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Short Communication

EVALUATION OF *IN-VITRO* LITHOLYTIC ACTIVITY OF PLANT *BOUGAINVILLEA SPECTABILIS*

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ABSTRACT

The aim of present investigation is to evaluate the *in-vitro* anti urolithiatic activity of the leaves of the plant *Bougainvillea spectabilis* (Willd.). Ethanolic and aqueous extracts of *Bougainvillea spectabilis* fresh leaves were prepared. Homogenous precipitation methods were used to prepare artificial stones of calcium oxalate. Semi-permeable membrane of eggs was used as dissolution bags. Different *in-vitro* anti urolithiatic studies were used such as method of mineralization and titrimetric method to evaluate the inhibition and dissolution of stone crystal using standard drug Cystone. In inhibition as well as dissolution models, the ethanolic extract of *Bougainvillea spectabilis* has shown better capability to dissolve calcium oxalate. Cystone exhibited strongly inhibitory action in both, the method of mineralization and titrimetric assay.

Thus, it was concluded that the ethanolic extract of *Bougainvillea spectabilis* exhibited inhibitory action in both of the methods to a significant level and the bioactive natural products present in leaf extracts of *Bougainvillea spectabilis* can be used in the development of new pharmaceuticals that enhances therapeutic use in renal calculi.

Keywords:*Bougainvillea spectabilis,* Calcium Oxalate*, In-vitro*, Titrimetric, Mineralization.

1. INTRODUCTION

The urolithiasis is well known from quite a while in the ancient times and it has been demonstrated to be indissociable from human history. The stone development is related to the reduced urine volume or the increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine and phosphate. Urolithiasis is a common health problem with increasing occurrence of up to 20% all over the world. The increased prevalence of the disease is due to the changes in lifestyle such as insufficient dietary intake of vegetables or fruits, higher consumption of meat, salt, sweetened beverages and insufficient fluid intake [1].

Urolithiasis is defined as the presence of one or more calculi in any location within the urinary tract [2]. It is a frequent disorder estimated to occur in approximately 12% of the population, with a reappearance rate of 70-80 % in male and 47-60 % in female [3]. Majority of the stones are calcium containing stones, especially calcium oxalate 80 % and others are 20% [4]. The medical treatment of urolithiasis involves treatment with drugs and extracorporeal shock wave lithotripsy (ESWL). The various therapy including diuretics and alkali citrate are used to prevent the reappearance of hypercalciuria and hyperoxaluria, which induce stone formation, but confirmation for their efficacy is less [5]. The surgical endoscopic removal of stone and extracorporeal shock wave lithotripsy, have revolutionized the treatment of urolithiasis but does not prevent the possibility of reoccurrence [6]. Besides imposing the expensive shock waves in therapeutic doses may cause acute renal damage, renal function impairment and increased possibility of stone reappearance. The reappearance of stone formation is also very high (50-80 %). In addition, persistent residual stone fragments and the possibility of infection after ESWL represent a serious problem in the treatment of kidney stones. Thus, medical management of urolithiasis is either expensive or poses severe side effects. The crystallization of the stone begins with increased urinary super saturation, with the subsequent formation of the solid crystalline particles within the urinary tract. This is followed by nucleation, by which stone-forming salts in supersaturated urinary solution combine into clusters that then increase in size by the addition of new constituent [7]. These crystals then grow and aggregate with other crystals in solution, and are ultimately retained and accumulated in the

kidney [8]. Therefore, if this development of crystallization can be prevented, then lithiasis can also be prevented.

There is rising awareness of herbal medicine in community, particularly in the treatment of urolithiasis because of limited choice in the pharmacotherapy. Data from *in-vitro*, *in-vivo* and clinical trials reveal that phytotherapeutic agents could be helpful as either an alternative or an adjunctive therapy in the treatment of urolithiasis. Many Indian plants are also reported to be useful as anti lithiatic agents [9-14]. Hence, the medicinal plants are continuously being investigated for possible anti lithiatic effects.

Bougainvillea is a popular ornamental plant in tropical and sub-tropical gardens of the world. The colourful bracts and wide adaptability of *Bougainvillea* into different agro-climatic situation are the main reasons for its popularity. Being a popular plant in horticultural nursery trade and grown in all types of gardens, greater variation in its colour, size and form would be of more use. *Bougainvillea spectabilis* (*B. spectabilis*) belonging to Nyctaginaceae family is commonly known as "paper flower" due to the bracts are thin and papery. *B. spectabilis* is used in several countries to prepare extracts with antibacterial activity [15-17]. Its aqueous and methanolic extracts show good oral glucose tolerance and significantly reduce the intestinal glucosidase activity. Study on leaves revealed its potential as anti-inflammatory, anti-diabetic, anti-fertility, anti viral, anti bacterial and so on [18-24].

