



INHIBITION OF CRYSTALLIZATION OF CALCIUM OXALATE BY THE EXTRACTION OF *MIMOSA PUDICA* (L)

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ABSTRACT

Objective: *Mimosa pudica* is used in the treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leucoderma, fatigue and blood diseases. The present study is designed to evaluate the antiurolithiatic activity of aqueous and ethanolic extracts of different parts of *M.pudica*. **Methods:** The *in-vitro* inhibition of calcium oxalate crystal formation by aqueous and ethanolic extracts of leaf, root and flower extracts of *M. pudica* at different concentrations (200, 400, 600, 800 and 1000 µg) were investigated by the time course measurement of turbidity changes due to the crystal nucleation and aggregation in the synthetic urine at 620nm by means of a spectrophotometer. **Results:** The aqueous and ethanolic extracts of different parts of *M. pudica* remarkably inhibit the crystal formation in a dose dependent manner. The aqueous extract of leaf, root and flowers have more antiurolithic activity as compared to ethanolic extracts. Leaf extract of *M. pudica* showed maximum antiurolithic activity when compared to other parts. **Conclusion:** Aqueous extract of leaf, fruit and flower of *M. pudica* showed maximum inhibitory effect on calcium oxalate crystallisation in synthetic urine than ethanolic extract. Results guide us for the further detailed investigation and development of new drugs from this medicinal plant for urolithiasis.

Keywords: *Mimosa pudica*, antiurolithic, calcium oxalate, leaf, root, flower

1. INTRODUCTION

Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia (Hiatt and Friedman, 1982). The worldwide incidence of urolithiasis is quite high and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi (Anderson *et al.*, 1967). Most patients still have to undergo surgery to be rid of this painful disease. Hyperoxaluria is the main initiating factor for urolithiasis, most calculi in the urinary system arise from a common component in urine e.g. calcium-oxalate, representing up to 80% of analyzed stones (Robertson and Peacock, 1980). Calcium oxalate urolithiasis has become a rising problem in many countries due to geographical/genetic variations and modern lifestyles. People living in drought and arid area suffer more from intestinal hyper absorption of calcium oxalate leading to kidney stones. It is estimated that 12% of the world population is affected by

kidney stones with a recurrence rate from 70 to 80% in males and from 47 to 60% in females (Curhan *et al.*, 1996). The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes nucleation, crystal growth, crystal aggregation and crystal retention. The stone formation requires supersaturated urine. Supersaturation also depends on urinary pH, ionic strength, solute concentration and complexations (Basavarajet *et al.*, 2007). In spite of substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. The treatment modalities like surgery and drug therapy are practiced in the management of kidney stones but have some limitations. Surgical procedures like (extracorporeal shock wave lithotripsy) ESWL have increased risk of stone recurrence and are also not affordable to poor sufferers. The armamentarium of therapeutic agents also does not have any effective drugs except for some diuretics like thiazides and furosemide (Amolet *et al.*, 2013). This warranted a search for new drug therapy which will be cost effective and can target multiple etiological risk factors in urolithiasis. Ayurveda, an indigenous system of Indian medicine, offers vast scope for the successful treatment of urolithiasis.

A number of plant drugs have been used in India and elsewhere which claim efficient cure of urinary stones. *Mimosa pudica* is a short lived ever green shrub which can be treated as an annual or perennial herb. Peculiar movement of leaflets that are sensitive to touch, makes it as an interesting plant (Ghani, 1998; Vaidyaratnam, 2013). *M. pudica*, invites attention of the researchers worldwide for its pharmacological activities such as anti-diabetic, antitoxin, anti-hepatotoxin, antioxidant and wound healing activities. It is reported to contain alkaloid, glycoside, flavonoid and tannin. It is used to suppress kapha and pitta, heal wounds, coagulate blood and sexual weakness (Joseph *et al.*, 2013). All parts of plants are considered to possess medicinal properties and used in the treatment of biliousness, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma, blood diseases (Bhagirath *et al.*, 2009). Based on these findings and others, in the present study, an effort has been made to establish the scientific validity for the anti-urolithiatic effect of aqueous and ethanolic extracts of leaf, root and flowers of *M. pudica* by *in vitro*.

