

Inhibition of calcium oxalate crystallisation in vitro by an extract of *Lantana camara*

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Abstract

To study the potency of aqueous extracts found from *Lantana camara* (family *Verbenaceae*) on calcium oxalate crystallization in vitro and the present study is aimed to determine the phytochemical screening Calcium oxalate crystallization was induced by the addition of 0.01 M sodium oxalate solutions. The effect of extract (0.2, 0.4, 0.6, 0.8 and 1 mg/ml) was studied by the measuring of turbidity in presence or absence of extract at 620 nm of a spectrophotometer. The herb extract of *Lantana camara* promoted the nucleation of calcium oxalate crystals, raising their amount but decreasing their size. It also promoted the formation of calcium oxalate dehydrates crystal, despite the presence of calcium oxalate monohydrate particles. Preliminary phytochemical screening of different extracts of *Lantana camara* revealed the presence of Alkaloids, Saponins, Glycosides, Carbohydrate, Tannins, Flavonoids, Steroids, Triterpenoids, fixed oil and fates. An outcome obtained showed that aqueous extract of leaf has the higher capacity to inhibit the crystal organisation and aggregation as compared to cystone we expected this investigation would provide encouragement for further exploration into new drugs for the prevention and treatment of urolithiasis, this plant may be explored therapeutic agent in future.

Keywords:

Lithiasis, Nucleation , *Lantana camara*, Urolithiasis, phytochemicals

1. Introduction

Lithiasis (Kidney stone) is constitution of urinary calculi at any level of urinary tract. It is approximated that 12% of global population experiences renal stone disease with a recurrence rate of 70%- 80% in male and 47%-60% in female (Parmar et al., 2012 and Sundararajan et al., 2006). Urolithiasis formation or kidney stone is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys.

The recurrence of urolithiasis represents a serious trouble as patients who have organized one stone are more likely to form another (Khan et al., 2010).

Calcium oxalate (CaOx) is the most common type of human kidney stone, of which hyperoxaluria is the major risk factor. The mechanism by which a CaOx stone is formed is complex, and many factors are believed to be involved. However, the exact mechanism of renal stone formation is poorly understood (Khandrika et al., 2012). Although it involves a cascade of events including one or more of the following: urinary super saturation, crystal nucleation, growth, and aggregation; retention of crystals in the renal tubules. (Fig.1) Oxalate (Ox) is a naturally-occurring, highly oxidized organic compound with powerful chelating activity that can cause death at high concentrations in animals and occasionally humans due to its toxic corrosive effects on cells (Joshi et al., 2012). A higher concentration of Ox in human fluids can cause a variety of pathological disorders, including hyperoxaluria, cardiomyopathy, cardiac conductance disorders, renal failure and, in particular calcium oxalate (CaOx) nephrolithiasis.

Many remedies have been employed during ages to treat renal stones most of which were taken from plants and proved to be useful (Al-Attar 2010). However, the rationale behind their use is not well established except for a few plants and some proprietary composite herbal drugs which were reported to be effective without any side effects (Aslam Khan., 2011). Now a days, the management of urolithiasis with open renal surgery is unusual and rarely used only since the introduction of extracorporeal shock wave lithotripsy which is a standard procedure to remove kidney stones, however it may leave persistent stone fragments and cause acute renal injury, a decrease in renal function and an increase in stone recurrence (Aslam Khan., 2011, Srisubat et al., 2009, Aboumarzouk et al., 2011). The procedure is not widely available and very costly to the people in developing countries. Using of herbal plant extracts for the nephroprotective activity is the best method in the traditional medicine. Because when we use the chemical compounds may induce the side effects for the many organs of our body. But plants are having lot of phytochemicals which heal the kidney damages without and side effects.

