

Diuretic activity of *Bixa orellana* Linn. leaf extracts

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The present investigation was aimed at investigating the diuretic activity of *Bixa orellana* Linn. leaves. The dried leaf powder was subjected to successive Soxhlet extraction with petroleum ether, chloroform, ethyl acetate, methanol and water. Among these, petroleum ether, methanolic and aqueous extracts were investigated for diuretic activity in Wistar rats using standard methods. The diuretic activity was assessed in terms of urine output and levels of sodium, potassium and chloride in urine. The results obtained revealed that the methanolic extract showed significant diuretic activity at a dose of 500 mg/kg body weight by increasing the total volume of urine and levels of sodium, potassium and chloride in urine when compared to standard drug, Furosemide and Arachis oil as control and vehicle for the extracts.

Keywords: *Bixa orellana*, Annatto, Soxhlet extraction, Diuretic, Furosemide, Acute toxicity, Arachis oil.

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Introduction

Diuretics are the drugs that increase the rate of urination. There are several classes of diuretics and they increase the excretion of water from the body by acting through different mechanisms. Diuretics are capable of increasing the flow of urine and are useful in the treatment of diseases related with the retention of fluids.

The plant *Bixa orellana* Linn., belonging to family Bixaceae is a shrub or bushy tree commonly known as Annatto tree. It grows from 3 to 10 m in height¹. It is bestowed with many medicinal uses in traditional systems of medicine including Ayurveda. Roots and leaves of the plant are useful in the treatment of sore throat, jaundice, dysentery, gonorrhoea and liver diseases and as diuretic and antipyretic agent². Previously published scientific reports proven that the plant was reported to possess antivenom, antimicrobial, anticonvulsant, analgesic and antidiarrheal, enzyme inducing, hypoglycemic and antimutagenic activities²⁻⁷. The plant is used in traditional Filipino cooking as a coloring agent. It is also used in coloring butter, margarine, cheese, beverages and meat and fish products. The Bixa dye,

also known as Annatto dye, is extracted from the outer covering of the seeds of the tree⁸.

The review of the scientific literature did not reveal any information on the diuretic studies of this plant. In this investigation, an attempt was made to assess the efficacy of this indigenous plant for its diuretic activity in terms of urine output and sodium, potassium and chloride levels in urine in experimental animals using standard methods.

Materials and Methods

Procurement of chemicals

Furosemide was obtained from Khandelwal Laboratories, Mumbai. The solvents used were of Laboratory grade obtained from EMerck Ltd., Mumbai. Groundnut or Arachis oil was purchased from local market. All other chemicals of highest available purity were obtained from HiMedia Laboratories, Mumbai.

Collection of plant material

The leaves of *B. orellana* were collected from the plant grown in the botanical garden of Department of Biotechnology, Kakatiya University, Warangal after identification by a taxonomist. A voucher specimen is deposited in the laboratory herbarium. The collected plant material was thoroughly checked and freed from foreign matter and shade dried, powdered with laboratory mixer and sieved.

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Preparation of the extracts

The dried leaf powder was Soxhlet extracted successively with petroleum ether (60-80°C), chloroform, ethyl acetate, methanol and water each for 6 hours. The obtained solvent extracts were concentrated in vacuo using rotary vacuum evaporator and dried in desiccator.

Animals

The healthy Wistar rats of either sex, approximately the same age and weighing about 150-180 g used for the study were obtained from Mahaveer Enterprises, Hyderabad. They were fed with standard chow diet and water *ad libitum*. The animals were housed in polypropylene cages maintained under standard environmental conditions (12h light/12h dark cycle; 25±3°C, 35-60% relative humidity). The animals were treated strictly according to the CPCSEA guidelines and the study was conducted after obtaining permission from Institutional Animal Ethics Committee (IAEC).

Acute toxicity and gross behavioral study

The rats were fasted overnight, divided into groups (n=6) and were orally fed with increasing doses (250, 500, 750 and 1000 mg/kg body weight) of petroleum ether, methanol and aqueous extracts suspended in Groundnut (Arachis) oil. After administration of the extracts, the animals were observed during first 2h for their gross behavioral changes and once in 30 min for next 4h and then once in 24h for next 72h to find out percentage mortality⁹⁻¹¹.

