

Screening of Aerial Parts of the Plant *Clerodendrum Paniculatum* Linn for Diuretic Activity

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Abstract: The present study was undertaken for investigating and validating the traditional claims of *Clerodendrumpaniculatum* Linn for its diuretic activity. *Clerodendrumpaniculatum* Linn belongs to verbenaceae family. Ethno medical importance of various species of *Clerodendrum* genus has been reported in various indigenous systems of medicines and folk medicines. It is used as a medicine for the treatment of sore eyes, urinary tract, gonorrhoea and kidney problems. Also pharmacological properties of plants have not yet been extensively studied, it is therefore thought worthwhile to study about the unrevealed properties of this plant. The aerial parts of plant are used for preparing extraction using ethyl acetate, petroleum ether and aqueous as a solvent. Shade dried, powdered (40 mesh size) leaves of *Clerodendrumpaniculatum* Linn were macerated with Ethyl acetate, Petroleum ether and Distilled water. On using solvent extraction phytochemical screening was analyzed, it reveals the presence of flavanoids, tannins, glycosides, saponins, and terpenoids like compounds. Diuretic activity of ethyl acetate extract shows significant activity comparable with standard drug, furosemide. Petroleum ether and aqueous extracts shows little diuretic activity.

Keywords: *Clerodendrumpaniculatum* Linn , Diuretic activity, Flavanoids, Furosemide, Ethyl acetate

1. Introduction

Clerodendrumpaniculatum Linn. (Family Verbenaceae) commonly known as 'Red Pagoda plant' is a semi woody shrub of 1-2 m height growing naturally in shady places throughout India. It is used traditionally in India, China and Japan, in the treatment of rheumatism, neuralgia, ulcer, inflammation, and for healing wounds[1-3]. Preliminary phytochemical screening showed the presence of terpenes, flavonoids, tannins, alkaloids, phenolic acid, sterols, glycosides, phenolic acid, sterols, and glycosides. The plant has got immense medical importance which is used for treatment for inflammation ulcer, vitratedvata, pitta wound and skin disease. A decoction of root is used as tonic for aches and pains. It is used as a medicine for the treatment of sore eyes, urinary tract, gonorrhoea and kidney problems (4,5)

The objective of this work is to evaluate the phytochemical analysis and diuretic properties of ethyl acetate, petroleum ether and aqueous extract of aerial parts of *Clerodendrumpaniculatum* Linn as a part of exploration of new and novel bio-active compounds.

2. Materials and Methods

2.1 Experimental details

Plant Materials

Clerodendrumpaniculatum Linn plant was collected from the local areas of Shivamogga and it has been authenticated by Dr. Rudrappa, Dean and Botanist, Department of Biological sciences, S.R.N.M. College, Shivamogga.

The aerial parts of *Clerodendrumpaniculatum* Linn were shade dried and reduced to a coarse powder in a Pulverizer (Sunbeam, Munger, India) using Mesh No. 3 and passed through a Sieve No. 40 to obtain about 2 kg of powder for further analysis.

Preparation of Plant Extracts

Various extracts of the plant material were prepared by soxhlet extraction method. The powdered material of *Clerodendrumpaniculatum* Linn was extracted with different solvents (ethyl acetate, petroleum ether and aqueous,) in a soxhlet extractor for 48 hrs in 8 batches of 50g each. The extract was concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and finally desiccator dried. The extract, so obtained was labelled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered aerial parts of the plant. These extracts are then used for the activities.

Preliminary screening

The presence of bio- active compounds was screened for saponins, flavanoids, terpenoids, saponins, glycosides and tannins.(6,7)

Animals

Healthy young rats (150 – 200 g) of Wistar strain of either sex were used for the acute toxicity and pharmacological studies (Diuretic activity) using ethyl acetate, petroleum ether and aqueous extracts of the aerial parts of the plant *Clerodendrumpaniculatum* Linn. The animals were procured from Central Animal House, National College of Pharmacy, Shivamogga, Karnataka. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry conditions.

Room temperature $27^{\circ} \pm 30^{\circ}\text{C}$, Relative humidity $65 \pm 10\%$, 12 hours – Light/dark cycle

All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd.,) and water was allowed *ad-libitum* under strict hygienic condition. Ethical Clearance (NCP/IAEC/CL/04/2016-17) for performing experiments on animals was obtained from Institutional Animal Ethical Committee (IAEC).