The plant *Lantana camara* Linn family *Verbenaceae* is available throughout central and south India in most dry stony hills and black soil. A large scrambling evergreen, strong smelling shrub with stout recurved prickles; leaves opposite, often rugose, scabrid on both sides; flowers small, normally orange but often white to dark red, in heads which are prominently capitate; bracts conspicuous and persistent. Fruits are small, 5 mm diameter, greenish-blue, blackish, drupaceous, shining with two nutlets almost throughout the year and dispersed by birds. Seeds germinate very easily. The chemical constitution for lantana camara is caryophyllene, 1- α -phellandrene, lantadene A, lantadene B, lancamarone quinone, lantanine. The plant is vulnerary, diaphoretic,

carminative, antilithiatic, antispasmodic and tonic, wounds, ulcers, swelling, tumours and rheumatism (venkatachalam et al., 2011) the aqueous extract of the leaf of *Lantana camara* showed activity. It also contains rich polyphenols which is found to be active to induce diuresis (Sasikala et al., 2013) In light of the above probe, *Lantana camara* has been selected for urolithiatic activity

2. Materials and methods

2.1. Collection of Plants Sample

The *Lantana camara* were collected from Tamil Nadu Sri paramakalyani center for environmental sciences Alwarkurichi, Tirunelveli.. The leaf was cleaned and cut into small pieces and shade dried. The dried leafs were powdered and passed through the coarse sieve (0.2mm). The powdered samples were extracted using Soxhlet apparatus. An individual extraction of leafs samples were carried out using solvents of different polarity from non polar to polar (petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and water).

2.2 Preparation of Extract

The leaf samples were extracted by hot percolation method. The extraction was repeated until the plant material becomes colourless. The extract was evaporated in a water bath at 60°C. The residue was stored in an airtight container in a refrigerator (Vimalin Hene J, 2010).

2.3 Preparation of Cystone Extract

The tablet of cystone was powdered and from this about 35mg of the powdered sample was dissolved in 900µl of distilled water and added 100 µl of Tween 80. The extract was stored in an air tight container in the refrigerator.

2.4 Preliminary phytochemical screening

For preliminary phytochemical screening, the extracts was screened for the presence of Alkaloids, Saponins, Glycosides, Carbohydrate, Tannins, Flavonoids, Steroids, Triterpenoids, fixed oil and fates following the standard procedures (Harborne 1973)

2.5 Experimental protocol

The effect of extracts on Calcium oxalate crystal was determined by the time course measurement of turbidity changes due to the crystal nucleation, Growth and aggregation. The precipitation of calcium oxalate at 37 °C and pH 6.8 has been studied by the measurement of turbidity at 620 nm. A spectrophotometer UV/Vis (Shimadzu) was employed to measure the turbidity of the formation of calcium oxalate (Bensatal and Ouahrani 2008)

2.6 Nucleation assay

We preferred the classical framework for the examiner of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor in order to evaluate the inhibiting capacity of any chemical species 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 herb extracts at the different concentrations (0.2, 0.4, 0.6, 0.8, 1 mg/ml). Crystallization was started by adding 950 mL of sodium oxalate solution. The temperature was maintained at 37°C. The OD of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control (Hennequin et al 1993; Atmani and Khan 2000)

2.7 Aggregation assay

The method used was similar to that described by (Atmani and Khan 2000) with some minor modifications. 'Seed' CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60°C in a water bath for 1 h and then cooled to 37°C overnight. The crystals were harvested by centrifugation and then evaporated at 37°C. CaOX crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37°C in the absence or presence of the plant extract after stopping the stirring.

2.8 Phytochemical investigation

The individual extracts like Ethanolic, Petroleum ether, Hydroalcoholic extract Chloroform and water was subjected to qualitative chemical investigation for the identification of different phyto-constituents.

3. Results

Herbal drugs are now receiving great attention for their therapeutics and because of this extensive research are now being carried out in this area. However, herbal drugs being a complex mixture of several phyto-constituents, it becomes difficult to decide that which component plant extract. Incubation of the 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 comparison to the control, showing that oxalate crystallization was less in the presence of extract (Fig. 2 and 5) showed the percentage inhibition of the crystallization of calcium oxalate (CaOx) with plant extract concentrations.

The increase in the concentration of plant extract increases the inhibition of nucleation. Maximum inhibition of nucleation observed at concentration of 1µg/ml. the plant extract has good potential no decrease

the nucleation process, compared to cystone. The present study is in agreement with the studies reported regarding the anti-urolithiatic potency of aqueous extract of *Lantana camara*. This nucleation property of plant extract could be significant in preventing kidney stone formation.