Assessment of diuretic activity

The diuretic activity of the extracts was assessed by the method previously described by Lipschitz *et al*¹². For the assessment of diuretic activity, the urine output and sodium, potassium and chloride levels in urine were measured. The animals were divided into eight groups with each group containing six animals. The animals were deprived of food and water for 16h prior to the experiment. Before the oral administration of test drugs, the animals were dosed with 25 ml/kg body weight of normal saline. Among the eight groups of animals, Group I received Arachis oil (control, vehicle for the extracts) and Group II received the standard diuretic drug Furosemide at 20 mg/kg body weight. Each proposed extract was studied at two concentrations. Groups III to VIII received petroleum ether (250 and 500 mg/kg body weight), methanol (250 and 500 mg/kg body weight) and aqueous (250 and 500 mg/kg body weight)

extracts, respectively in Arachis oil as suspension/solution.

Immediately after administration, the animals were placed in fabricated metabolic cages individually to allow separation of urine and faeces. The bottom of the metabolic cage was fixed with a glass funnel and the stem of the funnel was inserted in a measuring cylinder containing mineral oil. The presence of mineral oil in the measuring cylinders prevents loss of urine through evaporation. The urine was collected for six hours after administration of control, standard and extracts. The bladder was emptied by pulling the base of tail of each rat¹³. Diuretic assay parameters were observed for each rat. The observed parameters were total urine volume and Na⁺, K⁺ and Cl⁻ levels excreted in the urine. The concentration of Na⁺ and K⁺ were measured by flame photometry¹⁴ and Cl⁻ concentration was estimated by titration¹⁵ with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate as indicator. Data are presented as Mean ± SEM. The data was analysed using two way analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Dunnett's multiple comparison test.

Results and Discussion

In acute toxicity study, all the animals were found to be surviving after 72h. This indicates that the extracts were found to be safe up to the dose levels studied. Since, all the animals survived at a dose of 1000 mg/kg body weight, the LD₅₀ of the extracts will be >1000 mg/kg body weight. No major behavioral changes were observed during this period of study. The animals showed mild sedative effect upon administration of all the extracts.

The results obtained in diuretic assay of petroleum ether, methanol and aqueous extracts are shown in Table 1. From the results it can be observed that methanol extract has shown significant diuretic activity by increasing urine output and increased excretion of sodium, potassium and chloride levels when compared to control. The effect of methanol extract was found to be dose dependent, i.e., among the two doses studied, higher dose produce more effect. A comparison was made with the standard diuretic drug Furosemide. The diuretic effect observed was found to be significant in terms of urine output. The levels of Cl⁻ didn't show much variation on the administration of extracts. The petroleum ether extract was found to be least potent. Determination of urinary electrolyte concentration revealed that

Table 1—Diuretic activity of *Bixa orellana* Linn. leaf extracts

Name of the drug/extract	Dose (mg/kg)	Urine volume (ml)	Concentration of ions (mEq/l)		
			Na ⁺	K ⁺	Cl ⁻
Control	-	0.7±0.04	62±2.01	11.4±0.37	56±1.90
Standard (Furosemide)	20	3.5±0.44*	94±3.88	13.8±0.59	101±4.01
Petroleum ether	250	1.1±0.13	64±2.05	11.1±0.38	48±1.62
	500	1.2±0.06*	68±2.08	11.8±0.40	59±1.97
Methanol	250	2.0±0.04*	69±2.08	12.1±0.46	62±2.05
	500	2.4±0.02*	82±3.07	12.3±0.47	71±2.52
Aqueous	250	1.3±0.03*	73±2.61	11.8±0.43	49±1.47
	500	1.5±0.06*	32±1.47	12.2±0.47	47±1.52

All the values are expressed as mean ± SEM (n = 6); *P<0.05 compared with control

methanol extract was most effective in increasing urinary electrolyte concentration for all the three ions tested (Na⁺, K⁺, Cl⁻) followed by aqueous extract while petroleum ether extract did not show significant increase in either urinary volume or electrolyte concentration.

Conclusion

From the above results it can be concluded that the methanolic extract of *B. orellana* leaves possess significant diuretic activity by increasing the total urine output and increased excretion of sodium, potassium and chloride levels. However, the activity was not comparable in terms of quantitative activity elicited by standard drug. This could be due to the use of crude extracts. Hence, isolation of active principles will be advantageous to produce novel bioactive constituents from these extracts which may possess more significant activity.

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