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Diuretic activity and In-vitro anti-urolithiasis activity of *Bauhinia racemosa L* Leaf extract

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ABSTRACT

The main objective of this study was to determine the diuretic and anti-urolithiasis activities of the *Bauhinia racemosa* leaf extract. The extracts' in vitro anti-urolithic activity was determined by their ability to neutralize synthetic calcium oxalate crystals using a homogenous precipitation method, nucleation assay method, and aggregation assay method that increased the concentration of the test drug, and Cystone tablets as a standard. The results showed that the aqueous extract of *Bauhinia racemosa* dissolves calcium oxalate crystals and inhibits the growth of crystals; this effect increased with an increase in the concentration of the extract, which is compared with the poly-herbal formulation Cystone. The diuretic activity of *Bauhinia racemosa* extract was evaluated using the Lipschitz model. Urine volume and urine pH were noted; the concentration of sodium and potassium was estimated by flame photometry; and diuretic index, Saliuretic index, and Lipschitz values were calculated from the results. Furosemide was used as a positive control. It also demonstrates that increasing smooth muscle contraction plays an important role in treatment by increasing urinary output and the percentage of urinary stones that pass through the urinary tract. As a result, the study concluded that an aqueous extract of Bauhinia racemosa has significant anti-urolithiasis and diuretic activity.

Keywords: *Bauhinia racemosa, Diuresis, Antiurolithiasis, Calcium oxalate crystals, Cystone.*

INTRODUCTION

World Health Organization manifests that approximately 75% of the global population, of the developing world, depends on botanical medicines for their basic healthcare needs. (Khan *et al.* 2012). Exact identification and quality of the starting materials are essential prerequisites to ensure the reproductive quality of herbal medicine which will contribute to its safety and efficacy (Masao *et al.* 2000).

Urolithiasis otherwise urinary calculi a pathogenic continues to be more or less ambiguous and the predicament is found to be an ancient and worldwide distribution. The different calculi are painful urinary disorders that start as salt/chemical crystals that precipitate out from urine. Under normal circumstances, the urine contains substances that prevent crystallization but for patients with this condition, these inhibitory substances are ineffective. Tiny crystals will pass out along with the urinary flow without causing problems. At least a few people will pass a kidney stone during their lifetime, producing some of the most severe pain possible, by increasing the stone concentration in the kidney. If the stone is large enough to block the tube (ureter) and stop the flow of urine from the kidney, it must be removed by surgery or other methods. It is also called Renal Calculus. Symptoms usually begin with intense waves of pain as a stone moves in the urinary tract. Typically, a person feels a sharp, cramping pain in the back and side in the area of the kidney or the lower abdomen. Sometimes nausea and vomiting occur. Later, pain may spread to the groin. The pain may continue if the stone is too large to pass, blood may appear in the urine and there may be the need to urinate more often or a burning sensation during urination. If fever and chills accompany any of these symptoms, which may lead to infections. (Rathod *et al.* 2013).

Plants have always played a major role in the prevention and cure of diseases in humans worldwide. The use of medicinal plants is increasing day by day in both developed and developing countries due to an increase in the recognition of natural products. (Pandey *et al.* 2011) Genus Bauhinia has played a significant role in human civilization since ancient times. Genus Bauhinia is comprised of trees and shrubs which grow in a warm climate. About 300 species of Bauhinia are found in tropical regions with 5-7 m tall trees in deciduous forests. It is generally planted in gardens and along the roadsides for its beautiful flowers. It is a useful species for filling blanks in forest plantings and helps in preventing soil erosion (Memona *et al.* 2021).

Bauhinia racemosa L. (Caesalpiniaceae), is widely distributed throughout India, Ceylon, China, and Timor. The bark and leaves of this plant are traditionally used for the treatment of inflammation, headache, fever, tumors, skin infection, disease of the blood, dysentery, and diarrhea. (Sing *et al.* 2013).