The next step in calculus formation is aggregation that constitutes the most effective mechanism to increase the size of particle and structure of urinary stones (Fig. 3 and 6) indicates the percentage inhibition of the leaf aqueous extract against CaOx crystal aggregation. Maximum inhibition was observed at 1 mg/ml concentration. When compared with cystone showed better activity against CaOx crystal aggregation. Crystal aggregation inhibition was found low at either lower concentrations or higher concentrations of the extracts.

The microscopic examination of growth inhibitory potential of the leaf aqueous extracts was shown in (Fig.4 and 7). Addition of leaf extracts in the reaction mixture inhibited the crystal aggregation drastically. The growth clusters of crystals were observed in the control group. Maximum crystal growth inhibition was found at 1 mg/ml compared to other concentrations of leaf and cystone extracts.

3.4. Qualitative phytochemical analysis

Petroleum ether, chloroform, ethanol Hydro alcoholic and chloroform extracts of *lantana camara* Linn leaves were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening (Venkatachalam et al., 2011) The result obtained in the present investigation of petroleum ether, chloroform, ethanol Hydro alcoholic and chloroform extracts of the fruits of *lantana camara* Linn Showed in the table no: 1

Table 1: Data showing preliminary Phytochemical screening of the leaf extracts of *Lantana camara* Linn.

S.No	Tested group	Ethanolic	Petroleum ether	Hydroalcoholic extract	Chloroform
1	Alkaloids	++	++	--	--
2	Saponins	++	--	--	++
3	Glycosides	++	--	--	++
4	Carbohydrate	++	--	++	++
5	Tannins	++	++	++	++
6	Flavonoids	++		++	++
7	Steroids	++	++	++	++
8	Triterpenoids	++	++	++	++
9	Fixed oil and fates	--	--	--	--

(+ +) = indicates presence, (--) = indicates absence,

4. Discussion

Kidney stone function is a complex process that results from a succession of several physico-chemical events including the super saturation of urine with calcium oxalate is an important factor in crystallization, with later factors being nucleation, growth and aggregation. Thus if super saturation or initial stages in crystallization can be prevented, then lithiasis could be avoided.

The *in-vitro* results revealed that the extract of *Lantana camara* promoted the formation of Calcium oxalate crystals. The extract might contain some photochemical that inhibit the growth of Calcium oxalate crystals. The *Lantana camara* extract also contains some substances that inhibit the aggregation of Calcium oxalate crystals. Crystal aggregation is the most critical step, as it occurs very rapidly and has a considerable effect on particle size, and aggregated crystals are commonly found in urine and renal stones (Masao 2008).

The leaf of *Lantana camara* Linn was collected and analysed the various standardisation parameters. Preliminary phytochemical screening was performed in the leaf of *Lantana camara* Linn. The leaf extracted with different solvents and the percentage yields are tabulated in the table no: 1. Quantitative phytochemical analysis is performed in the all extracts and the results showed the presence or absence of certain phytochemicals in the drug. Phytochemical test revealed the presence of Alkaloids, Saponins, Glycosides, Carbohydrate

Tannins, Flavonoids, Steroids, Triterpenoids and Fixed oil and fates.

5. Conclusion

The consumption of the herbals as medicine is a lifetime old custom which will serve as a source of alternative medicine in succeeding also and help to overcome the toxic effects of the synthetic drugs. The results have come out to be very prompting and further pharmacological field could be carried out on the samples to reveal an efficient drug for the renal stone. The aqueous extract of *Lantana camara* leaves have inhibitory effect on CaOx crystallization thus may be beneficial in the treatment of urolithiasis but there is a need of detailed investigation in elaborated preclinical experimentations and clinical trials to establish the use of plant as antiurolithiatic agent, From the above results, it is concluded that aqueous extracts of leaves of *Lantana camara* showed significant antilithiatic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as Antilithiatic activity. The plant may be further explored for isolation of the active constituent accountable for antilithiatic activity.

