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Study On In Vitro Antiurolithiatic Activity of Aqueous Extract of Eichhornia Crassipes (Mart.) Solms Leaves by Turbidity Method

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ABSTRACT
A kidney stone, which is sometimes referred to as a renal calculus, is a solid concretion or crystal aggregation that develops in the kidneys as a result of dietary minerals passing via the urine. A number of physicochemical processes, such as super-saturation, nucleation, growth, aggregation, and retention within the kidneys, result in the complex process of urolithiasis. The management of urolithiasis may benefit from the use of phytotherapeutic drugs as an adjuvant or alternative therapy, according to data from in-vitro, in-vivo, and clinical trials. Natural goods are better for the body since they support the mending process naturally. There have been claims that Eichhornia crassipes (Mart.) solms have antiurolithiatic properties. Eichhornia crassipes (Mart.) solms powder's leaves were extracted in water. The antiurolithiatic effects of the plant were investigated in vitro using the common medication cystone and the aqueous extract and fresh juice of the leaves. The method of turbidity was used to determine what prevented stones from forming. Using the turbidity method, a microscopic investigation was conducted to compare the crystal density and size in each treatment. By using the turbidity method, it was determined that the aqueous extract (60.96%) and fresh juice (60.15%) of Eichhornia crassipes (Mart.) solms leaves had the same potential to block calcium oxalate as the common medication Cystone (61.24%).

Key words: Kidney stone, turbidity method, supersaturation, aggregation, Eichhornia crassipes.

I. INTRODUCTION

About 80% of people continue to use traditional medicine for basic medical issues all across the world, especially in underdeveloped nations. Therefore, research has concentrated during the last ten years on the scientific assessment of conventional medications with plant origins. A thorough assessment of the plants utilised in traditional medicine is urgently needed. Such study may result in the discovery of novel drugs or the development of treatments using locally available herbal remedies. The present prevalent idea that green medicine is safer and more dependable than the pricey synthetic treatments, many of which have side effects, is mostly to blame for the resurgence of interest in plant-derived medications. [1]

In the urinary tract, hard, solid particles known as kidney stones develop. The majority of the time, the stones are relatively small and can easily exit the body. However, if a stone (even a small one) prevents the passage of urine, it may cause agonising agony and necessitate immediate medical attention. The treatment of patients with stone illness frequently includes recurrent stone development. The most frequent calcium-containing stones are those made of calcium oxalate monohydrate, calcium oxalate dihydrate, and basic calcium phosphate, which account for 75–90% of all cases. Next in frequency are stones made of magnesium ammonium phosphate (Struvite), which account for 10–15% of cases, uric acid (3–10%), and cystine (0.5–1%).The reduction of calculi through an increase in urine volume, pH, and anti-calcifying activity balances the promoter and inhibitor of crystallisation in urine, affects crystal nucleation, aggregation, and growth (crystallisation inhibition activity), relieves the calculi's binding mucin (lithotriptic activity), and improves renal function. Herbs and herbal medications have powerful physiological effects and effective pharmacological actions. The overuse of synthetic medications, which increases the likelihood of unpleasant drug reactions, has also encouraged people to look to nature for risk-free cures. [2]

Water hyacinth (Eichhornia crassipes) is a free-floating perennial aquatic plant (hydrophyte)

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and herbaceous monocotyledonous member of the pickerelweed family (Pontederiaceae)[3] used as Sedative, CNS depressant, analgesic, immunostimulant, antioxidant, antibacterial, anti-epileptic, anti-anxiety, anti-psychosis, anti-depressant, memory enhancing agent, anti-diarrhoeal and induced urination. [4-8]

Eichhornia crassipes (Mart.) solms leaves were used in the study to assess the antiurolithiatic effect whereas Cystone was used as the reference substance. To determine the inhibition of stone formation, the turbidity method was used.

II. MATERIALS AND METHODS

Plant collection

The leaves of Eichhornia crassipes were collected from their natural habitats in Vaigai River, Madurai, Tamilnadu. The Plant material was authenticated by Dr.D.Stephen, M.Sc, Ph.D, Assistant Professor, Department of Botany, The American College, Madurai - 625002.

Plant Extraction

A. Preparation of Fresh Juice

The fresh leaves of Eichhornia crassipes to be crushed and mechanical grinder. Then 150ml of water added then filtered, the filtrate can be used for the nature of phytoconstituents present in Eichhornia crassipes leaves.

B. Preparation of Aqueous Extract

The Eichhornia crassipes leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water, leaf material was then air-dried on sterile blotter under shade. The shade dried leaves of Eichhornia crassipes (at 25^{o} C) were powdered in a mechanical grinder. Leaf powder was weighed; 150 ml of distilled water was added and kept for 2 days. Whatmann No. 1 filter paper was used to filter the extract, and the filtrate was then collected. The aqueous extract was prepared and stored in air tight bottles for subsequent analysis.

