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Comparative Study on Diuretic Effect of *Buchanania angustifolia* Roxb., and *Buchanania lanzan* Spreng. Fruit Extracts and Fractions

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ABSTRACT

The present study was attempted to evaluate diuretic potency of total alcoholic extracts and its polar and non polar fractions of Priyala fruits (*Buchanania angustifolia*, *Buchanania lanzan* Fam: Anacardiaceae) in rats. Healthy adult Wistar albino rats weighing about 150-200 g were used for the study. Acute toxicity study was performed to determine therapeutic dose of the extracts and fractions. Lipschitz method was employed for the assessment of diuretic activity, the control group received normal saline (25ml/kg P.O), and standard group received Frusemide (20mg/ kg, P.O). The remaining groups were treated with two doses (250 and 500mg/kg) of *Buchanania angustifolia* and *Buchanania lanzan* total alcoholic extracts, n-hexane insoluble and n-hexane soluble fractions. Total alcoholic extracts, n-hexane insoluble and n-hexane soluble fractions of the both fruits have shown significant diuresis when compared with control at 5 hrs. Out of two doses 500mg/kg has shown significant diuretic property resulting in the superior urine excretions of Na^+ and K^+ ions which can be compared to that of standard. With the same dose *Buchanania angustifolia* was found to be better diuretic than *Buchanania lanzan*. Priyala fruit extracts and n-hexane fractions produced significant diuretic effect at a dose of 500 mg/kg which appeared to be comparable with that of the standard drug Frusemide. However, further studies are encouraged to isolate the active phytochemical constituent for exploring exact mechanism of diuresis.

INTRODUCTION

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in variety of clinical situations. Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, renal failure, hypertension, and pregnancy toxemia (Agunu et al., 2005). Most diuretic drugs have the adverse effect on quality of life, such as diabetes, hypokalemia and others, fatigue and weakness (Zillich et al., 2006). Currently there is growing interest in herbal remedies due to side effects associated with synthetic drugs.

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Fruit and seeds of *Buchanania angustifolia* Roxb. (Syn. *B. axillaris* (Desr.) Ramam.), *Buchanania lanzan* Spreng. (Syn. *B. latifolia* Roxb.), family-Anacardiaceae (Figure 1, 2) are commonly known as Priyala in Ayurveda and are considered to be tonic and diuretic and used traditionally by Indian tribes for a wider range of ailments, including nutritional disorders, skin diseases, gravel, and other urinary problems. Their powder applied externally to wounds.

The gum dissolved in cow milk, is used internally in rheumatic pain (Siddiqui et al., 2014). In Unani medicine these are used as tonics for sexual debility (Chaudhari, 1996). Number of scientific publications is available regarding the various biological activities of these two plants, but the diuretic potential of these plants has not been proved scientifically. The present study was undertaken to assess the acute diuretic activity of two species of Buchanania fruits.

Fig. 1: Plant and fruits of *B. angustifolia.*

Fig. 2: Plant and fruits of *B. lanzan.*

MATERIALS AND METHODS

Plant material

Fruits of *B. angustifolia* and *B. lanzan* were collected from in and around Tirupati and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Dept. of Botany, Sri Venkateswara University, Tirupati, and A.P. A voucher specimen of both *B. angustifolia* and *B. lanzan* fruits has been deposited at the Dept. of Pharmacognosy, (Voucher specimen No. NCP/10/2010-11 and NCP/11/2010-11), National College of Pharmacy, Shivamogga.

Preparation of extracts

About 100gms of air dried, coarsely powdered fruits of *B.angustifolia* and *B. lanzan* were weighed accurately and separately macerated with 500 ml of ethanol (90%) and the filtrate was concentrated in rotary flash evaporator (Buchi). The marc of the above is subjected to Soxhlet extraction with ethanol and the extract was collected after removing the solvent using Rota evaporator.

The total alcoholic extracts (BATE and BLTE) were subjected for phytochemical screening by using standard procedures and are fractionated using n- hexane. Both n-hexane soluble (BAHS and BLHS) and insoluble (BAHI and BLHI) fractions were collected and concentrated under reduced pressure. Extracts and fractions were suspended in 2% Tween-80 and used for oral administration.

