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IN-VITRO ANTI-UROLITHIATIC ACTIVITY OF LEAVES OF *CUCUMIS SATIVUS* (LINN.)

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ABSTRACT

Urolithiasis is one of the serious renal disorder that require a well targeted therapeutic strategy. Number of drugs are available for the treatment of lithiasis, but clinical evaluation of these drugs has shown incidences of relapses, side effects and drug interactions. This has been the rational for the development of new anti-urolithiatic drugs and search for novel molecules. Also assumed that, if the remedy from herbal source that offer better protection and decreased relapse, because they promote the repair mechanism in natural way. After a survey, leaves of *Cucumissativus* were selected for the present study. Aqueous and alcoholic extract of leaves of *C.sativus* was screened for its antiurolithiatic activity by *in vitro* model. Aqueous extract showed significant antiurolithiatic activity when compared to the reference standard (Cystone).

KEYWORDS

Anti-urolithiatic and *Cucumissativus*.

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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. Many medications and remedies have been used during the past many years to treat urinary stones. Endoscopic stone removal and extracorporeal shock wave lithotripsy have revolutionized the treatment of nephrolithiasis, but do not avoid the possibility of new stone formation. Various therapies including thiazide diuretics and alkali citrate are being used in an attempt to prevent the recurrence of hypercalciuria and hyperoxaluria induced calculi, but scientific evidence for their efficacy is less

convincing. Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for the majority of the world's population^{1,2}. Several medicinal plants were used in traditional anti-urolithiatic therapy, from that, the leaves of *Cucumis sativus* plant were evaluated for its anti-urolithiatic activity through the scientific manner.

The plant

Cucumis sativus L, is a widely available plant, grown throughout the world, belonging to the family Cucurbitaceae. The plant is unbranched tendrils up to 30 cm long. Leaves are alternate and simple, with 3-7 palmate lobes and serrated margins. Yellow serous flowers bearing either female or male organs. Hairless cylindrical fruits are warty, yellow to green, and up to 50 cm long. Traditionally, it is used as Anti-diarrhoeal, Anti-inflammatory, Anti-hypertensive, proteolytic, Anti-fungal, Antioxidant and Anti-Panic activities. The seeds of the plant are cooling and diuretic, the fruit juice is used as nutritive and as a demulcent in anti-acne lotions^{3,4}.

Collection and Identification of plant material

The leaves of *Cucumis sativus* were collected during May 2017 from the coastal regions of Kanyakumari District, Tamil Nadu. The specimen of the plant material was identified and authenticated by Prof. V. Chelladurai, Research officer-Botany, Central Council for Research in Ayurveda and Siddha (Government of India), Tirunelveli, Tamil Nadu.

Preparation of the extract

The leaves of *Cucumis sativus* were dried under shade and powdered into a coarse state using a hand-operated mill. The coarse powder was extracted by heating with distilled water and also extracted with ethanol using a cold percolation method. The extracts obtained were used to determine the in-vitro anti-urolithiatic activity^{5,6}.

Anti-urolithiatic activity

Anti-urolithiatic activity of aqueous and alcoholic extracts of leaves of *Cucumis sativus* was screened by titrimetric and spectroscopic in-vitro models.

Preparation of molybdate-sulphuric acid reagent

Molybdate-sulphuric acid reagent was prepared by 5% w/v of sodium molybdate solution, 13 ml of conc. H₂SO₄ in 80 ml of distilled water. Finally, the volume was adjusted to 100 ml with distilled water.

Preparation of reducing solution

1 gm of p-phenylene diamine was dissolved in 100 ml of 3% w/v of sodium meta-bisulfite solution.

Preparation of artificial kidney stones

Usually, the human kidney stone is an aggregation of solute materials from urine such as calcium oxalate, calcium phosphate and uric acid. The kidney stones required for the present study were prepared artificially by a homogeneous precipitation method.

