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## Screening of selected plants for their effectiveness in the treatment of kidney stone

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### Abstract

Kidney ailments have increased several folds in the last two decades. Kidney stones are one of the most common urinary tract problems to be found. It has been associated with increased risk of renal failure if not treated in the early stages. Kidney stones are well known as renal calculus. They are mainly composed of minerals like calcium oxalate. An interaction with the local practitioners in Raigad district of Maharashtra, India revealed that several plant species are being used as lithotriptic agents. A simple technique has been designed to evaluate the lithotriptic activity of extracts of leaves of *Bauhinia purpurea* L., seeds of *Celosia argentea* L. and seeds of *Macrotyloma uniflorum* (Lam.) Verdc. Cystoliths and raphides which are aggregation of minerals are used as target minerals and their dissolution by the extracts of these plants are used as technique for evaluating the efficacy of these plants as lithotriptic agents.

### 1. Introduction

India has a glorious past of traditional medical system. Botanically derived medicine has played a major role throughout the history. Traditional knowledge is a valuable system continuously developed over generations by tribal and rural communities and transmitted from one generation to the other orally. The documentation of the indigenous knowledge on the utilization of local plant resources by traditional practitioners is one of the main objectives of ethnobotanical research. As these traditional knowledge is fast disappearing with time due to lack of scientific research, analysis and written documents. The authentication of herbal drugs is the need of the day (Alok *et al.*, 2013; Talele *et al.*, 2012).

The information of medicinal plants properties have been gathered from centuries based on diverse medicinal systems like Ayurveda, Unani and Siddha. It was found by the WHO report, that 60 % of the world's population depends predominantly on the traditional medicine while 80 % population in developing countries mostly use traditional medicinal practices and herbal medicines as a primary healthcare. Exploration of traditional medicine is an interesting and scientifically significant task for the ethnobotanists (Ram *et al.*, 2015). The process of calculi or stone formation is known as lithiasis. Lithiasis is concretion of material mainly mineral salts in any tissue of the body. Antilithics are the agents known for prevention from the formation of stone or promote the dissolution of formed calculi. The main stream medical management of kidney stone problems is

expensive and may result in reoccurrence of the problems and the drugs used in the treatment also have a many side effect. Plant based medicines are effective and devoid of any such side effects (Choubey *et al.*, 2010; Arya *et al.*, 2017).

Herbal medicines are more effective with fewer side effects and reduce recurrence rate of kidney stone formation. Thus, it has gained much recognition for antilithiatic drug from natural sources. It is of greater importance and it is also future promising. Herbal medicines have many phytoconstituents which are helpful and beneficial in kidney stone treatment. Plant extracts contain phytochemicals that hinder stone formation by inhibiting synthesis or by agglomeration of crystals. Although, use of herbal medicine is popular and promising, it is fundamental to carry out further research to understand the pathophysiology of disease, mechanism of action of herbal medicines in order to develop an efficient and safe litholytic agent (Ram *et al.*, 2015).

An ethnomedicinal survey was undertaken to gather information from traditional healers of various parts of Maharashtra, India to cure kidney stone. Most kidney stones are calcium stones, combined with oxalate, phosphate and some time with uric acid (Choubey *et al.*, 2010; Arya *et al.*, 2017). Many plants were being used for effective treatment of kidney stones, out of which *Bauhinia purpurea* L., *Celosia argentea* L., *Macrotyloma uniflorum* (Lam.) Verdc. were selected in the present study.

Our study shows the efficacy of leaves of *B. purpurea*, *C. argentea* and *M. uniflorum* seeds in the treatment of kidney stones. To demonstrate the effectiveness of these plants on mineral stones, the *Ficus elastica* leaves (containing cystolith) and *Colocasia esculenta* containing raphides were used. Cystolith is a term used for inorganic concretions generally of calcium carbonate and calcium oxalate, which are formed in cellulose matrix in special

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cells called lithocysts, generally found in the leaves of many species of *Ficus*. Raphides are needle shaped crystals of calcium oxalate occurring in clusters occurring widely in arums.