Animals

Wistar albino rats weighing about 150-200 g were purchased from Sri Venkateshwara Enterprises, Bangalore They were acclimatized and maintained at Room temperature of 25 ± 1^0C; relative humidity 45-55% and a 12:12 hrs light/ dark cycle. Approval from the Institutional Animal Ethical Committee (IAEC) of National College of Pharmacy, Shivamogga (Karnataka) was taken prior to the experiments.

Acute oral toxicity

The acute toxicity of total alcoholic extracts and fractions of *B.angustifolia* and *B. lanzan* fruits were determined as per OECD guideline no. 423 (OECD, 2001). Based on the cut-off Value of the median lethal dose (LD_{50}), the therapeutically effective dose was derived.

Assessment of Diuretic Activity

Lipschitz *et al.*, method was employed for the assessment of diuretic activity (Lipschitz, 1943; Murugesan et al., 2000). In this method male *wistar albino* rats weighing between 150-200gm deprived of food and water for 18 hrs prior to the experiment and were divided in 14 groups of 6 rats each.

The first group of animals serving as control received 0.9% normal saline (25ml/kg BW) second group received standard Frusemide (20 mg/kg BW). Groups 3^{rd}, 4^{th} and 5^{th} received (250mg/kg BW) of *B. angustifolia* total alcoholic extract (BATE), n-hexane insoluble (BAHI) and n-hexane soluble (BAHS) fractions respectively with saline solution. Groups 6^{th}, 7^{th} and 8^{th} received (500 mg/kg BW) *B. angustifolia* total alcoholic extract (BATE), n-hexane insoluble (BAHI) and n-hexane soluble (BAHS) fractions, groups 9^{th}, 10^{th} and 11^{th} received (250mg/kg BW) *B. lanzan* total alcoholic extract (BLTE), n-hexane insoluble (BLHI) and n-hexane soluble (BLHS) fractions and groups 12^{th},13^{th} and 14^{th} received (500 mg/kg BW) *B. lanzan* total alcoholic extract (BLTE), n-hexane insoluble (BLHI) and n-hexane soluble (BLHS) fractions.

Collection and Analysis of Urine

Immediately after dosing, pair of two rats per cage was kept in metabolic cages and urine was collected at 1h interval for 5h. The volume of urine (ml/100 g BW), electrolytes (Na^+, K^+, Cl^- mEq/l/100 g) was estimated in the urine for assessment of diuretic index. Diuretic index was calculated using formula;;

$$\textbf{Diuretic Index} = \frac{\text{Urinary excretion of test drug}}{\text{Urinary excretion of control}}$$

Table. 1: Preliminary phytochemical investigation of *B. angustifolia* and *B. lanzan* fruit extracts and fractions.

Chemical Constituent	Total extract		n-Hexane insoluble fraction		n-Hexane soluble fraction	
	B. angustifolia	*B. lanzan*	*B. angustifolia*	*B. lanzan*	*B. angustifolia*	*B. lanzan*
Alkaloids	+ve	+ve	+ve	+ve	+ve	+ve
Glycosides	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	-ve	-ve	-ve	-ve	-ve	-ve
Phytosterols	+ve	+ve	-ve	-ve	+ve	+ve
Triterpenes	+ve	+ve	-ve	-ve	+ve	+ve
Phenolics And Tannins	+ve	+ve	+ve	+ve	-ve	-ve

Table. 2: Effect of *B. angustifolia* and *B. lanzan* fruit extracts and fractions on urinary volume and excretion of electrolytes.

Treatment	Dose (mg/kg)	Cumulative Urine vol. (ml)	Diuretic Index	Na^+ (mmol/lt)	K^+ (mmol/lt)	Cl^+ (mmol/lt)	Na^+/K^+ ratio
Control	_	4.2±0.56	1.00	88.7±0.49	83.4±0.15	88.1±0.67	1.06
Standard	20	17.6±0.45*	4.19	130.8±0.11*	93.6±0.43*	89.4±0.54	1.39
BATE	250	4.8±0.59	1.14	98.7±2.11	89.1±2.07	88.4±2.15	1.10
BAHI	250	4.6±0.66	1.11	94.7±0.97	84.4±1.92	88.6±1.73	1.12
BAHS	250	4.9±0.51	1.16	95.1±2.21	86.5±2.31	88.3±1.17	1.09
BATE	500	15.5±0.34*	3.69	120.4±1.93*	87.8±1.63*	88.8±1.77	1.37
BAHI	500	9.2±0.53*	2.19	110.9±2.30*	91.7±2.10*	88.9±2.01	1.20
BAHS	500	10.9±0.71*	2.59	119.8±1.51*	90.1±1.21*	89.1±1.19	1.32
BLTE	250	4.5±0.13	1.07	96.3±2.01	87.6±2.41	88.5±2.33	1.09
BLHI	250	4.8±0.75	1.16	95.1±2.21	86.5±2.31	88.3±1.17	1.09
BLHS	250	4.7±0.98	1.11	94.7±0.97	84.4±1.92	88.6±1.73	1.12
BLTE	500	15.2±0.23*	3.61	125.1±1.13*	91.6±0.93*	89.4±1.10	1.36
BLHI	500	9.8±0.61*	2.33	109.3±0.33*	89.3±0.54*	88.9±0.77	1.22
BLHS	500	11.3±0.81*	2.69	119.8±1.93*	91.4±1.55*	88.5±1.34	1.31

