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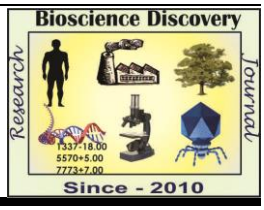

Study on *In vitro* antiurolithiatic activity of *Bryophyllum pinnatum* and *Ocimum gratissimum* leaves

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Abstract

Bryophyllum pinnatum and *Ocimum gratissimum* has been used to treat various disease including urinary stone disease since ancient time in India. In synthetic urine and nucleation method calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solution. In nucleation assay, the aim was to evaluate the effectiveness of different concentration of the aqueous extract (100-1000µg/ml) on calcium oxalate monohydrate crystal from artificial urine at different % concentration of extract (200-1000%) was investigated. *Bryophyllum pinnatum* and *Ocimum gratissimum* showed % inhibition for calcium oxalate crystal with maximum inhibition of 65.97% at 600µg/ml and 62.26% at 600 µg/ml respectively while in nucleation assay % inhibition for calcium oxalate formation was directly proportional to be increasing concentration of the plant extract with maximum inhibition was 89.28% and 86.62% at 1000µg/ml respectively. *Bryophyllum pinnatum* and *Ocimum gratissimum* was found to be potent and promising antiurolithiatic agent which is in accordance with its use in traditional medicine.

INTRODUCTION

Stone formation in the kidney is one of the oldest and most wide spread diseases known to man. In India people living in different states utilize different plants for curing urolithiasis (Chitme *et al*., 2010). It is belived that the urinary tract is the third most common affliction (Khan and Pradhan, 2012). All over the world, the most number of people, nearly 4-15% of the human populations suffer from urinary stone problem (Khare *et al*., 2014). The "stones belt" in India occupies parts of Maharashtra, Gujarat, Punjab, Haryana, Delhi and Rajasthan but south has fewer occurrences of urinary stone (Yashir and Waqar, 2011). Twelve percent of the population is expected to have urinary stones in India, out of which 50% may end up with loss of kidneys or renal damage (Ram *et al*., 2015). Since urolithiasis is a multifactorial disease, it is highly unpredictable and its etiology is very complex (Yashir and Waqar, 2011). A stone is an aggregation of solute materials from urine such as calcium, oxalate, phosphate and uric acid which forms stone. In India, the most predominant constituent of urolithiasis is found to be calcium oxalate (Agarwal *et al*., 2014). Stone formation is the culmination of a series of physiochemical events i.e supersaturation and nucleation, growth of the crystal and aggregation that occurs as the glomerular filtrate traverses through the tubules of nephron. Urine is normally supersaturated with most stone forming salt components, as well as contains chemicals that prevent or inhibit crystal development in urinary tract (Ram J *et al*., 2015).

However, the presence of certain molecules raise the level of super saturation of salts needed to initiate crystal nucleation or reduce the rate of crystal growth or aggregation and prevents stone formation (Singh, 2011). Calcium oxalate stones shows up to 80% of analyzed stones (Awari *et al*., 2009). Calcium phosphate account for 15-25%, while 10- 15% is mixed stones. The others are struvite 15-30%, cystine 6-10%, and uric acid stones 2 10% (Shashi *et al.*, 2013). Calcium oxalate stones are of basically two types, calcium oxalate monohydrate (whewellite) and calcium oxalate dihydrate (weddellite). The occurrence frequency of whewellite is 78% while that of weddellite is 43% (Rao *et al.*, 2011). Now-a-days, however, herbal medicine is very popular because, herbal medicines are more effective and have fewer side effects also. In ayurveda more plants having the quality of disintegrating and dissolving the stone are referred to as "pashanbheda". Ashok Kumar and his co worker in 2013 stated that Gokhsuradi churan possess an antiurolithiatic activity. *Bryophyllum pinnatum* has been used for treating cough, asthama, cold, fever and constipation. *Ocimum gratissimum* has also been used traditionally for treating cancer, inflammation, diarrhoea (Agrawal and Varma, 2014). Less information was available on antiurolithiatic activity of *Bryophyllum pinnatum* and *Ocimum gratissimum*. Keeping above knowledge in the mind, current study was done to find out the stone formation inhibitor effect of *Bryophyllum pinnatum* and *Ocimum gratissimum.*

Materials and Methods

Collection of Plant: The leaves of *Bryophyllum pinnatum* and *Ocimum gratissimum* were collected from Botanical Garden, Shri Shivaji College of Science, Akola, and Maharashtra during the month of September 2016.

Preparation of extract of plant: Fresh plant after collection was shade dried at room temperature then grinded in mixture grinder. The fine powder of plant was extracted with distilled water by Soxhlet apperatus for 72 hours. Then the extract was concentrated in vaccum to dryness at 30- 40º C. Then dried extract was stored in refrigerator for further use.