2.1 MATERIALS AND METHODS

2.1 Collection and preparation of plant extract

The medicinal plant *Mimosa pudica* was collected from in and around Mayiladuthurai, Tamilnadu, India, where it was found naturally in wet lands. Leaf, root and flower of this plant were washed thoroughly in running tap water to remove soil particles and other adhered debris then shade dried for two weeks and ground well into fine powder. The powdered materials were stored separately in different air tight containers until the time of use. 50 grams of this powdered material were soaked in 250 ml of various solvents (aqueous and ethanol) separately and kept at room temperature for 12 hours and kept at shaker for 3 hours. The samples were filtered through a single layer of muslin cloth, and then final filtrate was collected by passing it through a Whatman grade 1 filter paper in a Buchner funnel under vacuum. The filtrate was evaporated to dryness. The crude extract of *Mimosa pudica* was obtained. The various concentrations of the plant sample tested for their inhibitory potency were: 200 µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL and, 1000 µg/mL which were prepared at the time of experiment.

2.2 Calcium oxalate crystallization inhibition activity (Anti-urolithiasis)

In vitro anti-urolithiatic activity of aqueous and ethanolic extracts of different part of *M. pudica* was tested in terms of inhibition of calcium oxalate formation by turbidity method (Bensatal and Ouahrani, 2008).

2.2.1 Experimental protocol

The time dependent effects of turbidity changes in artificial urine on addition of 0.01M sodium oxalate solution alone and in combination with different concentrations of plant extracts were observed on calcium oxalate crystallization. The Precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620 nm using UV/Visible spectrophotometer (Kumar *et al.*, 2013).

2.2.2 Preparation of artificial urine

The artificial urine was prepared according to the method Burns and Finlayson (1980) and had the following composition: sodium chloride 105.5 mmol/l, sodium phosphate 32.3 mmol/l, sodium citrate 3.21 mmol/l, magnesium sulfate 3.85 mmol/l, sodium sulfate 16.95 mmol/l, potassium chloride 63.7 mmol/l, calcium chloride 4.5 mmol/l, sodium oxalate 0.32 mmol/l, ammonium hydroxide 17.9 mmol/l, and ammonium chloride 0.0028mmol/l. The artificial urine was prepared fresh each time and pH adjusted to 6.0.

2.2.3 Study without inhibitor

A volume of 1.0 ml of artificial urine 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.

2.2.4 Study with inhibitor

The extract was dissolved in distilled water, filtered through membrane filter and the concentration of 200µg/mL, 400µg/mL, 600µg/mL, 800µg/mL and 1000µg/mL was obtained. A mixture of 1 ml of artificial urine and 0.5 ml of plant crude 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 was calculated using the following formula:

$$\% \text{ Inhibition} = \{1 - [S_i / S_c]\} \times 100$$

Where; S_i : slope of graph in the presence of inhibitor (Extract),

S_c : slope of graph without inhibitor (Control).

3. RESULTS

Leaf, root and flower extracts of *M. pudica* treatment concentration dependent initial step rising in turbidity (nucleation) followed by decrease turbidity (aggregation) was seen in Figure 1-3 and 5-7.

3.1 Antiurolithic effect of aqueous extract of *M. pudica*

The aqueous extract of *M. pudica* leaf showed the inhibitory effect on the growth of calcium oxalate crystals as shown in Figure-1. When compared to the control (with no plant extract), the percentage of inhibition at the concentration of 200µg/mL was found to be 19.0%, which increased to 95.3% at the concentration of 1000µg/mL in aqueous extracts of leaf. Similarly, the percentage of inhibition at the concentration of 200µg/mL was found to be 16.0% and 20.0%,

which increased to 89.3% and 81.8% at the concentration of 1000 μ g/mL in aqueous extracts of root and flower respectively (Figure-2 and 3).

Figure-4. showed the maximum inhibition of calcium oxalate crystal formation 95.3% (turbidity) was seen by the aqueous extract of this plant leaves (1000 μ g/mL) which is highly different from aqueous extracts of root and flower. Aqueous extract of this plant root got second position among plant leaves extracts in bioactivity test which inhibited the calcium oxalate crystal formation 89.3% (turbidity) formation. Least inhibition in crystal formation was seen by aqueous flower extract which accounted only 81.8% inhibition.

3.2 Antiurolithiceffect of ethanolic extract of *M. pudica*

The ethanolic extract of *M. pudica* leaf showed the inhibitory effect on the growth of calcium oxalate crystals as shown in Figure-5. When compared to the control (with no plant extract), the percentage of inhibition at the concentration of 200 μ g/mL was found to be 16.3%, which increased to 89.4% at the concentration of 1000 μ g/mL (Figure 5). Similarly, the percentage of inhibition at the concentration of 200 μ g/mL was found to be 12.3% and 16.7%, which increased to 80.2% and 71.9% at the concentration of 1000 μ g/mL in ethanolic extracts of root and flower respectively (Figure -6 and 7).

