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# *Ocimum gratissimum* L.: A Medicinal Plant with Promising Antiurolithiatic Activity

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**ABSTRACT**

*Ocimum gratissimum* L. has been used to treat various diseases including urinary stone diseases, since ancient time in India. The inhibition of *in-vitro* calcium-oxalate crystal formation by *Ocimum gratissimum* L. extract was investigated by different methods i.e nucleation assay and synthetic urine assay. In nucleation assay, the aim was to evaluate the effectiveness of different concentrations of the extract (100-1000 mg/ml) on calcium oxalate crystallization *in-vitro* while in synthetic urine method the percentage inhibition and growth of the calcium oxalate monohydrate crystals from synthetic urine at different % concentrations of extract (25-100%) was investigated. In both the assay % inhibition for calcium oxalate crystal formation was found directly proportional to the increase in concentration of the plant extract with maximum inhibition of 66.08% at 1000 mg/ml, while in synthetic urine assay maximum inhibition was 62.07 % at 100% concentration of extract. Thus *Ocimum gratissimum* L. was found to be a potent and promising antiurolithiatic agent, which is in accordance with its use in traditional medicine.

**Keywords:** *Ocimum gratissimum* L., urolithiasis, calcium oxalate monohydrate (COM), *in-vitro*, calcium oxalate (CaOx).

## 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. [1] Urolithiasis is derived from the Greek words "ouron" (urine) and "lithos" (stone).It is considered as the third most common affliction of the urinary tract. [2]

The deposition or formation of stones in any part of the urinary system i.e the kidney, the ureters or the urinary bladder is called Urolithiasis. A stone is an aggregation of solute materials from urine such as calcium, oxalate, phosphate and uric acid which forms stone. In India, calcium oxalate is found to be the most predominant constituent of urolithiasis. 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. However, the presence of certain molecules raise the level of supersaturation of salts needed to initiate crystal nucleation or reduce the rate of crystal growth or aggregation and prevents stone formation. [3]

Calcium oxalate stones represent up to 80% of analyzed stones. [4] 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%. [5] Calcium oxalate stones are of primary two types, calcium oxalate monohydrate (whewellite) and calcium oxalate dihydrate (weddellite). The occurrence frequency of whewellite is 78% while that of weddellite is 43%. [6]

Though technological advancements have made dramatic improvement in the removal of urinary stones still some of the drawbacks of these methods exists which includes their being too costly for a common man and recurrence of stone formation along with a number of other side effects. [7] Hence search for new antilithiatic drugs from natural sources has assumed greater importance as herbal drugs are cost effective and cause least side effects. In ayurveda many plants having the property of disintegrating and dissolving the stone are referred to as "pashanbheda".

*Ocimum gratissimum* L. (Lamiaceae) is an herbaceous perennial plant commonly known as scent leaf. It is found in tropical Asia especially India. *Ocimum gratissimum* L. *var.* clocimum is a new hydrid strain of *Ocimum gratissimum,* developed by Sobti *et al.* from Indian Institute of Integrative Medicine formerly Regional Research Laboratory Jammu-Tawi. It has been used extensively in the traditional system of medicine in many countries. It has been reported to be rich

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in plant chemicals. The plant is known to contain alkaloids, tannins, flavonoids and oligosaccharides. [8]

It is used in the treatment of various diseases like cancer [9], antinociceptive, anti-inflammatory [10], antidiahorreal [11], antibacterial [12], antifungal [13], wound-healing [14] and as nephroprotective. [8]

Its ethanolic extract has shown various activities like analgesic [15], antifungal [16], aphrodisiac [17], hepatoprotective [18], antioxidant [19- 20] and anti-diabetic activity. [21]

Various reports have shown the use of *Ocimum* species in treating nephrotoxicity or urinary stone related problems. A study of ethanol extract of *Ocimum basilicum* showed nephroprotective activity against cisplatin. [22] In case of renal stone the juice of basil (*Ocimum sanctum*) leaves and honey, if taken regularly for 6 months, will expel them via the urinary tract. [23] Literature on traditional medicines show the use of fresh decoction of leaves of *Ocimum gratissimum* in treating urinary stones [24-26] but no such *in-vitro* study has been undertaken. Thus the aim of the present study is to evaluate the effectiveness of ethanolic extract of leaves of *Ocimum gratissimum* L. for its antiurolithiatic activity using two *in-vitro* methods; nucleation assay and synthetic urine assay.

In nucleation assay the aim was to evaluate the effectiveness of different concentrations (100-1000 mg/ml) of the extract on calcium oxalate crystallization *in-vitro* while in synthetic urine method the percentage inhibition and growth of the calcium oxalate monohydrate crystals in synthetic urine at different % concentration (25-100%) of extract was the object of investigation.

