

Evaluation of *In vitro* anti urolithiasis activity of herbal drugs

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Abstract

Urinary stone disorder has afflicted human kind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. The common component of urinary stone is calcium oxalate (CaOx). In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. In the indigenous system of medicine, the leaves *Mentha piperita* (family- Labiatae) *Elettaria cardamom* (zingiberaceae) and *Syzygium cumini* (family-Myrtaceae) are reported to be useful in the treatment of urinary stones. Hence, in the present study, the *Mentha piperita*, *Elettaria cardamom* and *Syzygium cumini* have been selected for their *In vitro* Antiurolithiatic activity on experimental kidney stones of calcium oxalate crystals.

Keywords: Urolithiasis; Plant extracts; *In vitro*; Calcium oxalate crystals; Neeri standard drug

1. Introduction to Urolithiasis

Urolithiasis also called Nephrolithiasis or kidney stones; Urolithiasis is the presence of calculi in the urinary track. The male – to- female incidence ratio is 4:1. Eighty percent of calculi are composed of calcium (either oxalate or phosphate), with others composed of struvite, uric acid or cysteine [1].

Annual incidences of kidney stones are about 0.1- 0.4% of the population and lifetime prevalence's in the USA and Europe range between 8 and 15%. Kidney stones occur more frequently with increasing age and among men. Within ten years, the disease usually recurs in more than 50% of people. Nowadays, about 85% of all kidney stones contain calcium salts (calcium oxalate and/or calcium phosphate) with respect to calcium salts as well as to uric acid, crystalluria is very common, i.e. healthy people excrete lower amounts or structurally defective forms of crystallization inhibitors which allows for the formation of large crystal aggregates as precursors of stones. Alternatively, crystal adhesion to urothelial surfaces may be enhanced in stone formers [2].

Urolithiasis the formation of calculi, or condition associated with urinary calculi. The term calculi are synonymous with uroliths, stones or crystals. These calculi are formed by deposits of polycrystalline aggregates composed of varied amounts of crystalloid and organic matrix. They can vary in size and may be found anywhere in the urinary track from the kidney to the bladder. For stone formation to occur it requires saturated urine dependent upon urine pH and the concentration of the solute. The presence of clinical signs depends on the obstruction of urine, the presence of infection and edema (swelling). Stones that cause obstruction to the flow of urine set up an environment of urine stasis and bacterial growth. The irritation caused by the stones results in secondary infections leading to pyelonephritis (inflammation of the kidney) an upper urinary tract infection, or cystitis (inflammation of the bladder) and urethritis (inflammation of urethra) a lower urinary tract infection. Though the cause of stone formation is hard to determine , some factors include a genetic predisposition , metabolic disorders such as diabetes, myeloproliferative disease like leukaemia, or hypocalcaemia (abnormally high amounts of blood calcium), diet imbalance, a poor intake of water,

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parasites such as bladder thread worm and bacterial infection such as *E. Coli*, *Klebsiella*, *Staphyococcus*, or *Mycoplasma*. Other conditions that can lead to stone formation due to the retention and stasis of urine are anatomical defects, or pocketing in the bladder wall known as diverticulas and immobility due to stroke and hind limb paralysis. Stones that are present, it is known that there is still a high rate of recurrence [3].

Stone disease is a common medical problem, frequently recurs and is associated with significant morbidity. Because appropriate medical therapy significantly decreases stone recurrence, this disorder must not be ignored by nonurologists. Even the single stone former should be offered a metabolic evaluation [4].

Compositional stone analysis should be an integral part of the metabolic evaluation. Of patients with nephrolithiasis. Moreover, stone analysis alone may provide guidance for therapeutic treatment and obviate a formal metabolic evaluation [5].

2. Literature search and criteria

[6],[7],[8] made an attempt to search various herbal drugs for urolithiasis and potential antiurolithiasis plant extracts. The *Mentha piperita*, *Elettaria cardamom* and *Syzygium cumini* have been selected for their *In vitro* Antiurolithiatic activity on experimental kidney stones of calcium oxalate crystals. This literature will help researcher for selection of suitable plant extracts in search of antiurolithiatic activity. We included studies that adopted at least one measurement relevant to renal urolithiasis, such as oxalate, calcium etc.

2.1. Phytochemical investigation

The leaves of *Mentha piperita* and *Syzygium cumini* were shade dried and powdered separately. The methanolic extract were prepared by Soxhlet extraction method.

The seeds of *Elettaria Cardamomum* were dried under shade and crushed and powdered. Was extracted with ethanol in Soxhlet apparatus.

2.1.1. Phytochemical analysis

The extracts were subjected to preliminary qualitative phytochemical investigation. The various tests and reagents used and observations are recorded in table [9].

2.1.2. Physicochemical analysis

The physical constant value will be determined by Pharmacopoeial method i.e., Total ash, Acid insoluble ash, Water soluble ash and Loss on drying. The alcohol (90v/v) soluble and water soluble extractive value will be determined by maceration process as per IHP 2000 [10, 11].

Crude drug procured for the study dried at room temperature, and subjected to physicochemical evaluation.