Figure 8. showed the maximum inhibition of calcium oxalate crystal formation 89.4% (turbidity) was seen by the ethanolic extract of this plant leaves (1000 μ g) which is highly different from ethanolic extracts of root and flower. Aqueous extract of this plant root got second position among plant leaves extracts in bioactivity test which inhibited the calcium oxalate crystal formation 80.2% (turbidity). Least inhibition in crystal formation was seen by aqueous flower extract which accounted only 71.9% inhibition.

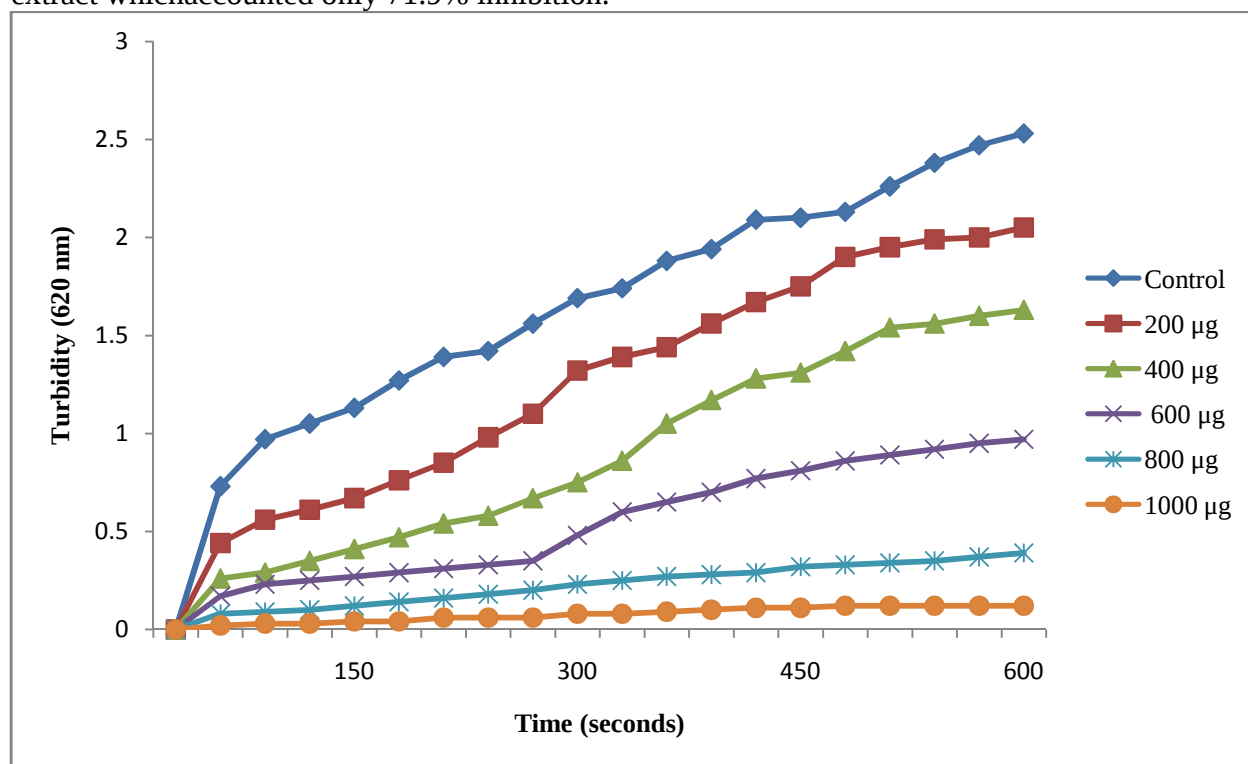


Figure-1: Shows change in turbidity without and with aqueous leaf extract of *M. pudica* at 620nm