Evaluation for Anti-urolithiatic Activity by turbidity method

Turbidity method [9, 10]

The aqueous extract of Eichhornia crassipes (Mart.) Solms leaves was examined for its in vitro anti-urolithiatic properties in terms of its ability to prevent the production of calcium oxalate in both the presence (with normal medications) and absence of inhibitors. By measuring turbidity using a UV/Visible spectrophotometer at 620 nm, calcium oxalate precipitation at 37^{o}C and pH 6.8 has been examined. It was used to quantify the turbidity brought on by calcium oxalate production. The comparison of uncontrolled growth of the stone nucleus for the comparison of growth in the presence of the standard medications and plant extracts was also detected in microscopical examination to assess calcium oxalate inhibition of plant extracts by absorptions.

Study without inhibitor

Volume of 1.0 ml of 0.025M calcium chloride dihydrate and 2 ml of Tris-buffer (pH7.4) were added in a test tube. Then 1.0ml of 0.025M sodium oxalate was added. After mixing the above solution immediately due to the formation of turbidity and then up to the period of 10 minutes to measure the turbidity of solution by UV/Visible spectrophotometer at 620 nm. This control experiment was done in three replications.

Study with inhibitor

In this experiment, 1 ml of 0.025M calcium chloride dihydrate, 2ml Tris-buffer and 1ml (10 mg/ml solution) of petroleum ether, ethyl acetate and ethanolic plant extracts were added in a four sets of test tubes. Two more test tubes were added 1 ml of 0.025M calcium chloride dihydrate, 2ml Tris-buffer and poly herbal formulation, Cystone. Then 1 ml of 0.025M sodium oxalate was added to each test tube and then up to the period of 10 minutes to measure the change in turbidity of the solution by UV/Visible spectrophotometer at 620 nm. Each procedure was done three times.

Inhibition in stone nucleus formation was calculated by graphical method using the following formula: Percentage inhibition = {1-[Si /Sc]} x 100

Where;

Si - Slope of graph in the presence of inhibitors (drugs/plant extracts)

Sc - Slope of without inhibitors (control).

The percentage inhibition values and IC_{50} values are shown in **Table.1** and **Figure.1** shows change in turbidity and percentage inhibition without and with plant extracts at 620 nm.

Microscopic study

Light microscopy of the crystals formed in the solution with and without was also done. Photographs of calcium oxalate were taken using the objective of 40X. (**Figure.2**)

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S.No	Concentration (µg/ml)	Percentage inhibition		
		Fresh juice	Aqueous extract	Cystone Standard
1.	Control	0	0	0
2.	200	13.31±0.34	14.68±0.87	14.37±0.35
3.	400	17.25±0.67	18.23±0.44	17.60±0.25
4.	600	40.67±0.062	22.83±0.75	22.34±0.07
5.	800	50.21±0.63	50.95±0.50	58.86±0.06
6.	1000	60.15±0.53	60..96±15	61.24±0.31

Table.1 The percentage inhibition of Eichhornia crassipes (Mart.) solms leaves extracts at different concentrations

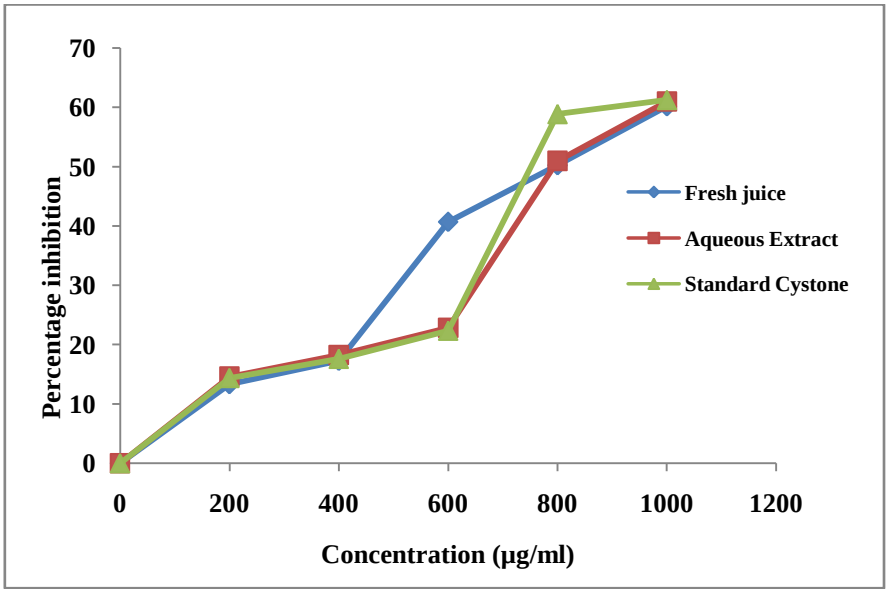
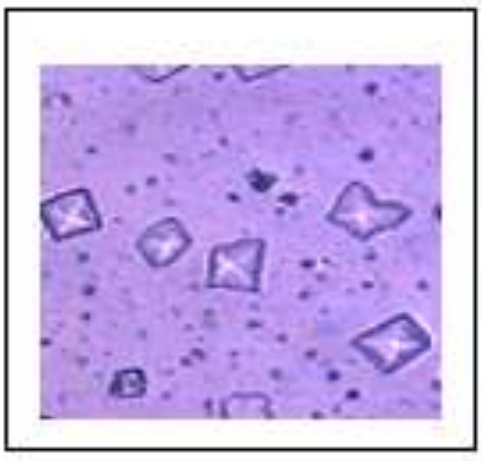


