STUDY OF ANTIUROLITHIC ACTIVITY OF TROPICAL FLORA OF WESTERN GHATS

Anokhee Bavishi[1], Nishita Gattani[2] and Dr. Shubhada Walvekar[3]*
1, 2 & 3 Biotechnology Department, Mithibai College, Mumbai.
3. Corresponding Author, Assistant Professor,

Abstract:

The study involved the analysis of antiurolithic potential of two monsoon seasonal plants from Western Ghats – *Tephrosia tinctoria* (Fabaceae) and *Boehmeria macrophylla* (Urticaceae). The methanolic extract of both the plants were used to check their ability to prevent the formation of calcium oxalate monohydrate crystals using artificial urine synthesised in the laboratory. The activity was estimated by turbidometric method and percentage inhibition with the graphical method was determined. Both the tropical species inhibited the formation of calcium oxalate crystals in artificial urine.

Index Terms – **Antiurolithic activity, *Tephrosia tinctoria, Boehmeria macrophylla,* Calcium Oxalate monohydrate**

I. INTRODUCTION:

Kidneys perform a variety of functions in human beings such as maintaining overall fluid balance of the body, filtering and regulating the mineral content and removal of waste materials from the blood, thereby regulation of blood pressure (Mikawlrawng, 2014), nephrolithiasis (the formation of kidney stones), ureterolithiasis (the formation of stones in the ureters) and cystolithiasis (the formation of bladder stones). Urolithiasis indicates formation of calcium, oxalate, uric acid stones in any part of urinary system such as kidney, urinary bladder, urethra or uretors (Arya, 2017). These are formed due to inadequate urinary drainage, presence of foreign bodies, microbial infection, diet rich in oxalate and calcium salts, deficiencie of Vitamin A as well as certain metabolic reasons such as hyperthyroidism, cystinuria and gout (Mekap et al, 2011). Stone formation occurs due to supersaturation, nucleation, growth and aggregation of crystals during the transport of glomerual fluid through the urinary tubules (Agarwal and Verma, 2014). Formation of uroliths leads to acute pain, bleeding with secondary infections. According to epidemiological studies, deposition of calcium oxalate appears to be the major reason for urolithiasis (Pareta et al., 2011). There are two basic types of Calcium oxalate stones - 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).

It is observed in individuals of all ages which results in blocking the flow of urine with renal colic. The patients also need a surgical treatment in severe conditions. It has a high frequency of recurrence after the first episode. There is an urgent need of prophylactic measures to prevent the recurrence and the painful episodes for the patient associated with it. Use of diuretics, alkali treatment act as remedies for hyperuricemia as well as urolithiasis but these are not cost effective and show side effects. The indigenous system of medicines with safe herbal remedies having no side effects appears to be a promising treatment of the above mentioned clinical conditions. Pathophysiology, most plant based therapy have been shown to be effective at different stages of stone development (Kishore et al, 2013). The study is carried out to check the efficacy of two tropical species, *Tephrosia tinctoria, Fig.1* (Fabaceae) and *Boehmeria macrophylla Fig.2* (Urticaceae) of Western Ghats for their antiurolithic activity using nucleation assay and synthetic urine assay. *Tephrosia* species are well known

Fig: 1. *Tephrosia tinctoria* (Fabaceae)

Fig:2. *Boehmeria macrophylla* (Urticeae)

The aim of the nucleation assay was to evaluate the effectiveness of various concentrations of extracts 50 – 250 µg/ml of plant extract on calcium oxalate crystallization *in vitro*. The assay using synthetic urine was carried out to study percentage inhibition and growth of calcium oxalate monohydrate crystals at various concentrations of plant extract (Kumar et al, 2014).

II. RESEARCH METHODOLOGY:

2.1.Collection of plant material and Identification:

The leaves and flowers of both the species selected were collected from Kudavale, Dapoli district during late monsoon and were identified with the help of regional flora as well as Taxonomists with confirmation using preserved herbarium specimens.

2.2.Preparation of extract:

The freshly collected specimens were cleaned, dried under the shade and then ground to make a fine powder. The powder was macerated defatted using petroleum ether and extracted in 80% Methanol for a period of 72 hrs and then subjected to hot percolation and distillation. The obtained solution was filtered, evaporated to dryness and dried in desiccators. It was stored in refrigerator for further use. The extract was dissolved in distilled water to obtain the concentration grades of 50 to 250µg/ml for further use for both the plants.