Preparation of artificial urine

The artificial urine (AU) was prepared according to the method Burns and Finlayson with slight modification and the following composition: sodium chloride 105.5 mM, sodium phosphate 32.3 mM, sodium citrate 3.21 mM, magnesium sulfate 3.85 mM, sodium sulfate 16.95 mM, potassium chloride 63.7 mM, calcium chloride 4.5 mM, sodium oxalate 0.32 mM, ammonium hydroxide 17.9 mM, and ammonium chloride 0.0028 mM. The AU was prepared fresh each time and pH adjusted to 6.0.

Study without inhibitor

A volume of 1.0 ml of AU was transferred into the cell and 0.5 ml of distilled water added to it and blank reading was taken. The 0.5 ml of 0.01M sodium oxalate was added, to the previous volume, and the measurement is immediately started for a period of ten minutes.

Study with inhibitor

The aqueous extract of *Bryophyllum pinnatum* and *Ocimum gratissimum* was dissolved in distilled water, filtered through membrane filter and the concentration of 200, 400, 600, 800 and 1000 µg/ml was obtained. A mixture of 1 ml of AU and 0.5 ml of plant extract solution is versed in the cell. A blank reading was taken and then 0.5 ml of 0.01M sodium oxalate solution was added and immediately the absorbance was measured for a period of ten minutes at 620nm.

The percentage of inhibition of calcium oxalate crystal formation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Where; Ab Test: Absorbance in the presence of inhibitor (Extract), Ab Control: Absorbance of without inhibitor (Control) (Shrinivasa *et al.*, 2013)

Nucleation assay:

Nucleation assay was done by following the method of Hennequin *et al.*, 1993. Solution of calcium chloride and sodium oxalate were prepared at the final concentration of 5mol/L and 7.5mmol/L respectively in buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at P^H 6.5 950µl of calcium chloride solution was mixed with100µl of herb extract at different concentration (100µg/ml to 1000µg/ml). Crystallisation was started by adding 950µl of sodium oxalate solution. The temperature was maintained at 37ºc.The OD of the solution was monitored at 620nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control (Atmani and khan, 2000; Masao *et al.*, 2000). The growth of the crystal was expected due to the following reaction.

$$CaCl_2+Na_2C2O_4 \rightarrow CaC_2O_4+ 2\ NaCl$$

The percentage of inhibition produced by the herb extract was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION

At different concentration the formation and growth of the calcium oxalate monohydrate crystals from artificial urine was studied. With certain urinary salts such as calcium oxalate stone formation is the result of super saturation of urine. The number of calcium oxalate monohydrate was found to be maximum in control. In order to estimate the inhibiting potential of plant extract for oxalate crystallization different percentages of plant extract was tested.

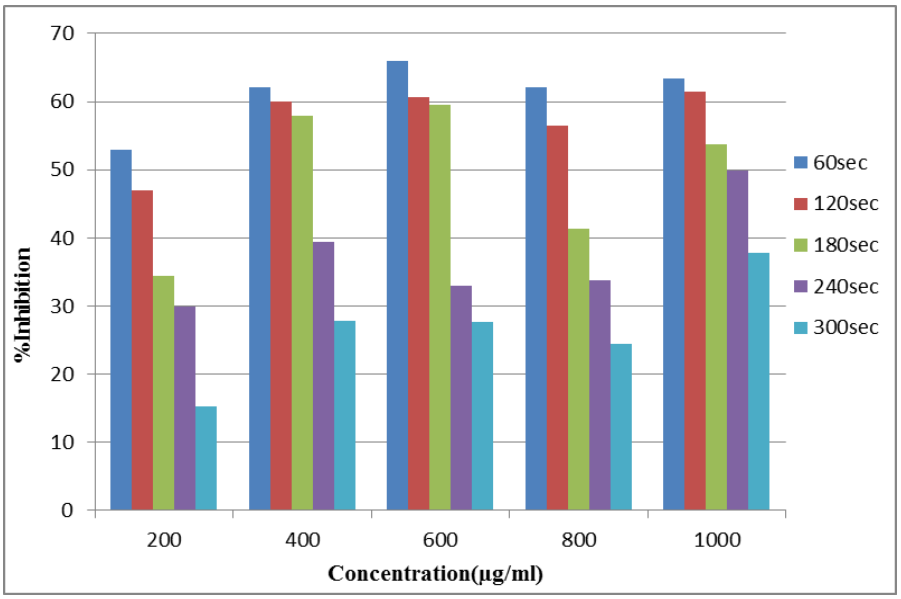

Figure 1: Effect of different concentrations of extract of *Bryophyllum pinnatum* on CaOx crystallization.

The results indicate that *Bryophyllum pinnatum* and *Ocimum gratissimum* showed % inhibition for calcium oxalate crystal with maximum inhibition of 65.97% at 600µg/ml and 62.26% at 600 µg/ml respectively (Fig. 1 and 2) while in nucleation assay % inhibition for calcium oxalate formation was directly proportional to be increasing concentration of the plant extract with maximum inhibition was 89.28% and 86.62% at 1000µg/ml respectively (Fig.3 and 4).