Fig 1: Pathogenesis of renal stone

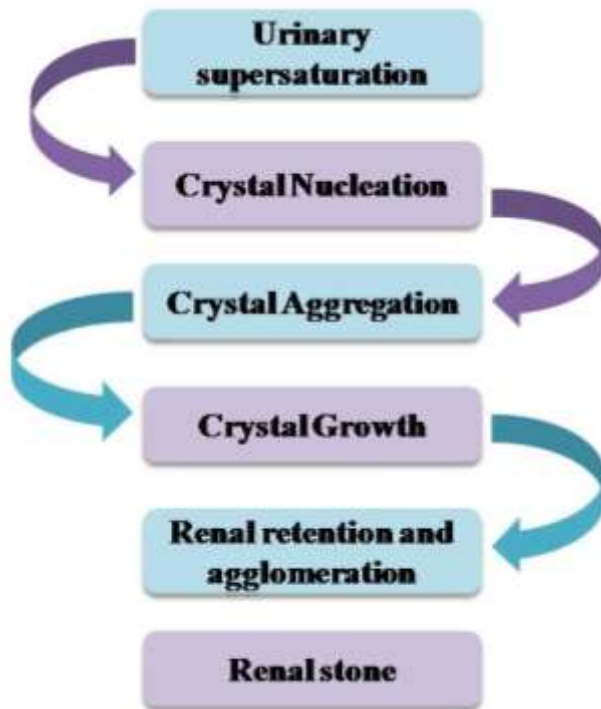


Fig 2: Microscopic evaluation of *Lantana camara* aqueous extracts on CaOx nucleation on different concentrations 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1 mg/ml.

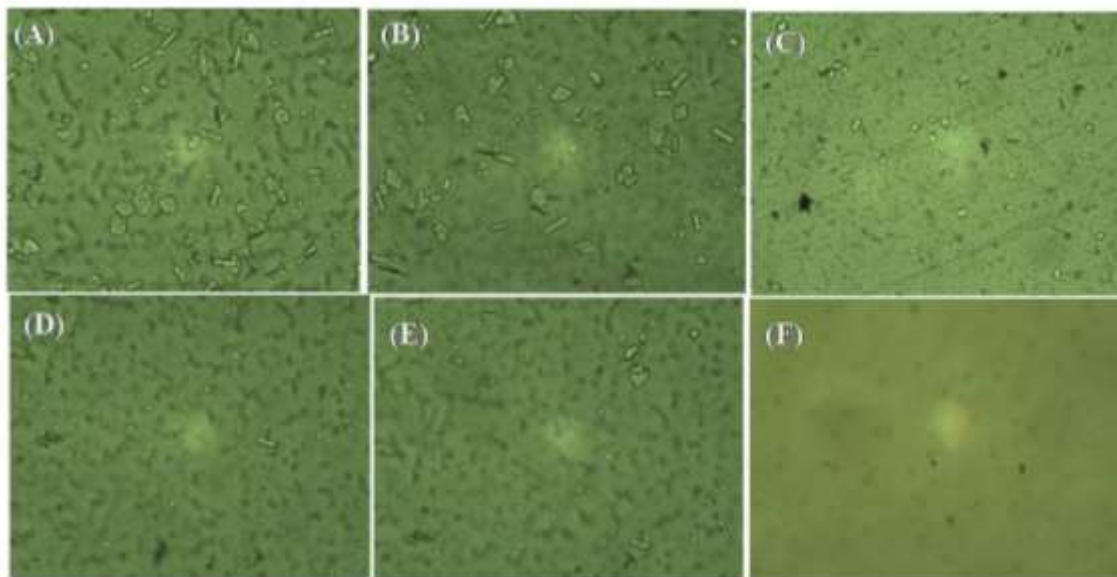


Fig 3: Microscopic evaluation of *Lantana camara* aqueous extracts on CaOx aggregation on different concentrations 0.2 mg/ ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1 mg/ml.