## 2. Materials and Methods

### 2.1 List of plant selected for testing lithotriptic activity

Target material for screening lithotriptic activity was cystolith of *Ficus elastica* leaves and raphides of *Colocasia esculenta* petiole.

Plant name	Family name	Parts used
<i>Bauhinia purpurea</i>	Caesalpiniaceae	Leaves
<i>Celosia argentea</i>	Amaranthaceae	Seeds
<i>Macrotyloma uniflorum</i>	Papilionaceae (Fabaceae)	Seeds

### 2.2 Phytochemical analysis

Leaves of *B. purpurea*, seeds of *C. argentea* and *M. uniflorum* were air dried for achieving the persistent weight. Extract was prepared by using dried powder of the plant material (size less than 180 micron) in ratio of 1:100 of solvent. For detection of phytochemicals, extracts were prepared in chloroform, acetone, 90 % methanol and water by sonicating 1 g in 100 ml of the respective solvent in an ultrasonic bath for 15 min at room temperature and corresponding filtrates were used for all phytochemical tests.

### 2.3 Effect of plant extract on cystolith and raphides

Extracts of *B. purpurea* leaves, *C. argentea* seeds and *M. uniflorum* seeds were prepared by using mortar and pestle.

The selected 1 g of plant material leaves were crushed in 10 ml of distilled water. The extracts were then filtered with whatmann filter paper no. 1. The filtrate was then used for further studies. Free hand sections of leaves of *F. elastica* showing cystolith and petioles of *C. sculenta* showing raphides were treated with the plant extracts. These sections were incubated in the extract at  $25 \pm 2^\circ\text{C}$  for 20-22 h. Effects of the extracts on the cystoliths were observed by comparing photomicrographs taken before and after treatment. Cystolith and raphides were treated with normal water as a control. Micrographs of *F. cystolith* were taken at 10 x magnification using a Motic digital Microscope and their size was determined using the Motic plus version 2.0 software. The results are based on observation of thirty sections of *F. elastica* leaves (cystolith) and *C. esculenta* petiole (raphides) treated with the extracts. All experiments were repeated thrice to ensure reproducibility of these results.

## 3. Results

The phytochemical screening of extracts of *B. purpurea* leaves, showed presence of flavonoids, glycosides, polyphenols, saponin and steroid mainly. *C. argentea* seed extracts showed presence of chalcones, emodins, glycosides and polyphenols, whereas extracts of seeds of *M. uniflorum* showed presence of chalcones, emodins, glycosides, polyphenols and tannins. These results have been compiled in Tables 1-3.

**Table 1:** Phytochemical screening of extracts of leaves of *Bauhinia purpurea* L.

Chemical constituents	Tests	Extracts			
		CH	AC	ME	WA
Alkaloids	Dragendroff reagent	-	-	-	-
	Wagner's reagent	-	-	-	-
Amino acids	Amino acids test	+	+	-	-
Anthocyanin	Anthocyanin test	-	-	-	-
Antraquinone	Antraquinone test	-	-	-	-
Carbohydrate	Felhing's test	+	-	-	-
	Benedict's test	+	-	-	-
Cardiac glycosides	Baljet's test	-	-	-	-
Chalcones	Chalcones test	+	-	-	-
Coumarins	Coumarin test	-	-	+	-
Emodins	Emodins test	+	-	-	-
Flavonoids	Pew's test	-	-	-	-
	Sodium hydroxide test	-	-	+	-
	Ammonium hydroxide test	-	+	+	-
Glycosides	Kedde's test	+	+	-	-
	Keller killani test	-	-	-	-
Leucoanthocyanin	Leucoanthocyanin test	-	-	+	+
Polyphenol	Folin's ciocalteau	-	-	+	+
Proteins	Proteins test	-	-	-	-
Saponin	Foam test	+	+	+	-
Steroids	Salkowski's test	+	+	+	+
Tannin	Tannin test	-	+	-	-
Terpenoids	Anisaldehyde reagent	-	+	-	-
	Vanillin-sulphuric acid reagent	-	-	-	-

(CH: Chloroform; AC: Acetone; ME: 90 % Methanol; WA: Water)

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Table 2: Phytochemical screening of extracts of seeds of *Celosia argentea* L.