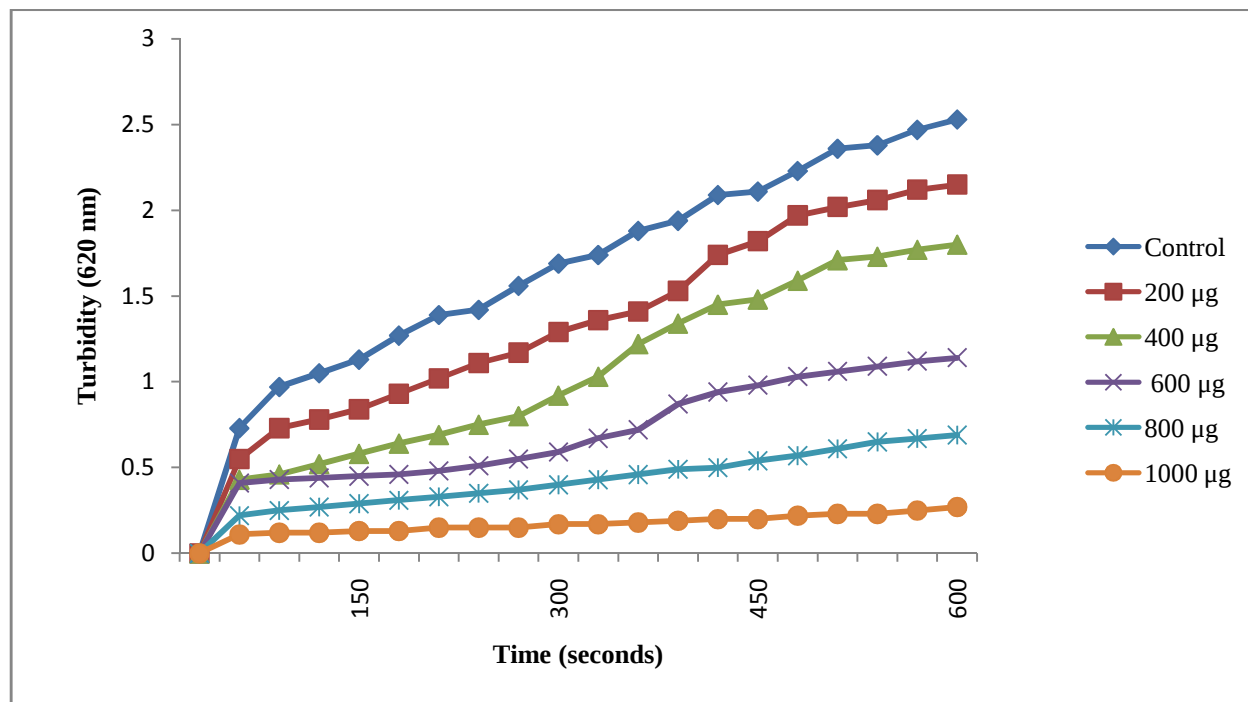


Figure-2: Shows change in turbidity without and with aqueous root extract of *M. pudica* at 620nm