Nutritional importance:

The seeds of *Bauhinia racemosa* are rich in calcium, potassium, magnesium, zinc, manganese, and iron. Glutelins are predominated whereas albumins and globulins are less in the seed protein of Bauhinia racemose. Essential amino acids like isoleucine, lysine, phenylalanine, and tyrosine are high whereas the contents of sulfur amino acids are limiting in the seed proteins. The fatty acids, linoleic, oleic, and palmitic acid are relatively higher in the seed lipids (Davey *et al.* 2011, Gupta *et al.* 2004, Nirmal *et al.* 2011). The leaves of the plant exhibited significant antilithiatic activity due to the presence of flavonoids and tannins in rich amounts (Surendra, 2011)

Fig. 1: ***Bauhinia racemosa***

MATERIALS AND METHODS

Material:

Plant collection: *Bauhinia racemosa* commonly known as the Bidi leaf tree is a rare medicinal species of flowering shrub with religious significance. It is a small crooked tree with drooping branches that grow 3–5 meters (10–16 ft) tall and flowers between winter and spring. It is native to tropical Southeast Asia. (https://www.picturethisai.com/wiki/Bauhinia_racemosa.html). It was collected from Yavatmal. It also gets from the local market. An aqueous extraction was performed by adding 200 ml boiling water to 10gm of dried leaf of Bauhinia racemose, filtering after 20 min, and then extract was filtered using filter paper and then the filter was collected. The remaining residue was re-extracted twice and then the two extracts were combined to get a concentrated extract. Then the solvent was removed and we will get a dried powder of extract. Store it in an airtight container. The powder was dissolved in distilled water for in-vivo and in-vitro studies.

Animals

Wistar rats of either sex of average weight 200-220 gms aged 3-4 months were used in the experiments. Rats were kept in metabolic cages under suitable conditions of housing, temperature, ventilation, and nutrition. These cages were specially designed to separate the urine and feces of animals. They were kept at a constant temperature of 26±2^{0}C and relative humidity of 30-70% under a 12 h dark/ light cycle.

Method:

The diuretic activity of* Bauhinia racemosa *extract:

The method of Lipschitz was employed for the evaluation of the diuretic activity. The male Albino rats weighing about 150 -200 g were divided into four groups of six rats in each and were fasted and deprived of food and water for 18 h before the experiment. On the day of the experiment, the Group I animals serving as control received normal saline (25 ml/kg, p.o), and the Group II animals received aq. extract (250 mg/kg), Group III before also received aq. extract (500 mg/kg,) and the Group IV animals received Furosemide (20 mg/kg, p.o), in normal saline. Immediately after the administration the animals were kept in metabolic cages (3 per cage) specially designed to separate urine and fecal matter and kept at room temperature (25 ± 0.5ºC) throughout the experiment. The total volume of urine was collected at the end of 5 h after dosing. During this period no water and food were made available to animals. Diuretic activity (Khandare *et al.* 2011)

Computation of diuretic parameters

Diuretic parameters were determined as in Eqs 1 - 4 (Danamma B *et al.* 2011, Abdala S *et al.* 2012)

Diuretic index = Vt/Vc …….. (1)

where Vt is the mean urine volume the of test group and Vc the is mean urine volume of the control group.

Lipschitz value = Vt/Vr …….. (2)

where Vt is the mean urine volume of the test group and Vr is the mean urine volume the of reference group.

Saliuretic index = Ct/Cc ……. (3)

where Ct is the concentration of electrolytes in the urine of the test group and Cc is the concentration of electrolytes in the urine the of control group.

Na+ /K+ ratio = Cn/Ck …….. (4)

where Cn is the concentration of Na+ the in the urine of a group and Ck is the concentration of K+ in the urine the of same group

In vitro antiurolithiatic activity:

Homogenous precipitation method

The experiment consisted of the following test of 10 ml capacity having 5 groups, each group has 6 test tubes, and in each tube 1ml of calcium chloride anhydrous and 1ml sodium oxalate was added to the tubes and 2 ml of tris buffer (disodium hydrogen phosphate and potassium dihydrogen phosphate) adjusted at 7.4 pH which to the kidney pH and incubated at 36.7°C overnight. The next day the test tubes were centrifuged for 10min to decant to remove the top liquid layer. The calcium oxalate crystal formed in the test tube was checked using the compound microscope under 45x magnification, the crystal formed was resembling the shape of the prism, this the extracts of plant Bauhinia racemose were induced to the tubes and at the same quantity the synthetic drugs

Spironolactone, Furosemide and the Poly herbal formulation Cystone were administered to the test tube, all the above-treating agents were administered as an aqueous suspension using tween 60 as suspending agent and again it was incubated 36.7°C for 3 days on the fourth day. All the test tubes were taken and checked under the microscope for observing whether the crystals were dissolved or not, by adding a drop of con HCl to separate the oxalate ion calcium and both ions were spectroscopically analyzed.