Statistical analysis

All the values were expressed as mean \pm S.E.M. Statistical analysis was carried out by performing one-way ANOVA followed by Pair wise comparisons of *Tukey's HSD* (honestly significant difference) test. A probability level of $P < 0.05$ was considered significant, $P < 0.01$ is considered as moderately significant and $P < 0.001$ is considered as highly significant.

Acute toxicity study⁸

Acute oral toxicity study for the proprietary formulation was carried out using OECD guideline 425 (modified, adopted 23rd march 2006). Tween-80 (1%) was used as a vehicle to suspend the extracts and was administered orally and the first animal receives a dose step below the level of the best estimate of the LD₅₀ and dose progression factor should be chosen to be the antilog of 1/(the estimated slope of the dose-response curve) and should remain constant throughout testing (a progression of 3.2 corresponds to a slope of 4). The testing samples were prepared by suspending the different extracts (ethyl acetate, petroleum ether and aqueous) in distilled water using tween 80 (1%) as suspending agent. The initial dose in this experiment was 200mg/kg body weight and there was no mortality or toxicity in animals hence this dose is considered as lethal. To study pharmacological activities the fraction was administered in the dose of 200mg/kg body weight which is equal to 1/10th of 2000mg/kg body weight.

Ethyl acetate, petroleum ether and aqueous extracts of *Clerodendrumpaniculatumlinn* were used to investigate the following diuretic activity at the dose of 200mg/kg body weight.

Diuretic activity

Diuretics are the drugs, which increase the flow of urine. They are useful in the treatment of the hypertension, edema, heart failure, kidney problem. Diuretic activity was carried

out by *Lipchitz et al.*, method and the parameters included were urine volume, pH and electrolytes. Furosemide was taken as the standard drug. In the present study albino rats of either sex weighing between 150-200 gm were used for screening diuretic activity. All the extracts, normal saline and standard drug were administered orally. The animals were maintained under a 12/12-light/dark cycle at the room temperature with free access to a standard pellet diet and water *ad libitum*.

The method of *Lipschitz et al.*, was employed for the assessment of diuretic activity. Five groups of six rats in each group were fasted and deprived of water for 18 hours prior to the experiment. All the animals received priming dose of normal saline solution of 25 ml per kg body weight. First group (negative control) received normal saline, second group (positive control) received the Furosemide (20mg/kg) and the remaining third, fourth and fifth group received the extracts in normal saline at the doses of 500 and 200mg/kg respectively. Immediately after the administration, the rats (two in each cage) were placed in metabolic cages specially designed to separate urine and faeces and kept at the room temperature of $25 \pm 0.5^\circ\text{C}$ throughout the experiment. The urine was collected in measuring cylinders up to 5 hrs after dosing. During the period, no feed or water is made available to the animals. The parameters taken for each individual rat was total urine volume, pH, concentration of Na⁺, K⁺ and Cl⁻ in urine. Finally the mean urine volumes were determined and used for their pH determinations using a pH meter (systronic Digital pH meter 333). Concentrations of Na⁺, K⁺ and Cl⁻ were measured by flame photometry (*Jeffery et al.*, 1989) and the diuretic potency was assessed by comparison of urine excretion due to the extracts with respect to the standard drug Furosemide^(9,10).

3. Results and Discussion

Table 1: Effect aerial parts of *Clerodendrumpaniculatumlinn* extracts on the volume of urine, its pH and electrolytes

Group	Dose (mg/ kg)	pH of the urine	Urine vol in ml/kg between 5 hr	Concentration of ions (m Eq/l)		
				Na+	K+	Cl-
Control	25	7.3 \pm 0.13	1.55 \pm 0.03	504.14 \pm 3.006	142.5 \pm 3.18	38.50 \pm 0.75
Standard	20	7.10 \pm 0.11	6.88 \pm 0.24****	702.5 \pm 3.619***	302.5 \pm 3.75***	59.67 \pm 0.51***
Ethyl Acetate	500	7.10 \pm 0.21	6.72 \pm 0.22*	637.5 \pm 3.62**	243.3 \pm 3.802*	46.34 \pm 0.07**
Petroleum Ether	500	7.0 \pm 0.21	4.72 \pm 0.22*	602.6 \pm 3.72**	207.5 \pm 3.919*	44 \pm 0.84**
Aqueous	500	7.0 \pm 0.15	4.01 \pm 0.32*	548.5 \pm 3.519*	226.8 \pm 2.89**	45.1 \pm 2.83**

Note: Data was analysed using one way ANOVA followed by pairwise comparison. Values are expressed as mean \pm S.E.M. $n=6$, **** $P < 0.001$ (highly significant), ** $P < 0.01$ (moderately significant) and * $P < 0.05$ (significant), ns=non significant.