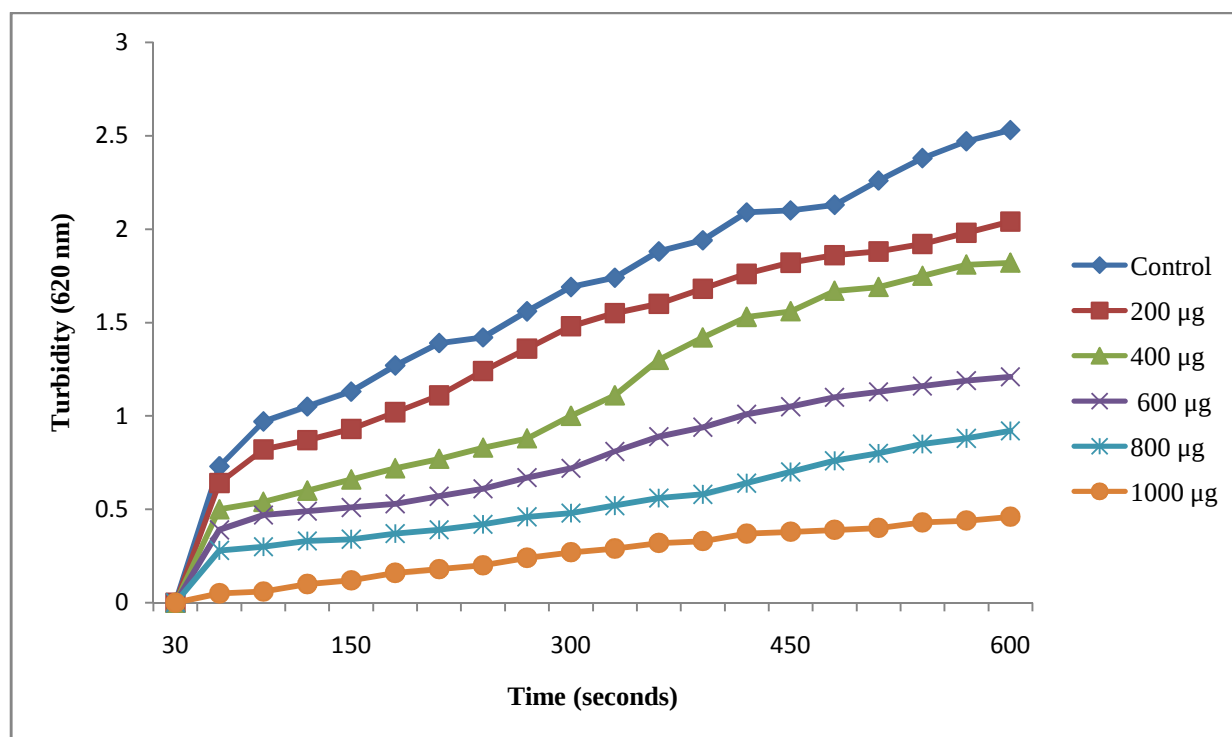


Figure-3: Shows change in turbidity without and with aqueous flower extract of *M. pudica* at 620nm

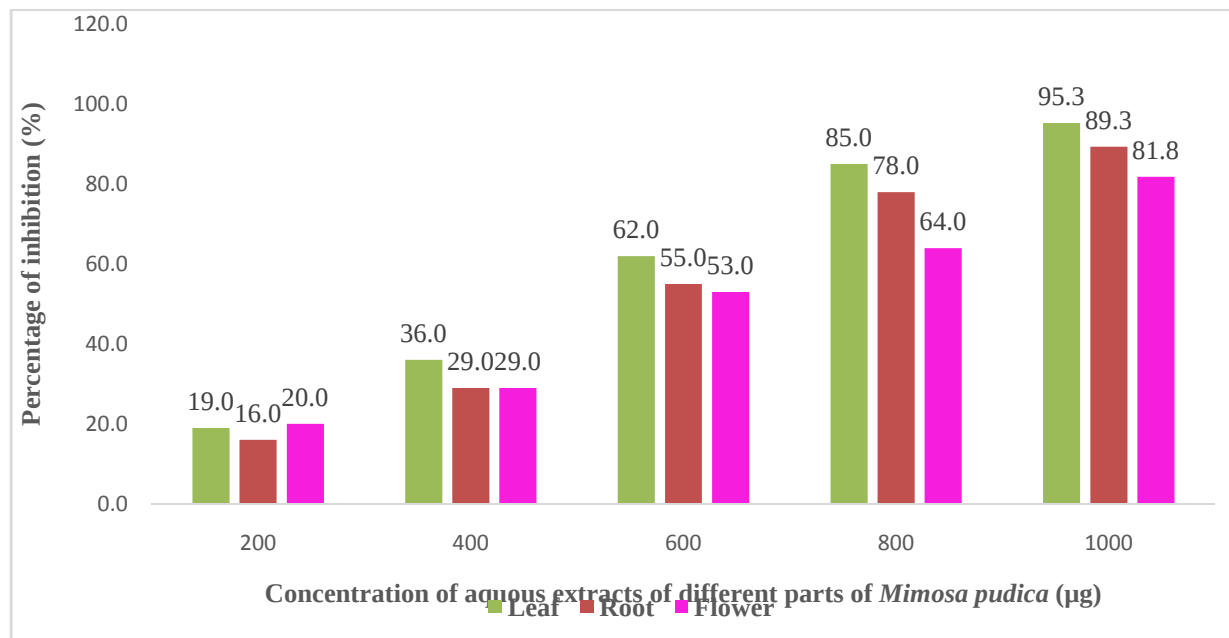


Figure-4. Effect of aqueous extract of *M. pudica* on nucleation and aggregation of calcium oxalate crystals inhibition.