Elemental Ions Analysis

Test for oxalate:

The determination of oxalic acid in 0.5ml of generated crystals samples, the co-precipitated oxalic acid with calcium sulfate, which is reduced to glycollic acid by boiling with dilute sulphuric acid and a zinc pellet and estimated calorimetrically with chronotropic acid at 570 nm.

Test for Calcium:

In an acid medium, calcium binds with O-Cresolphthalein Complex one (O-CPC) to produce a purple color, which absorbs at 570 nm and is proportional to the concentration of calcium.

Microscopical studies:

On revision, before adding the dilute HCl, the different extracts and the other agents treated on the regenerated calcium oxalate crystals in test tubes were observed under 10X magnification using the photographic microscope and pictures were taken and pragmatic effects of the agents were studied. the crystals formed are rated on assumption as per the scores ranging from 0-4 marks which in comparison to the control group-1 with the others treated groups and the formation of the crystals are denoted by the arrow marks (Satish, 2010, Rathod *et al.* 2013)

Nucleation Assay Method:

Preparation of Reagents: 10% Trichloro acetic acid was prepared by dissolving 10g of Trichloroacetic acid in 1000 ml of water (Trease *et al.* 2002).

Method:

It is the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor and with it, to assess the inhibiting capacity of any chemical species used. Solution of calcium chloride and sodium oxalate was prepared at the final concentrations of 5mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 µL of calcium chloride solution mixed with 100 µL of herb extracts at different concentrations (100 µg/ml– 1000 µg/ml). Crystallization was started by adding 950 µL of sodium oxalate solution. The temperature was maintained at 37 °C. The OD of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of the control. Cystone tablets are used as a standard solution. (Chernyseva *et al.*2002) The results were given in Fig and Table. The growth of crystals was expected due to the following reaction:

$CaCl2+NaC2O4 \rightarrow 2CaCO4+2NaCl.CaCl2+Na2C2O4 \rightarrow CaC2\ O4+2NaCl$

Aggregation Assay:

Preparation of Reagents: 50 mM CaCl2 was prepared by 50 g of calcium chloride dissolved in 1 liter of water. 50 mM of Sodium oxalate was prepared by dissolving 50 g of sodium oxalate was dissolved in 1 liter of water. 0.05 mM NaCl was prepared by dissolving 0.05 g of NaCl was dissolved in 1 liter of water. (Saha *et al.*2013)

Method:

CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60 °C in a water bath for 1 h and then cooled to 37 °C overnight. The crystals were harvested by centrifugation and then evaporated at 37 °C. CaOX crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37 °C in the absence or presence of the plant extract after stopping the stirring. The Cystone tablets are used as a Standard drug solution. (Atmani *et al.* 2000). The percentage aggregation inhibition rate (Ir) was then calculated by comparing the turbidity in the presence of the extract with that obtained in the control using the following formula: Ir= (1−Turbiditysample/Turbidity control) × 100.

RESULTS

Diuretic Activity

Table 1: the diuretic activity of *Bauhinia racemosa* extract:

Group	Extract & dose (mg/kg)	Volume of urine (ml/6 h)	pH	Diuretic index	Lipschitz value	Saliuretic index		Na+ /K
						Na+	K+	
1	Normal saline 10(ml/kg)	1.0±0.6	7.01	----	----	----	----	18.38
2	Furosemide	6.6±0.5*	7.78	6.6	-----	1.18	1.84	11.83
3	Test 1 (250mg/kg)	2.3±0.2*	6.96	2.3	0.34	1.00	1.23	15.03
4	Test 2 (500mg/kg)	3.1±0.1*	7.01	3.1	0.46	1.07	1.57	12.56