Preparation of Calcium oxalate stones

Equimolar solution of calcium chloride dihydrate in distilled water and sodium oxalate in 10 ml of 2N Sulphuric acid were allowed to react in a sufficient quantity of distilled water in a beaker to produce a precipitate of Calcium oxalate stones.

Preparation of Calcium phosphate stones

Equimolar solution of calcium chloride dihydrate in distilled water and Disodium hydrogen phosphate in 10 ml of 2N Sulphuric acid were allowed to react in a sufficient quantity of distilled water in a beaker to result in a precipitate of Calcium phosphate stones.

Both the precipitates (stones) were freed from traces of sulphuric acid by the treatment of ammonia solution, washed with distilled water and dried at a temperature not exceeding 60°C for four hours^{7,8}.

Preparation of semi-permeable membrane

The semi-permeable membrane was prepared with the aid of farm eggs. The eggs were placed in 2 M hydrochloric acid for an overnight, during which period the outer shell of the eggs was removed chemically, which caused complete decalcification. Further, the membrane-layered eggs were washed with distilled water. A hole was made on the top and the contents were squeezed out completely from the decalcified eggs carefully with a sharp pointer. Again, wash thoroughly with distilled water and place it in ammonia solution, in the moistened

condition for a while and rinsed it with distilled water and stored in refrigerator at a Ph of 7-7.4⁹.

Estimation of calcium oxalate (Titrimetric method)

Weighed accurately about one milligram of calcium oxalate and 10 mg of the extract/reference standard and packed it together in semi permeable membrane pouch by suture. This was allowed to suspend in a conical flask containing 0.1 MTRISS buffer. One group served as negative control, which contain only calcium oxalate. All the groups were kept in incubator (pre heated to 37°C for 2 hours) for about eight hours. The contents of semi permeable membrane pouches were transferred to test tubes. Each test tube was added with 2 ml of 1N sulphuric acid and titrated against 0.9494 N KMNO₄. Light pink colour was obtained as end point. Each ml of 0.9494 N KMNO₄ is equivalent to 0.1898 mg of Calcium.

Estimation of calcium Phosphate (Colorimetric Method)

Follow the procedure as above up to incubation. Transfer the contents of each group into the test tubes.

Each test tube was added with 2 ml of 1 N Sulphuric acid, 2.5 ml of Molybdic sulphuric acid reagent, 1 ml of reducing solution and made up the volume to 10 ml using distilled water.

Standard solutions of Calcium phosphate was prepared by adding 2.5 ml of Molybdic sulphuric acid, 1 ml of reducing solution and made the volume to 10 ml with distilled water. Absorbance was measured for groups and standard dilutions (200,400,600,800 and 1000µg/ml) using colorimeter at 750nm. The undissolved calcium phosphate was determined from the standard calibration curve by extrapolation method¹⁰.

RESULTS AND DISCUSSION

Anti urolithiatic activity of Aqueous and alcoholic extract of *Cucumissativus* was evaluated by titrimetric and spectrophotometric methods. Aqueous extract showed significant Anti urolithiatic activity when compared to the reference standard cystone (tablet). Alcoholic extract also showed anti-urolithiatic activity but less activity (dissolution effect) when compared to the aqueous extract and standard cystone.

Anti-urolithiatic activity of leaf extracts of *Cucumissativus*

S.No.	Group	% Dissolution of Calcium oxalate	% Dissolution of Calcium phosphate
1	Control	-----	-----
2	Standard (Cystone)	59.6	42.3
3	Aqueous extract of <i>C.sativus</i>	46.3	33.6
4	Alcoholic extract of <i>C.sativus</i>	32.7	28.2

CONCLUSION

The present study reveals that the leaf of *Cucumissativus* plant can be considered as an anti-urolithiatic (alternative) medicine. Usually, after collection of fruits from the plants, the farmers will throw out the Ariel part including leaves but this study creates utilisation of leaves in the beneficial manner. Also further investigation is needed to detect the causative matter responsible for the anti-urolithiatic activity being present in the leaf.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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