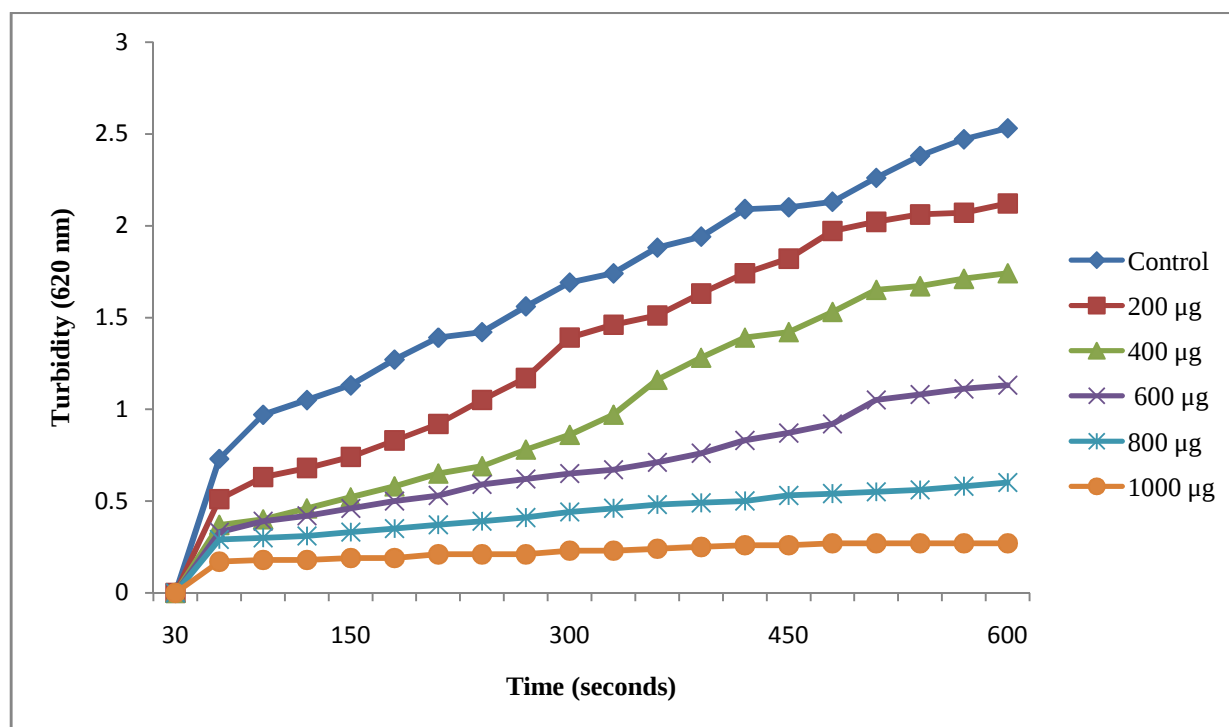


Figure-5: Shows change in turbidity without and with ethanolic leaf extract of *M. pudica* at 620nm

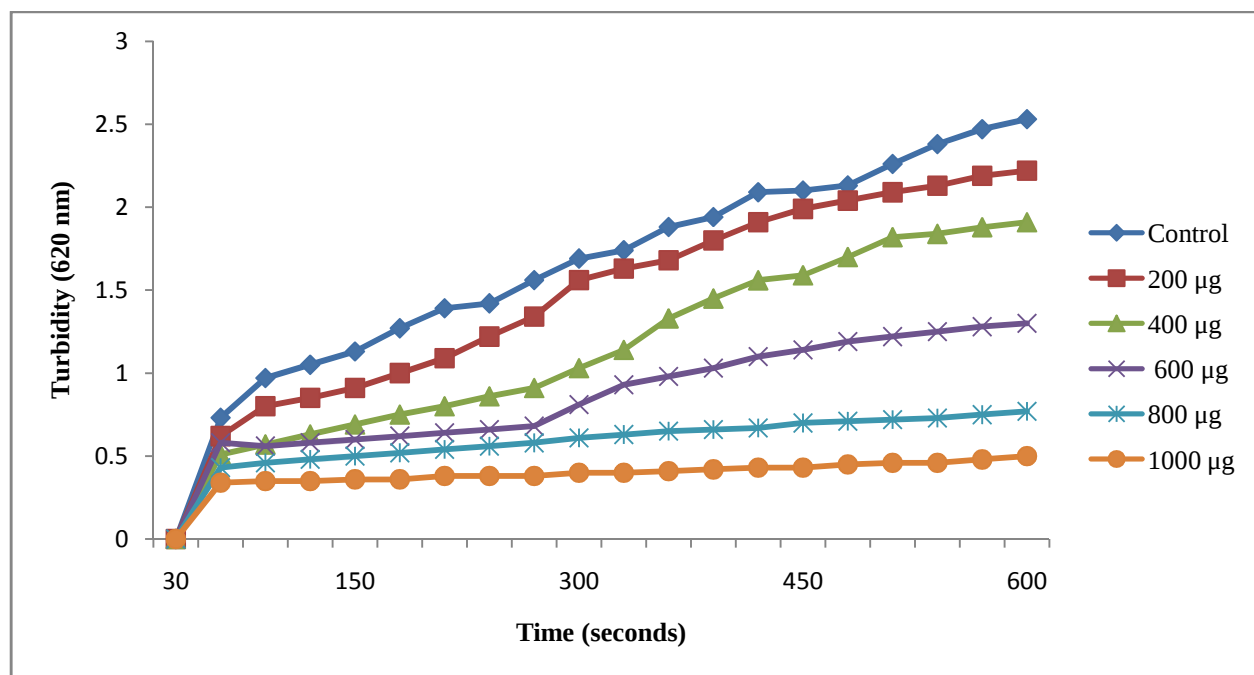


Figure-6: Shows change in turbidity without and with ethanolicroot extract of *M. pudica* at 620nm