2.3.Nucleation Assay:

The experiment was designed as specified by Atamani et al, (2000) This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of the plant extract used. The OD of the solution was monitored at 620 nm using spectrophotometer after every minute for 30 minutes. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. Data was represented in percentage inhibition. The growth of crystals was expected due to the following reaction:

$$CaCl_2 + Na_2C_2O_4 \rightarrow CaC_2O_4 + 2NaCl. \quad (2.1)$$

2.4.**Synthetic urine assay**:

2.4.1. **Preparation of synthetic urine:**

The model selected for checking antiurolithic activity includes the study of crystallization and with and without inhibitor, to estimate the capacity of the plant extract to inhibit crystallization. Two solutions of following composition were mixed: A: $Na_2C_2O_4$ (2 mmol/1) and B: $CaCl_2$ $2H_2O$ (10 mmol/1) as suggested by Beghalia et al, (2008). The two solutions were prepared along with adding NaCl 9 g to obtain the ionic force like the indoor environments. Synthetic urine is prepared by mixing and stirring two equal volumes of 50 ml of solutions A and B at constant temperature (37°C) in capped vessels to give final artificial urine. The mixture was agitated to prevent sedimentation.

2.4.2. **Simulation of the sedimentary crystal formation:**

The crystal size development was monitored in sample drops every five minutes by polarized microscope. A drop of sample was put was observed under microscope after 30 minutes. The number of crystals were observed and subsequently its photograph was taken. A series of experiments corresponding to the physiological concentrations of 25, 50, 75, and 100% of plant extract was conducted. The follow-up of the crystal size development by microscope was carried out after 30 minutes of formation of crystals and their photographs were taken.

The percentage of Inhibition (I %) was calculated with the help of following formula

$$I\% = [(TSI- TAI) / TSI] \times 100$$

TSI- represents the number of calcium oxalate monohydrate crystals without inhibitor.

TAI- represents the number of calcium oxalate monohydrate crystals after addition of inhibitor.

III. RESULTS AND DISCUSSIONS:

3.1. Effect of plant extracts on Nucleation Assay:

Mixing of calcium chloride and sodium oxalate results in the formation of calcium oxalate crystals. Nucleation per unit time was estimated by measurement of turbidity at 620nm after 30 minutes with and without plant extract for both the plant extracts separately. The addition of plant extracts reduces the optical density of the solution over time, which is lower than control due to inhibition of crystallization. The observations indicate that the percentage of crystallization was directly proportional to increase in concentration of plant extract. Both the plant extracts used were equally effective on reducing the number of crystals (Figure 3 to 5).