Values are in mean ± SEM. n= 6 in each groups; *p<0.001 was considered significant

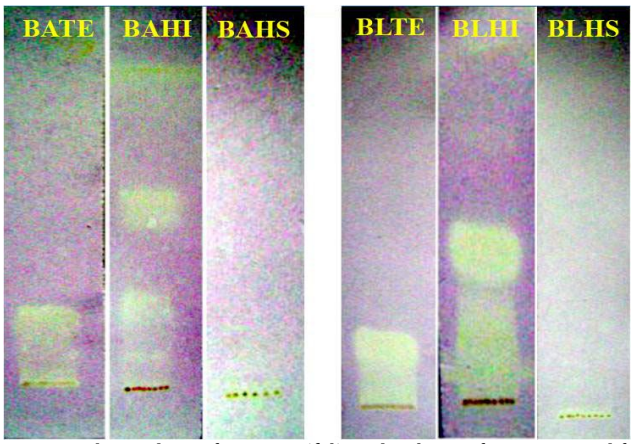

Fig. 3: Bioautographic analysis of *B. angustifolia* and *B. lanzan* fruit extract and fractions.

Bio-autography analysis

All extracts of fruits were dissolved in respective solvent and chromatographed on pre-coated silica gel G60 F_{254} plates. The plates were developed in selected solvent systems. The plates were dried in air flow for 3hrs then sprayed with 0.004% solution of DPPH in methanol using TLC sprayer. Plates were placed in dark for 20min for any reaction to be occurred. Anti-oxidant compounds were identified as white spots on dark background (Lihua et al., 2009).

Statistical Analysis

All the results are expressed as mean ± standard error of mean. The data was analyzed statistically using ANOVA at a probability level of $P < 0.001$.

RESULTS

Both the sources have shown the presence of alkaloids, Phytosterols, Triterpenes and polyphenolic compound including flavonoids. The hexane insoluble fraction (polar fraction) has indicated the presence of alkaloids and polyphenols, where as hexane soluble fraction (non polar fraction) showed the presence of phytosterols as well as Triterpenes. Alkaloids were present in both the fractions (Table 1). This result was substantiated by the Bioautographic analysis which showed the presence of poly phenols in total extract as well as in polar fraction, where as it was absent in non polar fraction (Figure 3).

The total alcoholic extracts of both *B. angustifolia* and *B. lanzan* at the dose of 500 mg/kg BW has shown significant diuresis with a diuretic index of 3.69 and 3.61 respectively. Whereas, fractions have shown moderate diuresis with the diuretic index ranging between 2.19 to 2.69 (Figure 1). The results are very much nearer to that of standard drug Furosemide (Diuretic Index 4.16) in compare to total alcoholic extracts. Surprisingly the extracts and fractions of both the plants have failed to elicit diuresis in animals at a dose 250 mg/kg BW. Neither total extracts nor fractions have shown significant diuretic response at the lower

dose.The effect of a single dose (20 mg/kg) of reference diuretic, Furosemide, induced a significant increase in the diuresis with total urinary output of 17.6 ml (Figure 2 & 3) when compared with that of the control. The urinary level of Na^+ was significantly increased in total alcoholic extract 500 mg/kg ($p< 0.001$), n-hexane insoluble and n-hexane soluble fractions 500 mg/kg ($p< 0.01$) of both fruits. There was also significant increase in K^+ level in the extract treated groups as compared to control animals. None of the extracts including standard had shown much changes in Cl^- level (Table 1). Out of both the doses 500 mg/kg has shown noticeable diuretic property resulting in the superior urine excretions of Na^+ and K^+ ions which can be compared to the standard. Comparatively total alcoholic extract of *B. angustifolia* with a diuretic index 3.69 shown better diuretic activity than *B. lanzan*.