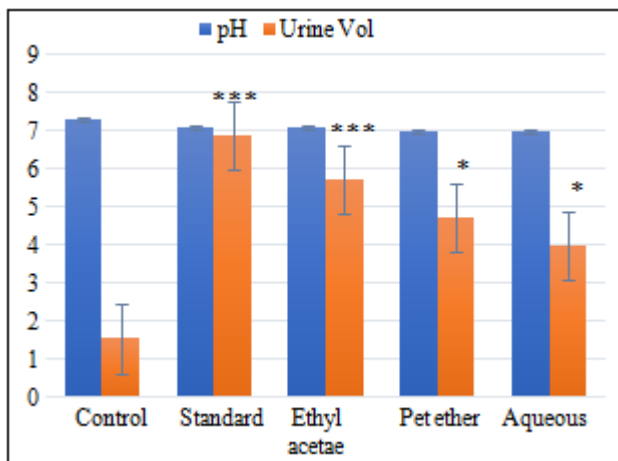


Figure 1: Histogram showing the effect of *Clerodendrumpaniculatumlinn* on pH and urine volume.

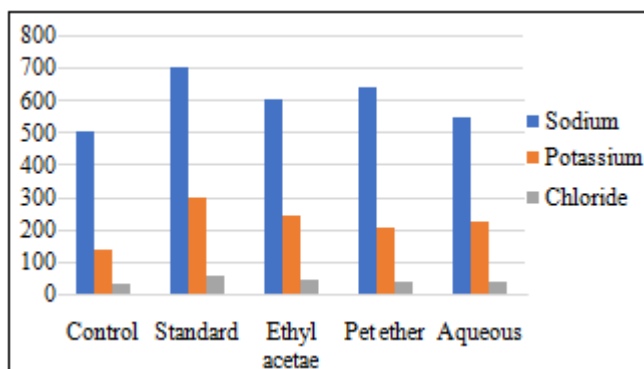


Figure 2: Histogram showing the effect of *Clerodendrumpaniculatumlinn* on electrolytes.

4. Conclusion

Diuretic activity study of ethyl acetate at the test concentrations shows significant activity ($P < 0.001$) comparable with standard drug, furosemide. Petroleum ether and aqueous extracts shows little diuretic activity.

Thus the study implicates that the aerial parts of *Clerodendrumpaniculatum Linn* possess significant dose dependant diuretic activity and therefore provides scientific base for its use in folklore remedies as an diuretic drug of natural origin. Hence there is need for further study to rationalize the active chemical entity.

References

- [1] PlantasMedicinales de Nigeria 2009. Available from: <http://www.scribd.com/doc/Plantas-Medicinales-de-Nigeria>. [Last accessed on 2009 Jan 20].
- [2] krishnakireeta-medicinal plants 2008. Available from: <http://www.ayurvedicmedicinalplants.com> [Last accessed on 2009 Dec 13].
- [3] The Wealth of India. 4th ed, Vol. 2c. New Delhi: Council of Scientific and Industrial Research; 1988. p. 231-2.
- [4] Neeta Shrivastava, Tejas Patel (2007).Clerodendrum and Healthcare: An Overview, Medicinal and aromatic plant science and biotechnology pp.143-150.
- [5] Barlow, S. M. (1990), Toxicological aspects of antioxidants used as food additives. In, Hudson, B.

J.F.(Ed.). Food antioxidants. Elsevier London: Applied Science. pp. 253-307.

- [6] Khandelwal. Practical Pharmacognosy. 1st edition, Pune: Nirali publications; 1995; 140-143
- [7] Kokate CK, Purohit AP, Gokhle SB. Practical Pharmacognosy. 4th edition Pune: Nirali prakashan 2005;108-11.
- [8] Prafulla P Adkar, Pranita P Jadhav, Shirishkumar D Ambavade, Tushar T Shelke, Vaidhun H Bhaskar. Protective effect of leaf extract of *Pandanus odoratissimus* Linn on experimental model of epilepsy, International Journal of Nutrition, Pharmacology, Neurological Diseases. 2014; 4(2): Page : 81-87.
- [9] Nwafor PA, Okwuasaba FK, Binda LG. Antidiarrhoeal and Antiulcerogenic effects of methanolic extract of *Asparagus pubescens* roots in rats. Journal of Ethanopharmacology 2000;72(3):421-427.
- [10] Piyush govtam and Ramesh Patel, An overview. European Journal of Complementary and Alternative Medicine. 2014;1(1):10-14.