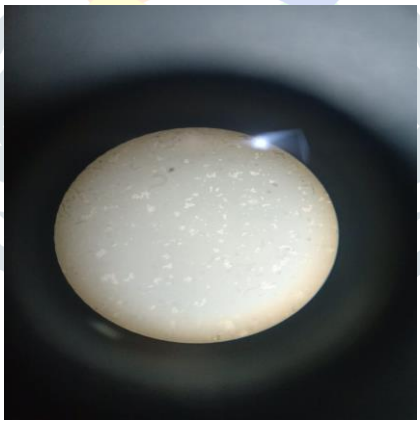


Figure 3. – Formation of Crystals wothout inhibitor

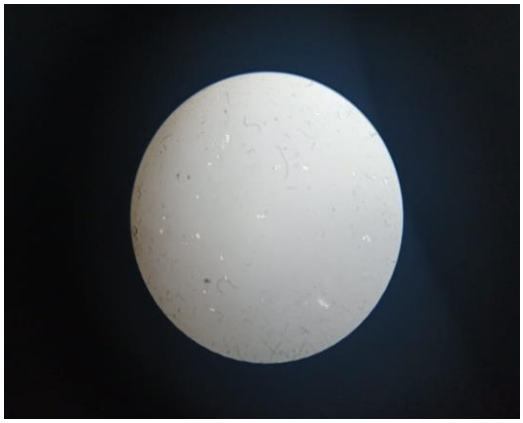


Figure 4. – Inhibition of Crystallization by 250µg/ml of *T. tinctoria*

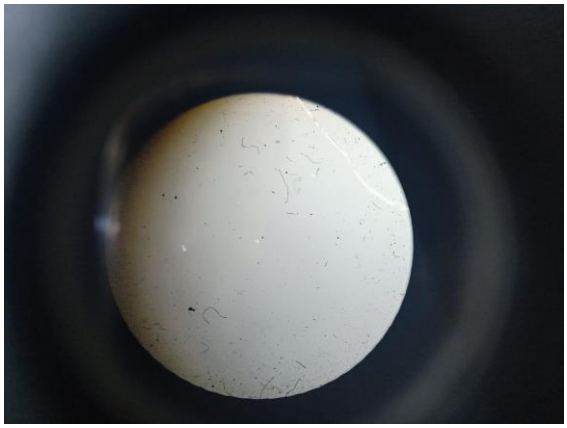


Figure 5. Inhibition of crystallization by 250µg/ml of *B. macrophylla*

3.2. Synthetic urine assay:

The effect of *T. tinctoria* and *B. macrophylla* extracts on the formation of calcium oxalate monohydrate crystals was studied for the duration of 10 minutes. The turbidity was measured at the interval of one minute and the percentage inhibition of crystallization was calculated. Both the plant extracts were effective in inhibiting the formation of crystals indicating antiurolithic ability (Graph 1). The inhibition of crystallization was higher in case of *B. macrophylla* for all the concentrations of plant extract (Table 1).

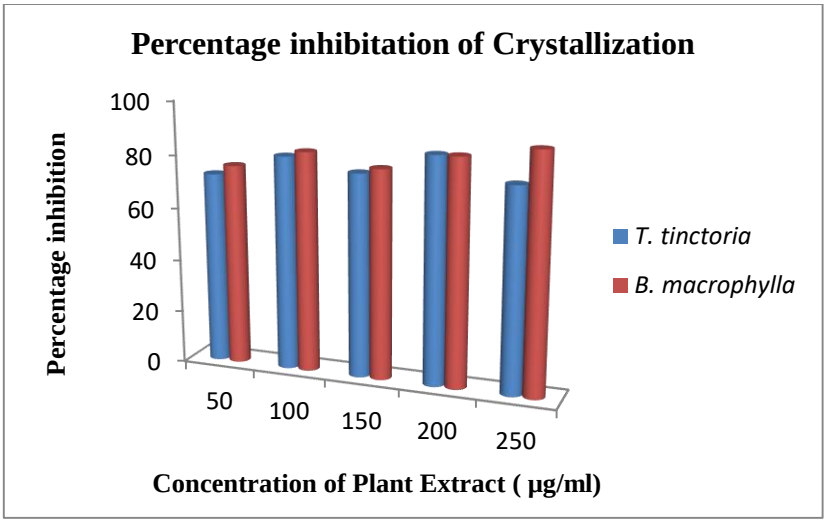
Use of herbal extracts to reduce the urinary crystallization is reported for various plants by researchers. Arya et al.,(2017) have carried out a research on kidney stones formation and use of medicinal plants as antiurolithiatic agents where a review of various types of stones and the factors leading to their formation is reviewed. Mikawlrawng, (2014) have reported antiurolithic action of herbs from Manipur. Khan and Pradhan, (2012) have reported effective use of *Ceropegia* bulbs using animal models. Vamsi et al, (2014) have reported similar activity in *Mucuna puriense*. Vyas et al.,(2011) have studied the effect of whole plant hydroalcoholic extract of *Pergularia demia* on nephrolithiasis in rats.

The study could be carried out further using various gradient extracts to find out the effective solvent for extraction. The plants selected could be studied for their xanthine oxidase inhibitor activity which is crucial in prevention of formation of uric acid crystals.

Table 1. Percentage Inhibition of Calcium oxalate monohydrate crystals

Conc. of extract (µg/ml)	**Percentage Inhibition of Crystallization**	
	T. tinctoria	***B. macrophylla***
50	72.34	76.00
100	80.85	82.97
150	76.59	78.72
200	85.10	85.10
250	76.59	89.36

Graph 1. Percentage inhibition of Crystallization due to extracts of *T. tinctoria* and *B. macrophylla*



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