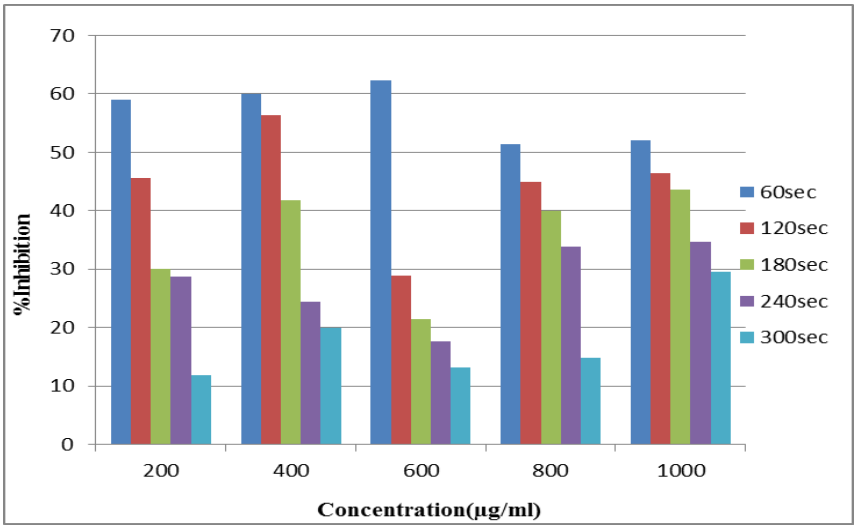

Figure 2: Effect of different concentrations of extract of *Ocimum gratissimum* on CaOx crystallization.

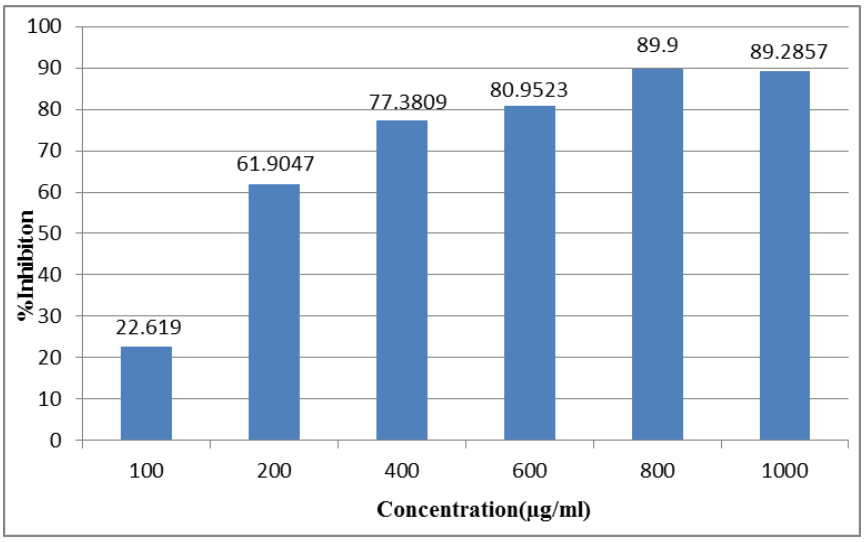

Figure 3: Effect of aqueous extract of *Bryophyllum pinnatum* on nucleation of calcium oxalate.

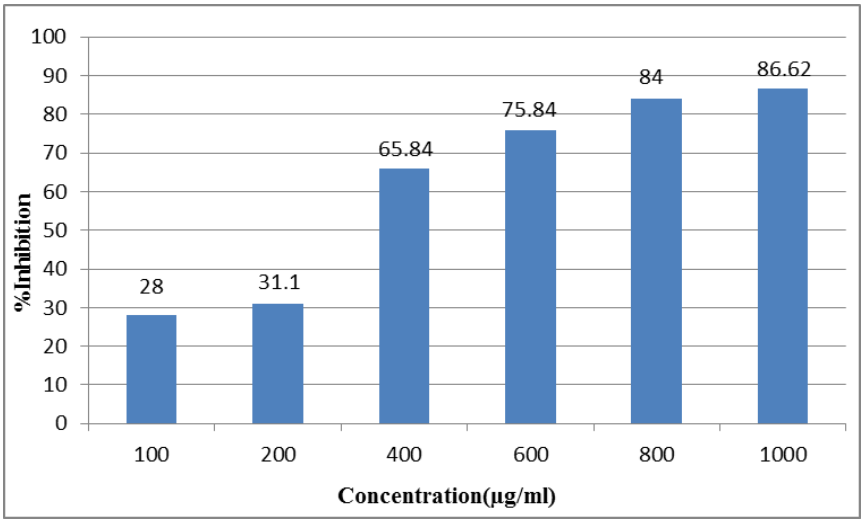

Figure 4: Effect of aqueous extract of *Ocimum gratissimum* on nucleation of calcium oxalate.

From the present study it was found that the aqueous extract of both the plant *Bryophyllum Pinnatum* and *Ocimum Gratissimum* has shown significant antilithiatic activity. However these *in-vitro* results should be confirmed *in-vivo* in order to develop a potent antilithic agent from this plant, as this property of the extract is advantageous in preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of their retention in the urinary tract.

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