2.2. Pharmacological investigation

2.2.1. *In vitro* anti urolithiatic activity test by titrimetry

Chemicals used: Neeri, sodium oxalate, Tris buffer, calcium chloride, potassium Permanganate (KMnO_4), sulphuric acid. (H_2SO_4)

Experimental kidney stones of calcium oxalate were prepared in the laboratory by taking equimolar solutions of calcium chloride dehydrate in distilled water and sodium oxalate in 10ml of 2N H_2SO_4 . Both were allowed to react in sufficient quantity of distilled water in beaker, the resulting precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60 °C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1mg of calcium oxalate and 10mg of extract, packed in together in semi permeable membrane of egg as shown in the model designed given below [11, 12].

This was allowed to suspend in a conical flask containing 100ml of 0.1M Tris buffer. First group served as blank containing only 1 mg of calcium oxalate. The second group served as positive control 1 mg of calcium oxalate and along with the 10 mg standard drug Neeri. The 3rd group along with 1 mg of calcium oxalate contain extract. The conical flask of all groups were kept in an incubator preheated to 37 °C for 2 hours. Remove the content of semi permeable membrane from each group into separate test tube, add 2ml of 1N H_2SO_4 to each test tube and titrated with 0.0494N KMnO_4 till light pink colour end point obtained.

The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various extracts [13].

3. Results and discussion

3.1. Phytochemical investigation

The Physicochemical constants of leaves of *Mentha piperita*, *Elletaria cardamom* and fruits of *Syzygium cumini*.

Table 1 Physicochemical Analyses of herbal drugs

Sr. No.	Physic-chemical parameters	<i>Mentha piperita</i>	<i>Elletaria cardamom</i>	<i>Syzygium cumini</i>
1	Foreign matter	1.5%	1.2%	1.1%
2	Ash values			
	Total ash value	8.5%	2.09%	25%
	Acid insoluble ash	1.5%	0.25%	2.5%
	Water- soluble ash	2.1%	1.45%	5.25%
3	Extractive value			
	Water soluble extractive	8.1%	53.23%	1.16%
	Alcohol Soluble Extractive	10.2%	51.7%	8.5%
4	Loss on drying	9.83 %	6.21%	7.5%

Table 2 Preliminary qualitative tests of various extracts of herbal drugs

Phytoconstituents	<i>Mentha piperita</i>	<i>Elletaria cardamom</i>	<i>Syzygium cumini</i>
Alkaoids	-	+	+
Amino Acids	-	-	-
Carbohydrates	-		-
Flavonoids	+	+	+
Glycosides	-	+	-
Proteins	-	-	+
Saponin	-	+	-
Steroids	-	-	-
tannins	+	-	-
Vitamins	+	-	-

"+"= Present "-"= Absent

The extracts of

- *Mentha piperita* shows Falvanoids, Tannins and Vitamins.
- *Elletaria cardamom* shows Alkaloids, Flavonoids, Glycosides and Saponnins
- *Syzygium cumini* shows Alkaloids, Flavonoid and Proteins.

3.2. *In -vitro* experimental model set up to evaluate anti urolithiatic activity



Figure 1 Decalcification of egg shell in 10% Acetic acid overnight

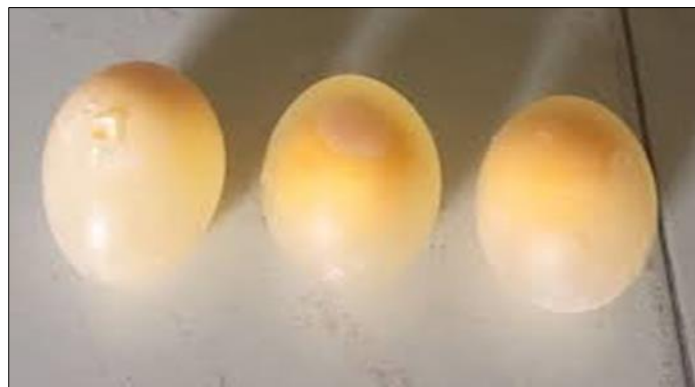


Figure 2 Decalcification of eggs



Figure 3 Egg membrane along with the content suspended in to the 0.1 M Tris buffer

3.3. *In vitro* anti urolithiatic activity test by Titrimetry

Table 3. Percentage Dissolution of CaOx by various Drugs

S no	Groups	% Dissolution of calcium oxalate		
		<i>Mentha piperita</i>	<i>Elettaria cardamom</i>	<i>Syzygium cumini</i>
1	Blank	0	0	0
2	Positive Control	81	81	81
3	Extract	98.1	99	98

4. Conclusion

In vitro urolithiasis has been performed on the selected medicinal plants *Mentha piperita*, *Syzygium cumini* and *Elettaria cardamom* by using standard drug, neeri. The work was performed by using *In vitro* antiurolithiatic model for calculating percentage dissolution of kidney stone.

This study evaluates the antiurolithiatic activity of Methanolic leaf extract of *Mentha piperita* found 98.1% of calcium oxalate dissolution. The seeds of ethanolic extract of *Elettaria cardamom* show 99% of calcium oxalate dissolution and in ethanolic extract of leaves of *Syzygium cumini* indicate 98% dissolution calcium oxalate crystal than standard drug neeri. From the study it was observed that ethanolic extract *Elettaria cardamom* shows highest dissolution calcium oxalate crystal with the standard drug neeri. This study has given primary evidence to possess lithothriptic property.

In vitro study was given data and shown various extract of plants were quite promising for further study in this regard.

Compliance with ethical standards

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Disclosure of conflict of interest

No Conflict of interest.

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