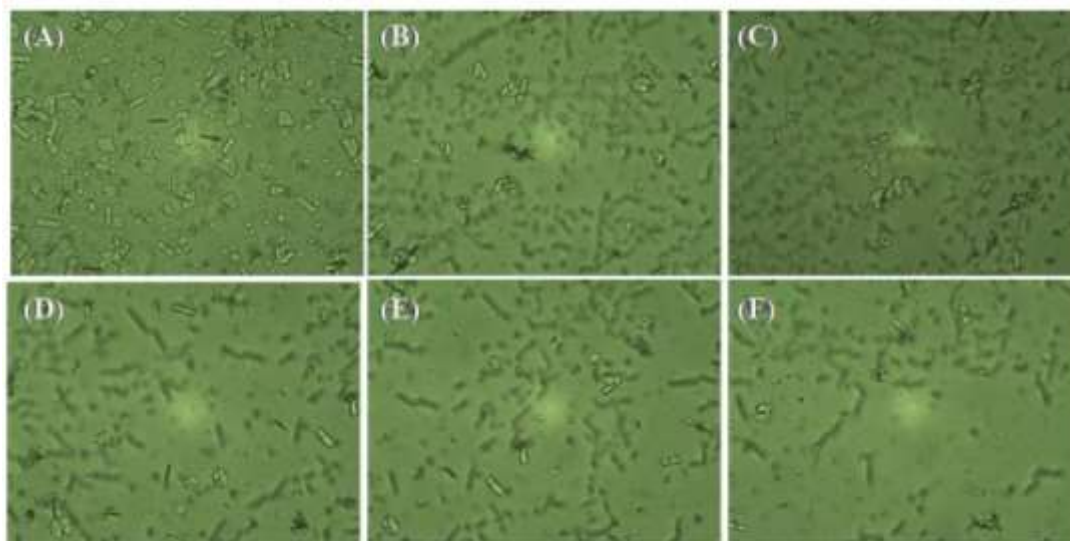


Fig 4: Microscopic evaluation of *Lantana camara* aqueous extracts on CaOx growth on different concentrations 0.2 mg/ ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1 mg/ml.