## MATERIALS AND METHODS

### Chemicals

All chemicals used were of high purity Merck grade. Sodium oxalate was obtained from Burgoyne reagents, while sodium chloride and calcium chloride dihydrate were procured from Sigma Aldrich.

The leaves of *Ocimum gratissimum* L. were collected from Kolar road, Bhopal, Madhya Pradesh, during the month of January 2013 and the plant was identified with the help of regional Floras [27] and taxonomists and finally confirmed with the herbarium of Botanical Survey of India (BSI), Allahabad, with voucher specimen No. 90459.

### Preparation of plant extract

Fresh plant, after collection was shade dried at room temperature and then grinded. The plant material (100 g) was extracted with alcohol by Soxhlet apparatus for 72 hours. Then the extract was concentrated in vacuum to dryness at 30-40°C temperature, obtaining 21.75% w/w of dried extract. The dried extract was stored in refrigerator for further use.

### Experimental Work

### Nucleation assay

We chose the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. [28] This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of the plant extract used. Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5 mmol/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 mL of calcium chloride solution mixed with 100 mL of extracts at different concentrations. Crystallization was started by adding 950 mL of sodium oxalate solution. The temperature was maintained at 37ºC. The OD of the solution was monitored at 620 nm using spectrophotometer (Systronics digital spectrophotometer 166) after 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:

$$CaCl2 + Na2C2O4 \rightarrow CaC2O4 + 2NaCl$$

### Synthetic urine assay

### Preparation of synthetic urine

We chose the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. [29] This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of the plant extract. Two solutions of following composition were mixed: A: $Na_2C_2O_4$ (2 m mol/1) and B: $CaCl_2$ $2H_2O$ (10 mmol/1). 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. Mixture agitation was maintained to prevent sedimentation.

### 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 on hemacytometer counting chamber and it was observed under microscope after 30 minutes. The number of crystals was calculated 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

$$\mathbf{I\% = [(TSI- TAI) / TSI] * 100}$$

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

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

## RESULT

### Effect on Nucleation assay

Incubation of the metastable solutions of calcium chloride and sodium oxalate resulted in the formation of calcium oxalate crystals. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. The O.D. was monitored at 620nm after 30 minutes. The turbidity of solution in the presence of herb extract was lower in comparision to the control, showing that oxalate crystallization was less in the presence of extract. Data represents that % inhibition for calcium oxalate crystal formation was directly proportional to the increase in concentration of the plant extract, with minimum inhibition of 42.29% at 100 mg/ml to a maximum inhibition of 66.08% at 1000 mg/ml extract concentration. (Fig. 1)

### Effect on synthetic urine assay

The formation and growth of the calcium oxalate monohydrate crystals from artificial urine at different concentration was studied. Stone formation is the result of

supersaturation of urine with certain urinary salts such as calcium oxalate. Since crystallizable oxalate species are pH independent, the crystallization of oxalate in the absence of inhibitor, led to the formation of calcium oxalate monohydrate crystals monitored by polarized light microscopy. The number of calcium oxalate monohydrate crystals in control, was found to be maximum. In order to assess the inhibiting potential of plant extract for oxalate crystallization different percentages of plant extract was tested. In the presence of different percentages of plant extract, the length and the width of the crystals were reduced. The average length of the crystals grown in the presence of the inhibitors was less than that of the control sample. It was found that the plant used in this study inhibited potently the crystal development with maximum number of crystals 225/mm$^3$ at 25% extract concentration (Fig. 2.) while minimum number of crystals 137.5/mm$^3$ was formed at 100% concentration of extract (Fig. 3). Results show that the decrease in number of crystal as well as % inhibition of the formation of calcium oxalate monohydrate crystals was directly proportional to the increase in percentage of plant extract, with minimum inhibition 37.93 % at 25% extract while maximum inhibition of 62.07 % at 100 % extract concentration (Fig. 4).