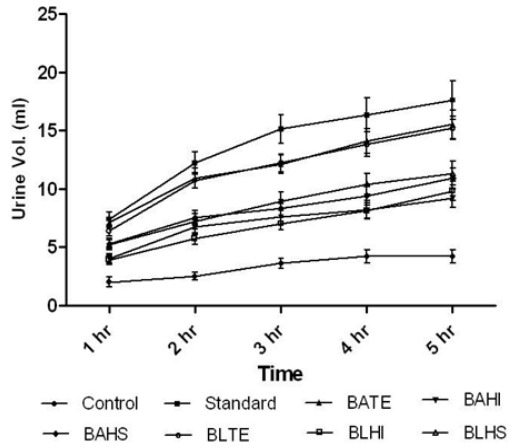

Fig. 4: Time course of diuresis in rats with extracts and fractions of *B. angustifolia* and *B. lanzan* fruits at 500 mg/kg BW.

DISCUSSION

The present study was aimed to evaluate diuretic potential of *B. angustifolia* and *B. lanzan* fruits. Diuretics are known to relive pulmonary congestion and peripheral edema. Diuretics reduce the blood pressure by reducing the volume overload, cardiac workload, oxygen demand and plasma volume [9]. *B. lanzan* is commonly used as diuretics in traditional medicine. The fruits of this plant are being used as cooling, liver disorder, fever and in thirst. It is also used in external application in skin diseases, pimples and also as tonic (Kirthikar and Basu, 1999). The kernel of *B. angustifolia* is considered to be the best among *Buchanania* sp. and uses are similar to that of *B. lanzan* (Khare, 2007). Total alcoholic extracts and their fractions of both fruits had shown significant diuretic action as compared with that of the control. In this diuretic action of *B. angustifolia* total alcoholic extract was found to be better than *B. lanzan* total alcoholic extract, Both polar and non polar fractions of these plants have shown significant activity compared to control group but it was much less as compared with that of the total extract. This may be attributed to the combined effect of the active constituents present in the total alcoholic extracts. Overall *B. angustifolia* extract has shown better diuretic activity than *B. lanzan* which is the official source of Priyala in Ayurveda (Anonymous, 2008). Significant increase in N^+/ K^+ ion excretion was observed in total alcoholic extract and n-hexane soluble fractions of both fruits but less in n-hexane insoluble fractions. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increases sodium ion excretion to a greater extent than potassium, this particular decreased hyper-kalaemic effect is one of the ideal qualities of a diuretic agent (Bose et al., 2006). The chloride ion excretion was not elevated significantly indicating that the extract is a potent natriuretic (Hemanth et al., 2007). The phytoconstituents such as terpenoids, polyphenols and flavonoids have been reported previously to be responsible for the diuretic activity in plants (Thambi et al., 2008).

The best diuretic effects could be associated to the flavonoids content, also it promote high levels of Na^+ and K^+ in urine. There is a direct relationship between the volume of urine and the concentration of Na^+, and through this mechanism diuretic effect is produced due to decreased re-absorption of Na^+ ion in renal tubule (Vishal et al., 2012), it produces the dragging of the osmotic equivalent of water (Gupta et al., 2012; Asif et al., 2013). As per the preliminary phytochemical investigation flavonoids, polyphenols are present in total alcoholic extract and n-hexane insoluble fractions of both fruits. The TLC Bioautographic analysis of extracts and fractions has revealed the presence of poly phenol content in extract and hexane insoluble fraction. Additional studies like isolation and characterization of diuretic principle needed to understand and confirm the exact mechanism of action.

CONCLUSION

Both *B. angustifolia* and *B. lanzan* fruit extract and fractions showed significant diuretic effect in experimental animals at a dose of 500 mg/kg BW. *B. angustifolia* extract showed slightly better diuretic potency than *B. lanzan* and also demonstrated superior urine excretions of Na^+ and K^+. The presence of flavonoids and Triterpenes could be the possible active constituents for this diuretic activity. *B. angustifolia* is a better drug compared to *B. lanzan* with respect to its diuretic potential and it can be used in place of *B. lanzan*.

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