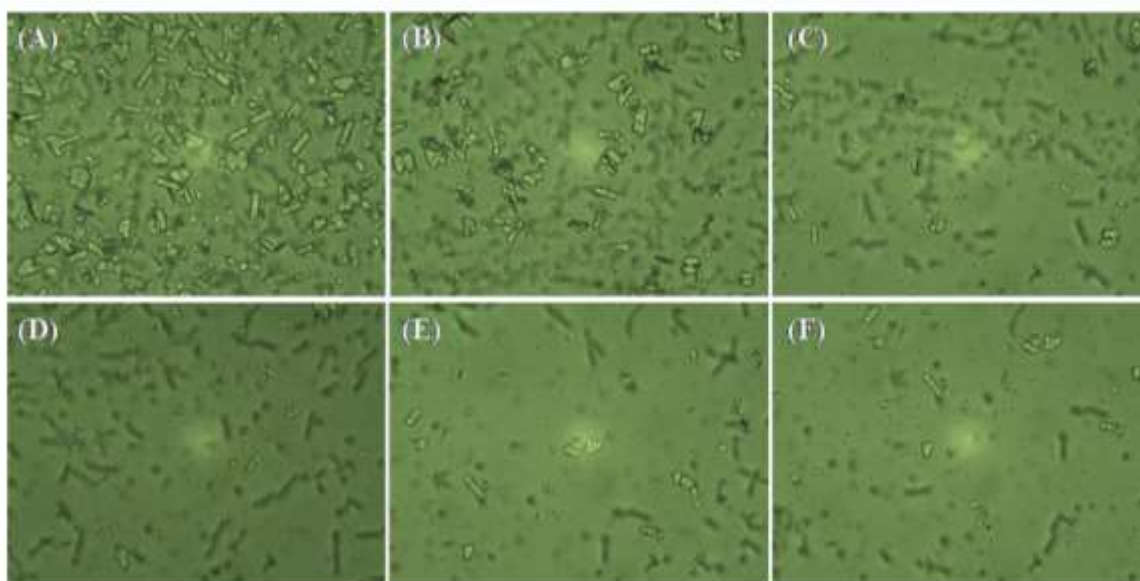


Fig 5: Effect of *Lantana camara* and cystone extracts on CaOx nucleation

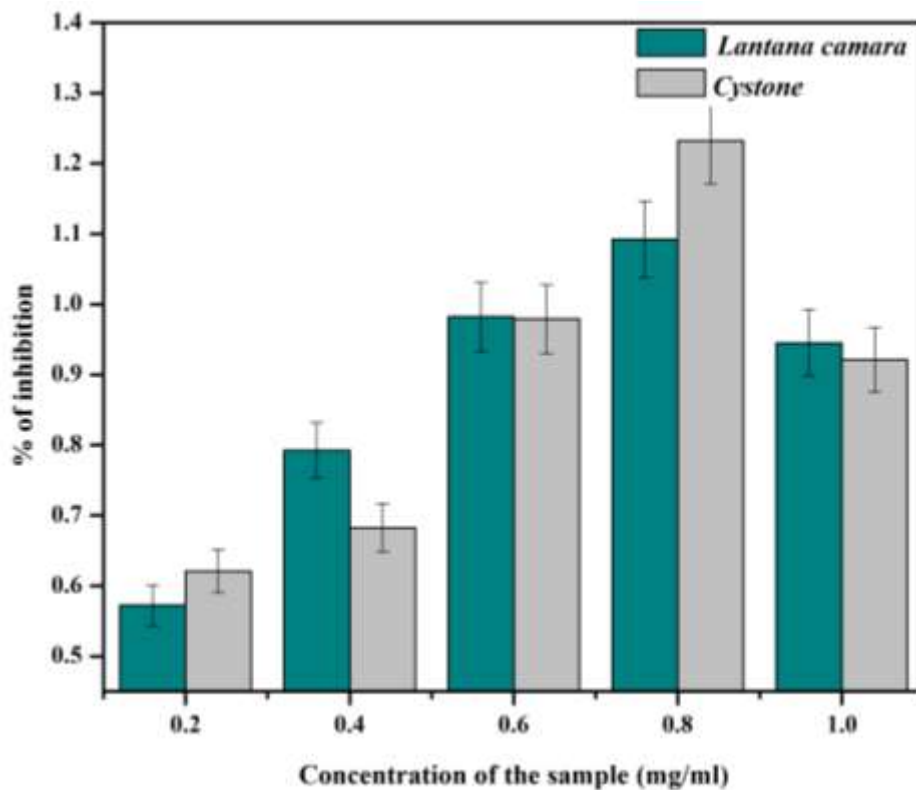


Fig 6: Effect of *Lantana camara* and cystone extracts on CaOx aggregation

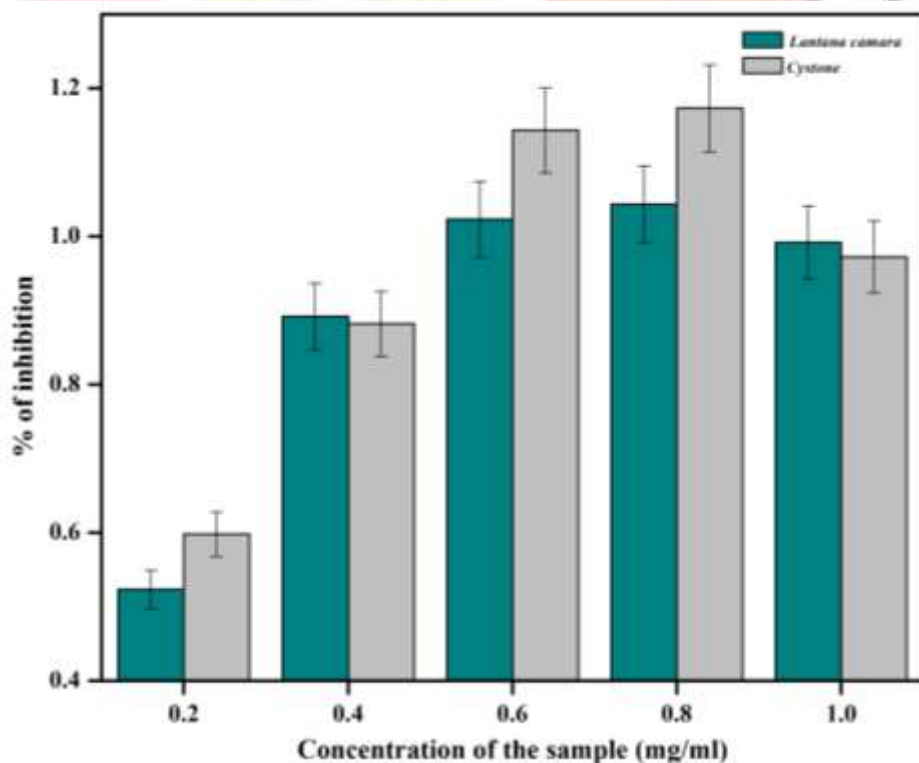
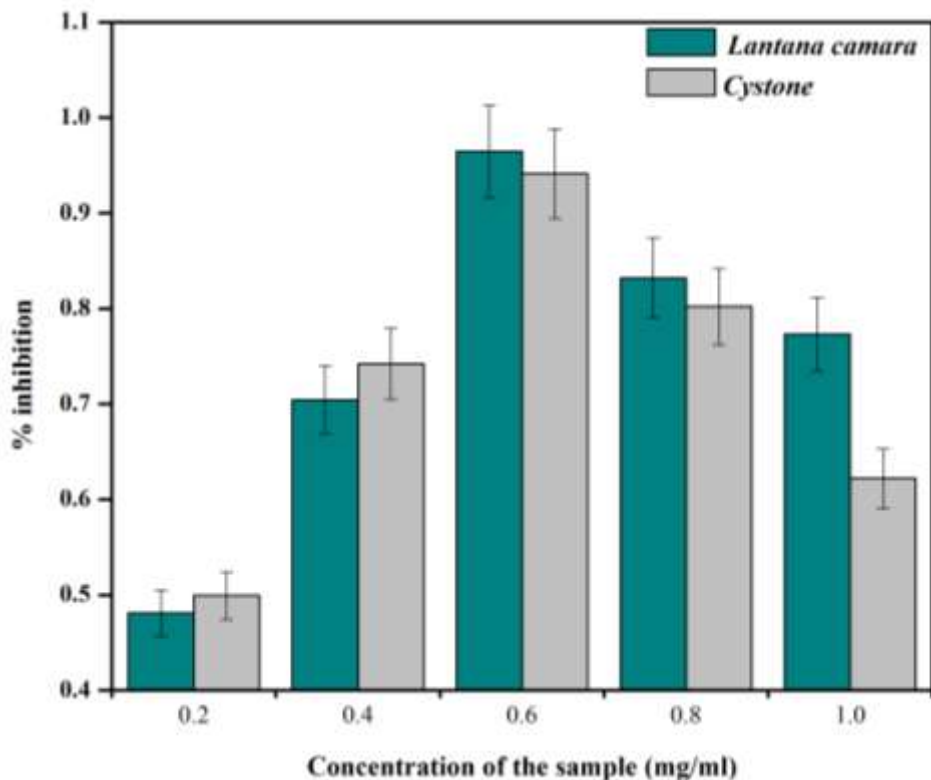