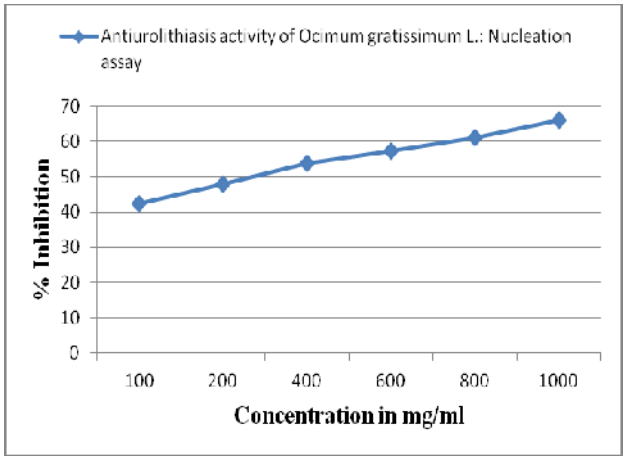


**Fig. 1: The effect of different concentrations of *Ocimum gratissimum* L. on Calcium oxalate crystal inhibition by nucleation assay**

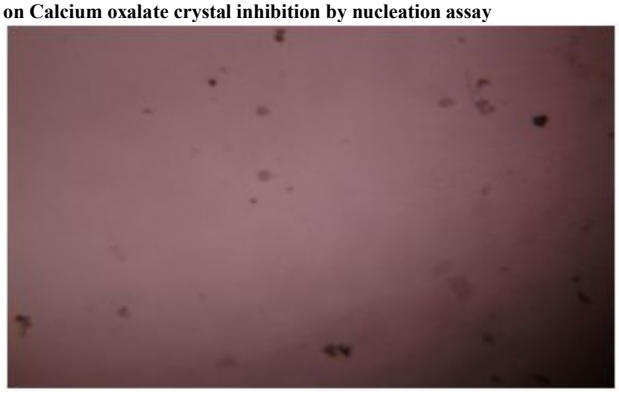

**Fig. 2: Calcium oxalate monohydrate crystal development in 25% extract concentration in synthetic urine assay**

## DISSCUSSION

The supersaturation of urine with calcium oxalate is an important factor in crystallization, with later factors being nucleation, growth and aggregation. Thus if supersaturation or initial stages in crystallization can be prevented, then lithiasis could be avoided. The *in-vitro* results revealed that the plant extract has potent antiurolithiatic ability in both nucleation assay (with maximum inhibition of 66.08% at 1000 mg/ml extract concentration) and synthetic urine assay (with maximum inhibition of 62.07% at 100% extract concentration). Along with this in synthetic urine assay maximum number of crystals was formed in control (362.5/mm$^3$) while minimum (137.5/mm$^3$) were formed at 100% concentration of extract. 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. The mechanism by which the plant exerts its effects remains unknown and could be the objective of study in future. The plant extract may contain phytochemicals that inhibit the growth of calcium oxalate monohydrate crystals, thus phytochemicals responsible for this activity could be analyzed in future studies. Although Ezeonwu *et al*., (2013) [8] reported, nephroprotective effect of a bi-herbal formulation containing *Ocimum gratissimum* in acetaminophen-induced toxicity but to the best of our knowledge and in accordance with the literature survey, this is the first report on potent antiurolithiatic activity in ethanolic extract of leaves of *Ocimum gratissimum* L.

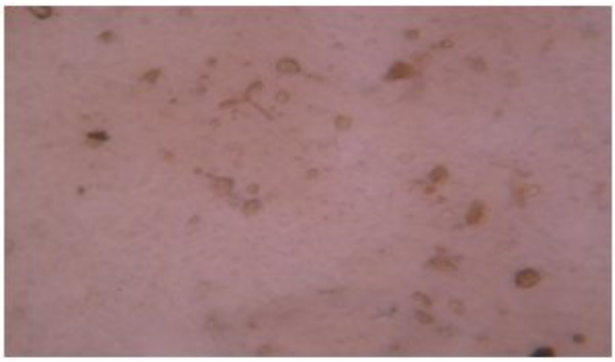

**Fig. 3: Calcium oxalate monohydrate crystal development in 100% extract concentration in synthetic urine assay**

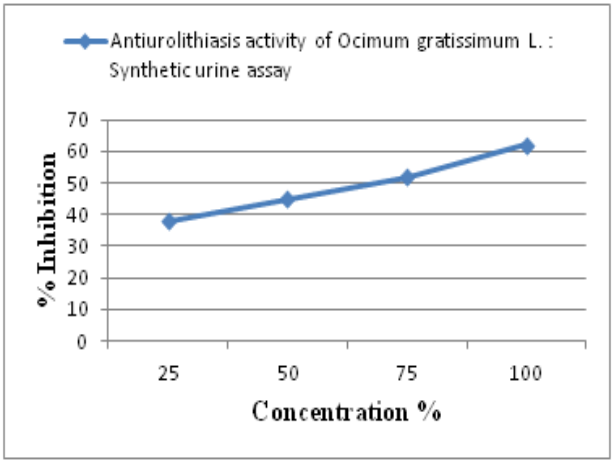


**Fig. 4: The effect of different percentages of *Ocimum gratissimum* L. extract on calcium oxalate monohydrate crystal inhibition in synthetic urine assay**

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