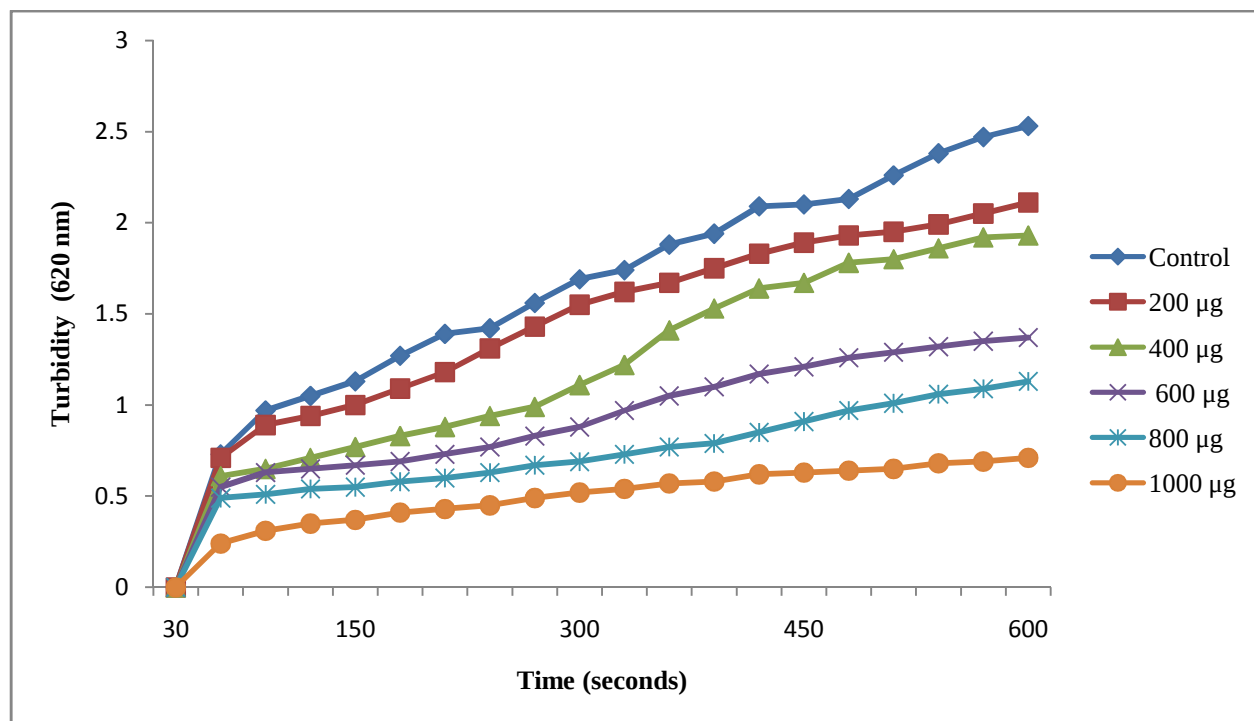


Figure-7: Shows change in turbidity without and with ethanolic flower extract of *M. pudica* at 620nm