From the reported results of the phytochemical screening, the plant extract showed the presence of various phytochemical constituents such as alkaloid, glycoside, Carbohydrate, Flavonoids, Saponins, Tannins and Phytosterols [25]. So in present study, we tried to evaluate anti urolithiatic effect of ethanolic and aqueous extract of *Bougainvillea spectabilis* taking Cystone as a standard drug. The *in-vitro* mineralization method and titrimetric method for dissolution of calcium oxalate were performed to access the inhibition of stone formation and dissolution of stone crystals respectively.

2. MATERIAL AND METHOD

2.1. Collection and authentication of plant material

The leaves of the plant of *Bougainvillea spectabilis* Willd. were collected in month of February from the medicinal garden of GRY Institute of pharmacy, Borawan, MP. The herbarium specimen of plant was deposited in the department of Pharamacognosy and it has been authenticated by Ex. Professor of Botany, Dr. S. K. Mahajan, Govt. PG College, Khargone. Plant authentication certificate registration number obtained was SKM/PGC/2018/X-10 for *Bougainvillea spectabilis.*

2.2. Processing of Plant material

Collected fresh leaves were washed, shade dried and was pulverized with a mechanical pulverized for size reduction. It was then passed through sieve and the fine power was collected.

2.3. Chemicals and Reagents

Cystone, Sodium oxalate, Tris buffer, calcium chloride, Potassium permanganate ($KMnO_4$), Sulphuric acid (H_2SO_4) etc. were used in the study. Cystone was obtained from Himalaya Drug Company. Sodium oxalate was obtained from Fisher Scientific, Mumbai. Calcium chloride, Potassium permanganate and Tris buffer were obtained from LOBA Chemie, Mumbai. All the chemicals and reagents used were of analytical grade.

2.4. Preparation of the plant extracts [26-27]

The dried and coarse leaves sample (50 gm) was extracted successively with petroleum ether, chloroform, ethyl acetate, ethanol and water in a soxhlet extractor by continuous hot percolation. Finally, the concentrated extracts were evaporated to dryness and the extracts obtained with each solvent were weighed.

2.5. *In-vitro* method of mineralization [28-29]

To investigate inhibition of calcium oxalate mineralization by *Bougainvillea spectabilis;* we used simultaneous flow static model (S.S.M.). We used ethanolic and aqueous extracts of *Bougainvillea spectabilis* (200mg/ml), Standard drug Cystone (200mg/ml), calcium chloride (0.1 M) and sodium oxalate (0.1 M) (for obtaining calcium oxalate) in separate burette (25 ml) and allowed to fall simultaneously into a 250 ml beaker with a slow and steady pace. After 30-40 Min, the mixture was kept in a hot water bath for 10 min, cooled to room temperature and precipitate was collected into a pre-weighed centrifuge tube. Supernatant fluid was discarded. Then, the tubes were dried in a hot air oven at 120°C, cooled to room temperature and weighed till constant weight was achieved. Weight of the precipitate was calculated. Then percentage inhibition was calculated by following formula: (Graph:1)

Inhibition (%) = (Weight of PPT in blank set - Weight of PPT in experimental set x 100) / Weight of PPT in blank set

2.6. Evaluation of *in-vitro* anti-urolithiatic activity by the Titrimetric method

2.6.1. Preparation of calcium oxalate crystals by homogeneous precipitation method

Calcium Chloride dihydrate (4.41g) dissolved in distilled water and Sodium Oxalate (4.02g) dissolved in 2N Sulphuric acid, were taken into separate beakers and both the solutions were mixed together to react with stirring until Calcium oxalate precipitate formed. Excess Sulphuric acid was removed by washing with Ammonia solution and distilled water respectively. It was allowed to dry at 60°C for 4 h [30].

2.6.2. Preparation of a semi-permeable membrane from farm eggs [31-33]

The semi - permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin and yolk. The shell was chemically removed from the eggs by placing the eggs in HCl (2M) overnight, which caused complete decalcification. After washing with distilled water, a hole was carefully made on the top, and the contents were completely squeezed out from the decalcified egg. Then the egg membranes were washed thoroughly with distilled water, placed in ammonia solution, and stored in moistened condition at a pH of 7-7.4 in a refrigerator.