Figure.1 Shows change in turbidity and percentage inhibition without and with Eichhornia crassipes (Mart.) solms leaves extracts at 620 nm

I. Without inhibitor



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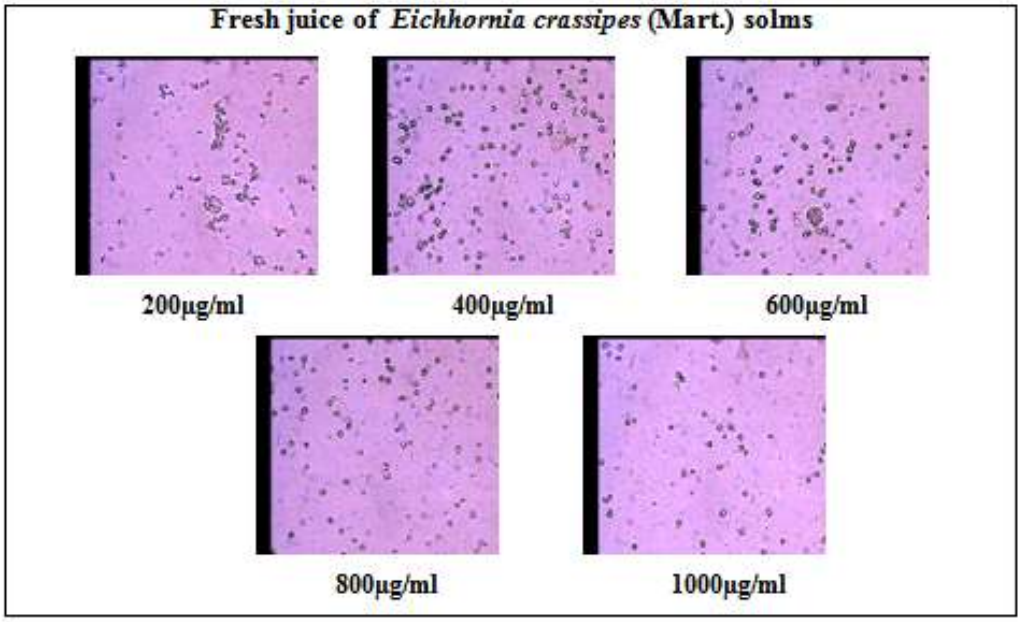
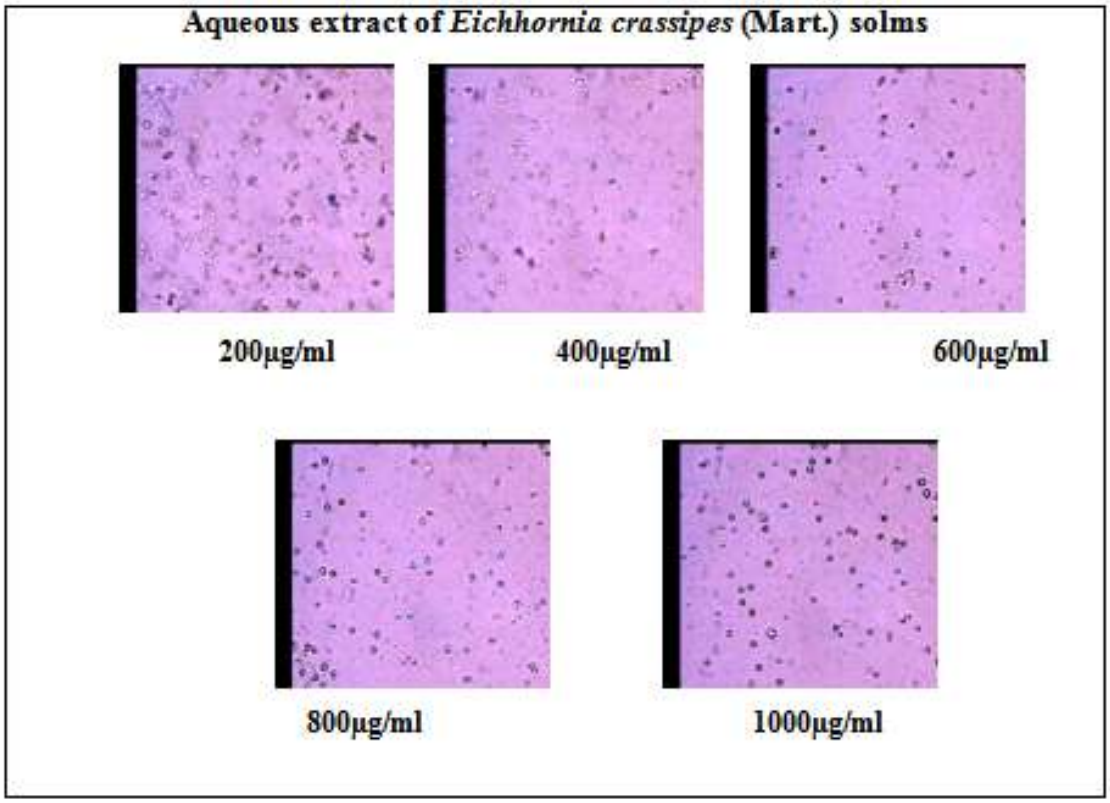
II. With inhibitor