Fig. 7: Effect of *Lantana camara* and cystone extracts on CaOx growth



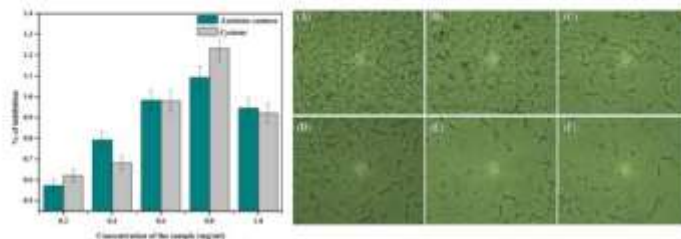
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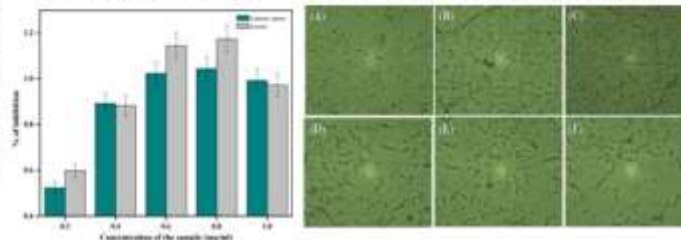
Phytochemical study



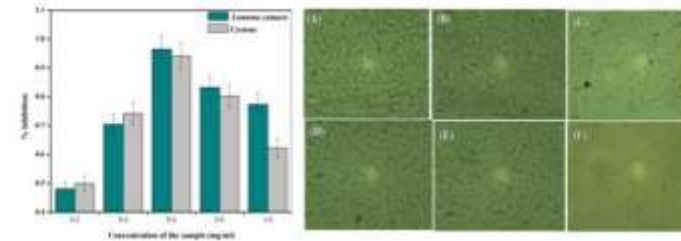
Effect of *Lantana camara* extracts on CaOx nucleation



Effect of *Lantana camara* extracts on CaOx aggregation



Effect of *Lantana camara* extracts on CaOx growth



In vitro studies

Conflict of interest statement

We declare that we have no conflict of interest.

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