2.6.3. Determination of calcium oxalate [31-33]

Titrimetric estimation of CaOx was determined according to Saso et al. (1998). The dissolution percentage of calcium oxalate was calculated by taking exactly 1 mg of calcium oxalate and 10 mg of ethanolic and aqueous plant extracts, Standard drug Cystone

(Positive control) 10mg packed together with egg semipermeable membrane. They were allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer. One group served as negative control (containing only 1 mg of calcium oxalate). All conical flasks were placed in an incubator, preheated to 37°C for 2 h (Fig. C). Next, the content of the semi-permeable membrane from each group was transferred into a test beaker. Finally, 2 ml of 1 N sulphuric acid were added and titrated with 0.9494 N $KMnO_4$ till a light pink color was obtained (end point). 1 ml of 0.9494 N $KMnO_4$ was equivalent to 0.1898 mg of calcium oxalate. The dissolution percentages of the calcium oxalate crystals were calculated for each sample to evaluate the activity.

The percentage dissolution was calculated as follows:

Dissolved calcium oxalate = (Undissloved calcium oxalate) - (Total quantity used in the Experiment in the beginning)

Percentage dissolution=Dissolved calcium oxalate X 100

3. RESULTS AND DISCUSSIONS

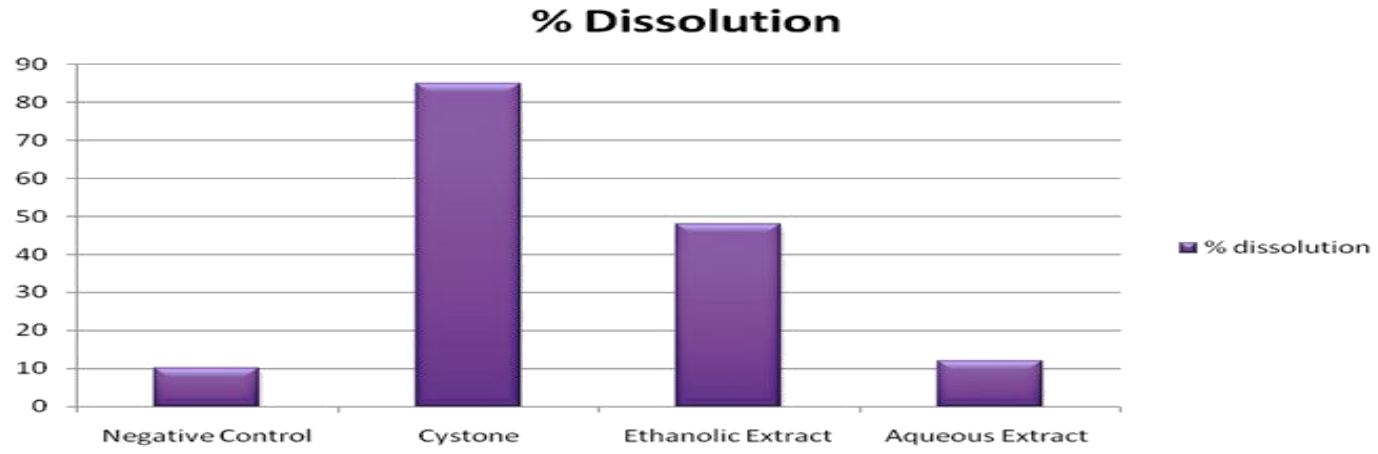
This study evaluates the anti urolithiatic activity of ethanolic and aqueous extract of *Bougainvillea spectabilis*. In both the method of *In-Vitro* studies, the highest percentage i.e. 81 % of inhibition and 85 % of calcium oxalate (CaOx) dissolution was observed in standard drug followed by significant result of 43% of inhibition and 52 % of calcium oxalate dissolution observed in ethanolic extract. Aqueous extract showed some positive but insignificant result of 15 % of inhibition and 12 % of calcium oxalate dissolution (Table 1 and 2, Graph:1 and 2). From this study, it was observed that ethanolic extracts of *Bougainvillea spectabilis* showed their significant potency in inhibition and dissolution of calcium oxalate compared to aqueous extract. This may be due to the presence of Flavonoids in ethanolic extract of the plant which is absent in aqueous extract. This study has given primary evidence for *Bougainvillea spectabilis* as the plant which possess lithotriptic property. This *in vitro* study has given lead data and shown that ethanolic extracts are quite promising for further studies in this regard.