*The values are significant, * p< 0.05; ** p < 0.01 when compared with the standard.*

Fig. 2 : Metabolic cage

Homogenous precipitation method:

Table: 2 Effect of the drugs and extracts treated on generated calcium oxalate analysis by Homogenous precipitation method

Parameters mg/ generated crystals in a test tube	Group- I Control	Group- II 5mg/ml Furosemide	Group- III 5mg/ml Spiranolactone	Group- IV 5mg/ml Cystone	Group- V 15mg/ml Aq. extract
Oxalate	15.02 ± 0.45	14.78 ±0.66***	10.53±0.66**	6.77±0.50*	5.4 ±0.44***
Calcium	6.20 ± 0. 29	5.24 ± 0. 20***	3.01 ±0. 25**	2.66 ±0.21*	1.84 ±0. 31***

The values are significant,* p< 0.05; **p < 0.01; ***p < 0.001 when compared with the standard.

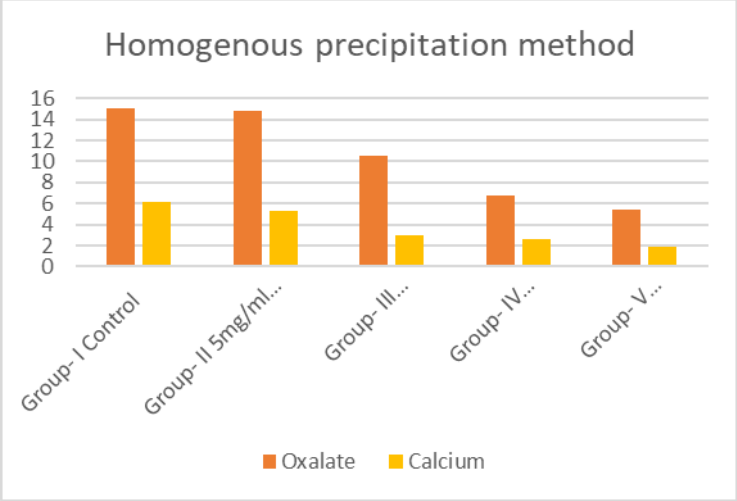

Fig. 3: Homogenous precipitation method

Nucleation Assay Method

Table: 3 Antiurolithiatic effect of different concentrations of aqueous extract of ***Bauhinia racemosa*** leaves by nucleation assay method

Sr no.	Concentration(ug/ml)	Aq. Extract of BR	Std Drug
1	200	63.1%±0.0029**	75.85%±0.0016*
2	400	75.5%±0.001**	79.23%±0.0012*
3	600	82.2%±0.078**	79.56%±0.0042*
4	800	86.2%±0.0018**	85.2%±0.004*
5	1000	94.2%±0.008**	91.2%±0.0011**

The values are expressed as Mean ±SEM, n= 6. The values are significant,* p< 0.05;**p < 0.01 when compared with the standard.

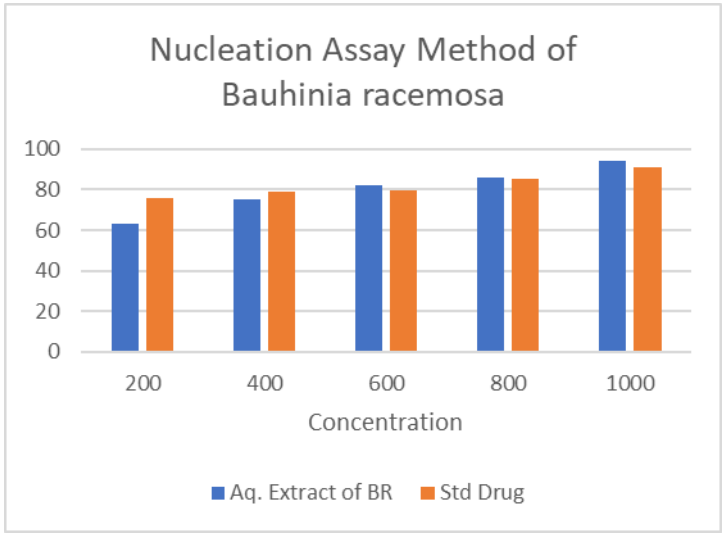

Fig. 4: Nucleation assay method

Aggregation Assay:

