 17) Yamunadevi M, Wesely EG, Johnson M. Phytochemical studies on the terpenoids of medicinally important plant *Aerva lanata* L. using HPTLC. Asian Pacific Journal of Tropical Biomedicine 2011; 220-25.
- 18) Yamunadevi M, Wesely EG, Johnson M. Chromatographic finger print studies on saponins of *Aerva lanata* L. Juss. ex schultes by using HPTLC. International Journal of Current Pharmaceutical Research 2012; 4: 52-57.
- 19) Ashoka babu VL, G Arunachalam, KN Jayaveera, V Madhavan, S Banu. Hepatoprotective activity of methanolic extract of *Ecbolium viride* Forssk Alston roots against carbon tetrachloride induced hepatotoxicity. International research journal of pharmacy 2012; 3: 251-53.
- 20) Kavimani S, Ilango R, Gurubatham J, Jaykar B, Majumber UK, Gupta M. Acetylcholine antagonistic action of aqueous extract of *orthosiphon thymiflorus*. Indian Journal of Pharmaceutical Sciences 1997; 59:271-2.