Chemical constituents	Tests	Extracts			
		CH	AC	ME	WA
Alkaloids	Dragendroff reagent	-	-	-	-
	Wagner's reagent	-	-	-	-
Amino acids	Amino acids test	+	+	-	-
Anthocyanin	Anthocyanin test	-	-	-	-
Antraquinone	Antraquinone test	-	-	-	-
Carbohydrate	Felhing's test	+	+	-	-
	Benedict's test	+	+	-	-
Cardiac glycosides	Baljet's test	-	-	-	-
Chalcones	Chalcones test	+	+	+	+
Coumarins	Coumarin test	-	-	-	-
Emodins	Emodins test	+	+	+	+
Flavonoids	Pew's test	-	-	-	-
	Sodium hydroxide test	-	-	-	-
	Ammonium hydroxide test	-	-	-	-
Glycosides	Kedde's test	+	+	+	+
	Keller killani test	-	-	-	-
Leucoanthocyanin	Leucoanthocyanin test	-	-	-	-
Polyphenol	Folin's ciocalteau	+	+	+	+
Proteins	Proteins test	-	-	-	-
Saponin	Foam test	-	-	-	-
Steroids	Salkowski's test	-	-	-	-
Tannin	Tannin test	-	-	-	-
Terpenoids	Anisaldehyde reagent	-	-	-	-
	Vanillin-sulphuric acid reagent	-	-	-	-

(CH: Chloroform; AC: Acetone; ME: 90 % Methanol; WA: Water)

Table 3: Phytochemical screening of extract of seeds of *Macrotyloma uniflorum* (Lam.) Verdc.

Chemical constituents	Tests	Extracts			
		CH	AC	ME	WA
Alkaloids	Dragendroff reagent	-	-	-	-
	Wagner's reagent	-	-	-	-
Amino acids	Amino acids test	-	+	-	-
Anthocyanin	Anthocyanin test	-	-	-	-
Antraquinone	Antraquinone test	-	-	-	-
Carbohydrate	Felhing's test	+	+	-	-
	Benedict's test	+	+	-	-
Cardiac glycosides	Baljet's test	-	-	-	-
Chalcones	Chalcones test	+	+	+	+
Coumarins	Coumarin test	-	-	-	-
Emodins	Emodins test	+	+	+	+
Flavonoids	Pew's test	-	-	-	+
	Sodium hydroxide test	-	-	-	-
	Ammonium hydroxide test	-	-	-	-
Glycosides	Kedde's test	+	+	+	+
	Keller killani test	-	-	-	-
Leucoanthocyanin	Leucoanthocyanin test	-	-	-	-
Polyphenol	Folin'sciocalteau	+	+	+	+
Proteins	Proteins test	+	+	+	+
Saponin	Foam test	-	-	-	-
Steroids	Salkowski's test	-	-	-	-
Tannin	Tannin test	+	+	+	+
Terpenoids	Anisaldehyde reagent	-	-	-	-
	Vanillin-sulphuric acid reagent	-	-	-	-

(CH: Chloroform; AC: Acetone; ME: 90% Methanol; WA: Water)

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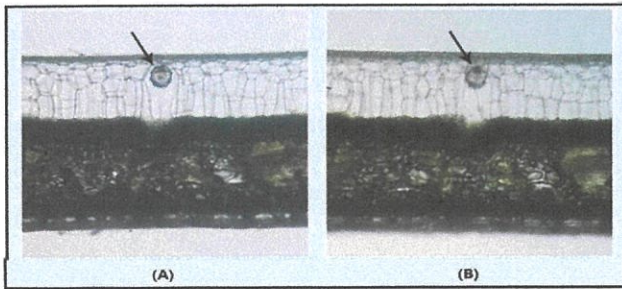


*F. elastica* leaf sections showing cystolith were used for the study. Extract of *B. purpurea* leaves, seeds of *C. argentea* and *M. uniflorum* were used to observe their lithotriptic activity on cystolith and raphides. It was seen that after  $20 \pm 2$  h of treatment, the cystolith had completely dissolved leaving behind the peg which is made of