Table 1:Percentage Inhibition of calcium oxalate {CaOx} by *Bougainvillea spectabilis* leaves extracts

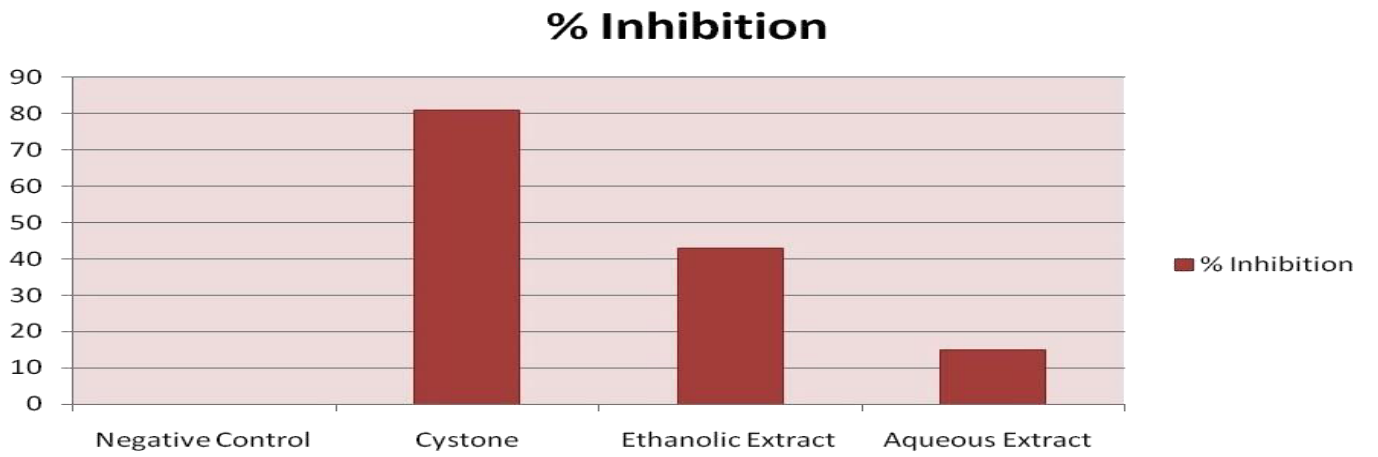
S. No	Treatment	Wt. of PPT (mg) (Blank Set)	Wt. of PPT (mg) (Exp Set)	% Inhibition
1	Negative Control	1.7	1.7	0
2	Cystone	1.7	0.31	81
3	Ethanolic Extract	1.7	0.96	43
4	Aqueous Extract	1.7	1.43	15

Table 2:Percentage dissolution of calcium oxalate by *Bougainvillea spectabilis* leaves extracts

Treatment	Dissolved calcium oxalate (mg)	% Dissolution
Negative Control	0.1	10
Positive Control (Cystone 10 mg)	0.85	85
Ethanolic Extract (10 mg)	0.52	52
Aqueous Extract (10 mg)	0.12	12
Treatment	**Dissolved calcium oxalate (mg)**	**% Dissolution**



Graph 1:Percentage Inhibition of Calcium Oxalate PPT



Graph 2:Percentage Dissolution of Calcium Oxalate

4. CONCLUSION

Findings of the present study clearly demonstrate anti urolithiatic potential of *Bougainvillea spectabilis* against calcium oxalate urolithiasis *in vitro*. The plant showed prominent inhibition and great efficacy in the dissolution of calcium oxalate crystals. Further, tests will be necessary to objectify a scientifically proven benefit of the extract tested on the solubilization of such calculi. Since mechanism of anti-urolitholytic activity in the extract is exact unknown till date, thus *in-vivo* studies should be further investigated to reveal the phytochemicals of the extract responsible for dissolving or disintegrating renal calculi and to know better understanding in the molecular mechanism of litholysis and provide more clarity as per its clinical significance and also its role as an herbal drug in human health and medicine. The study has given a basic scientific evidence for the traditional uses of the plant in the prevention and treatment of urolithiasis. Therefore, this plant could be a potential source of new drug molecules with anti-urolithiatic activity.

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Conflict of interest

Nil

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