Table 3: anti urolithiasis effect of different concentrations of aqueous extracts of ***bauhinia racemosa*** leaves by aggregation activity

Sr no.	**Conc. (ug/ml)**	**Aq. Extract of BR**	**Std Drug**
1	200	63.1%±0.0012**	62.85%±0.066*
2	400	75.5%±0.082**	72.23%±0.023*
3	600	80.2%±0.078**	83.2%±0.044*
4	800	89.2%±0.078**	94.2%±0.078**
5	1000	90.2%±0.078**	95.2%±0.078**

The values are expressed as Mean ± SEM, n= 6. The values are significant, * p< 0.05; ** p < 0.01 when compared with the standard.

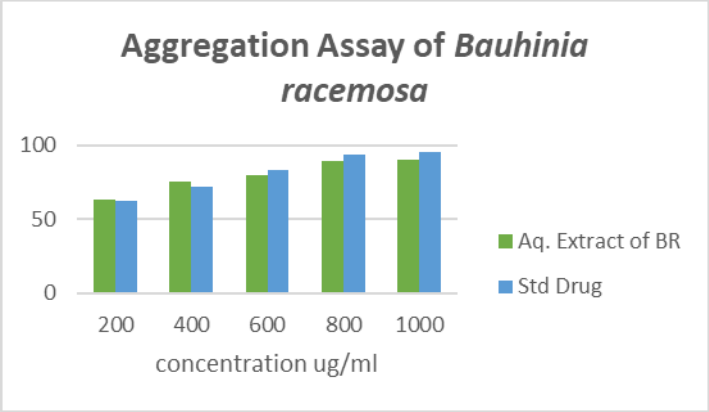

Fig. 5: Aggregation assay method

The literature survey has established the safety of *Bauhinia racemosa*. This study demonstrated that there were no adverse signs and symptoms and no fatalities in rats fed BR extract orally at doses up to 2000 mg/kg. According to the dose safety threshold up to 2000 mg/kg, (Kumar et al., 2017) two doses of BR extract, weighing 250 and 500 mg/kg each, were selected for the diuretic experiment.

The aq. extract of the leaf of *Bauhinia racemosa* treated groups at different dose levels (250 and 500 mg/kg) has seen a significant increase in the urine output and also significantly boosted the excretion of Sodium, Potassium ions in urine when compared to to the control group in the diuretic model. The homogenous precipitation method was employed to perform in-vitro lithiasis activity, where calcium oxalate crystals were produced by sodium oxalate and calcium chloride while incubating at 36.70C with tris phosphate buffer at 7.4 pH. In the experimental part, the produced crystals were divided into five groups and treated with the various agents indicated above. Group, I served as the control group, while the other four groups received the treatment, and calcium and oxalate concentrations were determined in each group. The aq. extract of *Bauhinia racemosa*, cystone, and spironolactone all demonstrated significant and improved action in the dissolution of the crystals, while furosemide was essentially similar to the control and was found to be non-significant when the ion content was measured. When compared to standard medicine Cystone tablets, the anti-urolithiasis activity by nucleation assay findings showed that the aqueous extract at 1000 g/ml had a higher percentage of inhibition. According to the anti-urolithiasis activity by aggregation assay results, % inhibition is equivalent to Cystone pills, a common medication.

CONCLUSION

The result shows that aq. extract of the leaf of *Bauhinia racemosa* significantly increased the urine output along with sodium and potassium concentration. It also shows the anti-urolithiasis activity of plants at various concentrations of plant extract by using a different method of in-vivo study**.** The aqueous extracts of Bauhinia recemosa leaf have an inhibitory effect on CaOx crystallization and thus may be beneficial in the treatment of urolithiasis. But there is a need for detailed investigation in elaborated pre-clinical experimentations and clinical trials to establish the use of the plant as an antiurolithiatic agent.

Conflict of Interest: None of the authors have any conflicts of interest to disclose. All the authors approved the final version of the manuscript.

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