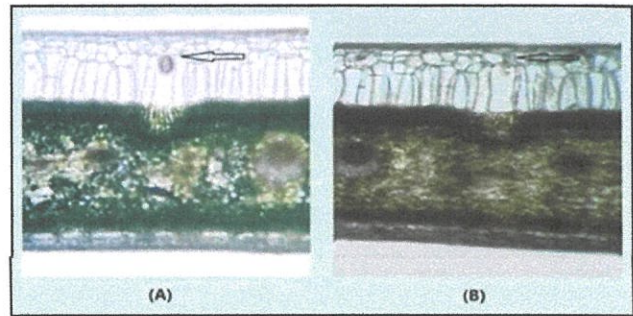
cellulose, whereas the raphides of *C. esculenta* petiole had completely dissolved. Control sections were treated with distilled water and in these sections, cystolith and raphides remained intact even after twenty four hour of treatment. These results are summarised in Table 4.

**Table 4:** Effect of plant extract on cystolith and raphides

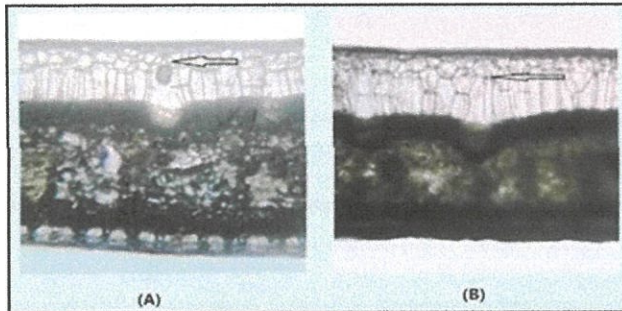
Plant extract	Extract of plant part used	Effect on <i>Ficus</i> cystolith and <i>Colocasia</i> raphides	Photoplate
Control (water)	-	No effect	No 1
<i>Bauhinia purpurea</i>	Leaves	Dissolution of cystolith	No 2
<i>Celosia argentea</i>	Seeds	Dissolution of cystolith	No 3
<i>Macrotylomauniflorum</i>	Seeds	Dissolution of cystolith	No 4
Control (water)	-	No effect	No 5
<i>Bauhinia purpurea</i>	Leaves	Complete dissolution of raphides	No 6
<i>Celosia argentea</i>	Seeds	Complete dissolution of raphides	No 7
<i>Macrotylomauniflorum</i>	Seeds	Complete dissolution of raphides	No 8



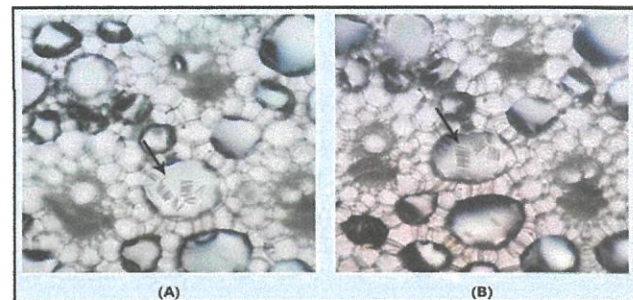
**Photoplate 1:** Control - *Ficus elastica* leaf section showing cystolith before (A) and after (B) treatment with water.



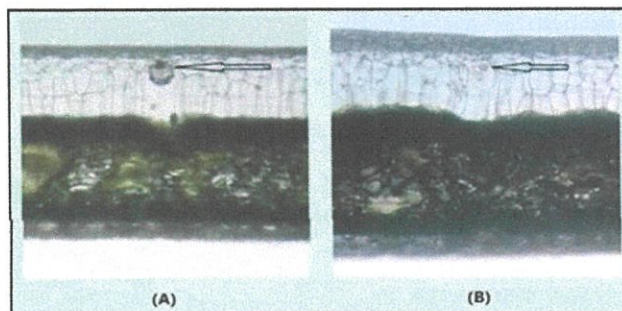
**Photoplate 4:** *Ficus elastica* leaf section showing cystolith before (A) and after (B) treatment with *Macrotyloma uniflorum* extract of seeds.