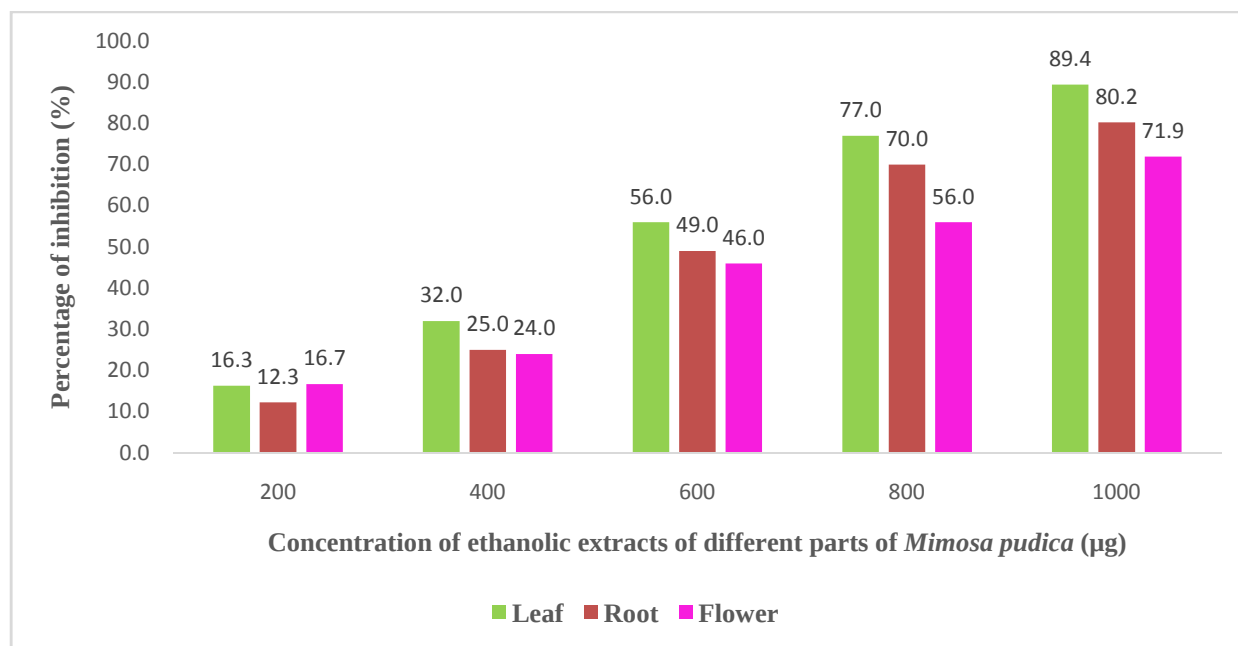


Figure 8.Effect of ethanolic extract of *M. pudica* on nucleation and aggregation of calcium oxalate crystals inhibition.

4. DISCUSSION

Urolithiasis is one of the important constraints in livestock as well as human health globally since last decades, irrespective of geographical, racial and cultural boundaries (Aggarwalet *al.*, 2013). It is also considered as the third most common problem of urinary tract (Bashir *et al.*, 2010; Sayanaet *al.*, 2010). Urolithiasis generally includes nephrolithiasis (Ureter calculi) ureterolithiasis (Ureter calculi) and cystolithiasis (Bladder calculi). Urinary stones are one of the major problems and an important cause of morbidity and end stage renal failure in India. Those composed of calcium oxalate, either alone or mixed with calcium phosphate, are forming the most common uroliths accounting for more than 80% of the stones (Tiselius, 2003).

The mechanisms of urolithiasis is a complex process that occurs due to imbalance between promoters and inhibitors in the kidneys. The factors involved in stone formation include urine output, concentration of urine, urine pH, infection or damage within the urinary tract (Khan, 1997 and Achilles, 1997). Various therapies like diuretics are being used in attempt to prevent recurrence of hypercalciuria- and hyperoxaluria-induced calculi but scientific evidence for their efficacy is less convincing (Hesseet *al.*, 2003). Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of world's population (Atmaniet *al.*, 2003). Several pharmacological investigations on the medicinal plants used in traditional antiurolithic therapy have revealed their therapeutic potential in the *in vitro* or *in vivo* models (Barros *et al.*, 2006; Khan *et al.*, 1992).

Antiurolithic activity observed in the plant crude extract might be due to the presence of these phytochemicals. For example, flavonoids are known to possess antispasmodic and Ca^{2+} channel blocking, antioxidant and antidiuretic activities (Vennila and Mariyal, 2015). Saponins are known to possess anti-crystallization property by disaggregating the suspension of mucoproteins, promoters of crystallization (Gurocak and Kupeli, 2006).

In the present study, antiurolithic activity of aqueous and ethanolic extracts of leaf, root and flower of *M. pudica* were evaluated. The aqueous extract of leaf, root and flower of *Mimosa pudica* have more antiurolithic activity as compared to ethanolic extract. The maximum antiurolithic activity was observed in aqueous and ethanolic leaf extracts as compared to root and flower extracts. Roots of this plant contain tannin up to 10 per cent. Gandhiraja *et al.* (2009) reported that the *M. pudica* leaf extract contains bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins. Antiurothiatic activity of this plant may be due to the presence of these phytochemical compounds.

CONCLUSION

From the above results and study we can conclude that, the antiurolithic activity of this plant extracts are dose dependant. Aqueous extract of leaf have maximum antiurolithic activity than root and flower extracts, since it has reduced the calcium oxalate crystal formation which were increased due to the development of stone in the kidney. Further analysis and fractionation of these extracts is needed to predict the phytochemical constituents responsible for the antiurolithic activity.

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CONFLICT OF INTERESTS

The authors have declared that there is no conflict of interests exists.

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