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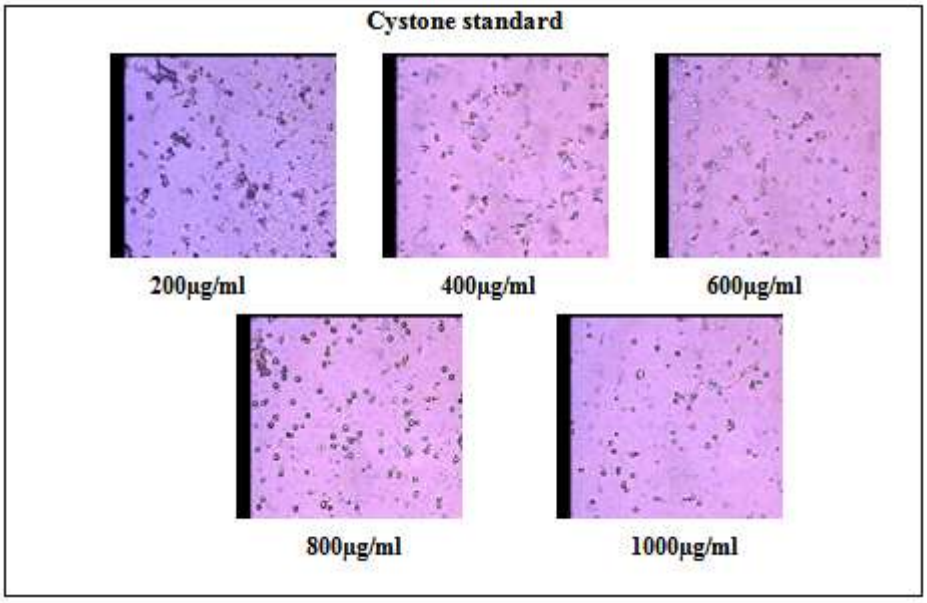

Figure.2: Formation of Calcium oxalate nucleus inhibition by plant extracts and standard drugs in turbidity method.

III. RESULT AND DISCUSSION

A wide range of plants and plant derived products are used in folk medicine for the treatment of urolithiasis as a prophylactic agent or as curative agent. The graphical representation showed percentage inhibition of calcium oxalate with different concentration of fresh juice and aqueous extracts of Eichhornia crassipes (Mart.) solms leaves. In all the treatments concentration dependent initial step rising in turbidity (nucleation) followed by decrease turbidity (aggregation) was seen. Maximum inhibition was found to be in aqueous extract of Eichhornia crassipes (Mart.) solms leaves (**60.96%)** at 1000µg/ml after 180 seconds of chemical reaction started as compared to the control. The fresh juice of leaves showed (**60.15%)** the percentage inhibition was more or less similar to the aqueous extract at the same concentration this standard drug Cystone inhibited **61.24%** in stone nucleation.(**Table1 and figure 1, 2**)

IV. CONCLUSION

The aqueous extract of Eichhornia crassipes (Mart.) Solms leaves have an inhibitory effect on calcium oxalate crystallization similarly to the cystone standard. Thus may be beneficial in the treatment of urolithiasis. Further evaluation of this plant with clinical trials may yield a solution for this urinary problem.

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