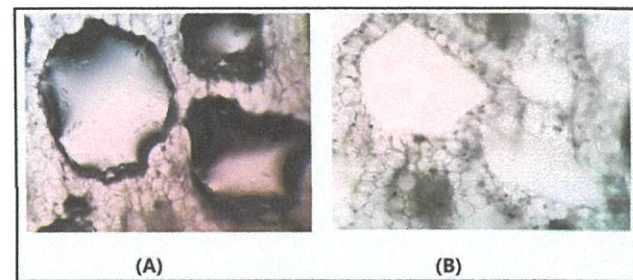
**Photoplate 2:** *Ficus elastica* leaf section showing cystolith before (A) and after (B) treatment with *Bauhinia purpurea* extract of leaves.



**Photoplate 5:** Control - *Colocasia esculenta* raphides before (A) and after treatment (B) with distilled water.



**Photoplate 3:** *Ficus elastica* leaf section showing cystolith before (A) and after (B) treatment with *Celosia argentea* extract of seeds.

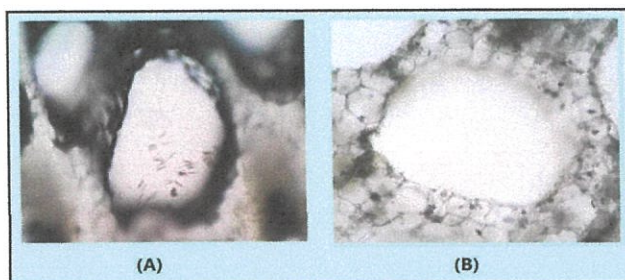


**Photoplate 6:** *Colocasia esculenta* section showing raphides before (A) and after (B) treatment with *Bauhinia purpurea* extract of leaves.

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**Photoplate 7:** *Colocasia esculenta* section showing raphides before (A) and after (B) treatment with *Celosia argentea* extract of seeds.

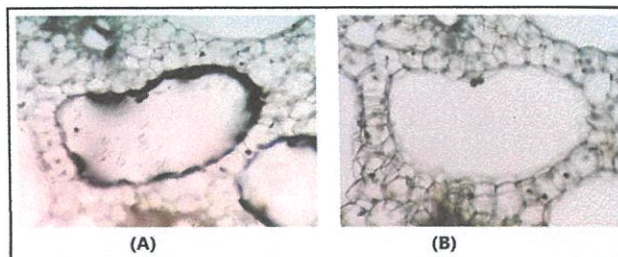
#### 4. Discussion

Herbal extracts are like diuretic in nature and have crystallization inhibiting activity, lithotriptic activity, analgesic and anti-inflammatory activity which can be used to prevent stone formation. *Celosia* roots are used for urolithic activity (Ahmed *et al.*, 2016), method used for the study is fruitful as it shows a visible results, hence it can be screened easily whether seeds also have same effect or not. A detailed further research is essential for the study of interactions and its adverse effects, it will also help in identifying the active principles compound from medicinal plants for assess their dosage and quality control.

Ethanobotanical studies have mentioned the use of many plants in treatment of kidney stone. Some of the plants like *Bauhinia acuminata* bark and leaf, *Celosia argentea* roots and many other have been listed (Arya *et al.*, 2017). Herbal medicine is popular and promising but still it is essential to carry out further detailed research to understand the disease and the action mechanism of herbal medicines in order to develop a best efficient and safe lithotriptic agent. Clinical trials on animals are a must for the dosage and quality control study. Substitution of animals is what most people think of when you say alternatives to animal testing. Mostly, it is tried to replace the animals either by methods that does not involve animals at all (absolute replacement) or only the cells or tissues of animals (relative replacement) are used. Our method provides absolute replacement as it involves plant cells having mineralisation as a test subject.

#### 5. Conclusion

Animals and plants follow the same process for the formation of calcium oxalate crystals. This method of treating plant cystolith and raphides with ethnomedicine can be used as a screening process for the drug claiming lithotropic activity in a simpler way. Hence, the mechanism of dissolution of crystals in plant cells by imaging technique can be used to validate the herbal medicine for the treatment of kidney stones.



**Photoplate 8:** *Colocasia esculenta* section showing raphides before (A) and after (B) treatment with *Macrotyloma uniflorum* extract of seeds.

#### Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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