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Original Article

EVALUATION OF *IN VITRO* ANTI-UROLITHIATIC ACTIVITY OF *SOLANUM TUBEROSUM* ON CALCIUM OXALATE CRYSTALS

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ABSTRACT

Objective: Present study was designed to evaluate the anti-urolithiatic activity of Indian species of *Solanun tuberosum* on calcium oxalate crystal *In vitro* model.

Methods: Potato powder was prepared by cutting, grinding and dried in hot air oven at 60-65 °C. *In vitro* model of Calcium oxalate crystal was used.

Results: It was observed that the sample prepared from *Solanumtuberosum*, both potato ash as well as potato powder, had ability to act as anti-urolithical drug due to the presence of saponin and potassium.

Conclusion: From the present study, we can conclude that the sample prepared from *Solanumtuberosum*, had the ability to act as anti-urolithical drug and *in vivo* study can be carried out for further investigation.

Keywords: Urolithiasis, *Solanumtuberosum,* Calcium oxalate crystal

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INTRODUCTION

Urolithiasis is one of the most common diseases of the urinary tract, which has been Afflicting humankind since antiquity. Urinary stones affect 10-30% of the population in industrialized countries. It occurs more frequently in men than women but rare in children [1]. Urolithiasis is associated with calculus formation at any level in the urinary collecting system, but calculus often arises in the kidney. Recurrent stone formation is probably the most important problem in the aftercare patients who have undergone operations for renal and ureteric calculi. Urolith formation is a multifactorial process which may relate to diet, urinary tract infection, altered urinary solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque and microliths etc [2].

When the urea-splitting organisms infect the urinary tract, bacteria disintegrate the urea excreted in the urine in the presence of urease enzyme, which subsequently trigger the formation of ammonia, rendering the urine alkaline. In alkaline state, urine leads to contain precipitated crystals of calcium oxalate, magnesium phosphate and calcium carbonate in large amount, thereby leading to a strong tendency to form calculi. Bacterial infection may induce stone formation by crystal adherence. Most of the urea-splitting organisms belong to species Proteus but, organisms such as Pseudomonas, Staphylococcus, *Escherichia coli* and even Mycoplasma were reported to be capable of producing urease [3]. Infected stones were associated with the organisms like E. coli, Proteus species, Streptococcus, Staphylococcus, Pseudomonas and Ureaplasma urealyticum [4]. There are increasing evidence that have been reported that the end products of urealysis damage the Glycosaminoglycon layer of the renal urolithial cells thus leading to bacterial adherence, biofilm formation and mineral encrustation. Exhaustive microbiological investigations are therefore necessary to diagnose and treat the infection responsible for the stone formation.

Urinary tract stone disease has been documented historically as far back as Egyptian Mummies [5]. The experimental intoxication induced by ethylene glycol is widely used for Kidney stone formation in rats. When ethylene glycol is metabolized by the body, it produces toxic metabolites like glycoaldehyde, glycolate and glyoxylate. These metabolites cause tissue destruction, primarily from calcium oxalate deposition and metabolic abnormalities, specifically a high anion-gap metabolic acidosis, lactic acidosis and hypocalcemia. Oxalic Acid combines with calcium to form calcium oxalate crystals, which deposit in the kidneys. This can result in hematuria and proteinuria, increased creatinine and renal failure [6].

Surgical operation, lithotripsy and local calculus disruption using high power laser are widely used to remove the calculi. Many remedies have been employed since ages to treat renal stones and most of them were from plants and proved to be useful. In Ayurveda and Folklore medicine many herbs are used in the management of urolithiasis [7].

Solanum tuberosum is locally known as potato it belongs to the family Solanaceae. The potato is a starchy tuber and is a root vegetable native to the Americas. The plant is a perennial in the nightshade. Potatoes are an excellent source of vitamin C, potassium, fibres, B vitamins copper, tryptophan, manganese and even lutein. People use potatoes for diabetes, heart disease, high blood pressure, indigestion (dyspepsia), and other conditions [8].

Aim and objective

To determine the Calcium oxalate crystallization inhibition by *in vitro* method.

Plant profile

- Plant name: Solanum tuberosum
- Synonym: Tuber, Potato, Aloo, Batata.
- Part used: Stem tuber
- Taxonomy:

Kingdom–Plantae

Division–Tracheophyta

Class–Magnoliopsida

Order–Solanales

Family–Solanaceae

Genus-Solanum

Species–Solanum Tuberosum L.

- Description: The potato is a starchy tuber of the plant *Solanum tuberosum* and is a root vegetable native to the Americas. The plant is a perennial in the nightshade family Solanaceae.
- Distribution: There are two main peaks in global potato distribution by latitude. The major peak is between 45 °N and 57 °N and represents potato production zones in temperate climates where potato is a summer crop [8].

Fig. 1: Whole plant of *Solanum tuberosum*

MATERIALS AND METHODS

Sample collection and authentication

- Collection: Potatoes were collected in January 2022 from Parner, Ahmednagar, Maharashtra-India.
- Authentication: Plant material was identified and authenticated from the Dept. of Botany, Chandmal Tarachand Bora College, Shirur-Pune Maharashtra-India.

Chemical used

Calcium chloride dehydrate, sodium oxalate, Potassium permanganate, oxalic acid, and sulphuric acid were taken from College laboratory, Cystone was purchased from Sukhakarta Pharmacy (Himalaya Drug Company). Shirur-Pune Maharashtra-India.

Sample preparation

- Potato Powder: Potato were peeled and chopped into small pieces, washed properly, and then grinded as fine as possible. It was filtered through filter paper. Air dried the residue and then dried in hot air oven for 2 h at 60-65 °C.
- Potato Ash: For making potato ash, the residue obtained was burned out in crucible for 5-6 h.

Cystone tablet

Himalaya Cystone Tablet contains ingredients that possess diuretic, demulcent, and antimicrobial properties. Pasanabheda is known to soothe and protect irritated or inflamed internal tissue. The formulation helps in the treatment and prevention of crystals in the urine. Moreover, it helps in the removal of kidney stones and prevents the recurrence of stones.

Fig. 2: Potato powder

Fig. 3: Potato powder

Key ingredients

- Pasanabheda (Saxifraga Ligulata)
- Shilapushpa (Didymocarpus pedicellata)

Key benefits

- As a diuretic, the herb helps to flush out small stones and gravel along with urine.
- Shilapushpa (Didymocarpus pedicellata) prevents the formation of urinary stones and helps dissolve kidney stones.
- It can be used as an adjuvant in chronic UTI, painful urination or blood present in urine and burning micturition (urination)

Directions for use

- Use as directed by the physician.
- Safety Information:
- Read the label carefully before use
- Keep out of reach of the children
- Store in a cool dry place

Use under medical supervision [10]

Pasanabheda (Saxifraga Ligulata), possesses diuretic, demulcent and antimicrobial properties. Due to a high content of mucilage, which renders the herb its demulcent property, Pasanabheda soothes and protects irritated or inflamed internal tissue. As a diuretic, the herb helps to flush out small stones and gravel along with urine.

Table 1: Test for phytoconstituent

Test	Observation	Result
Test for Carbohydrate: Molish's test-2 ml aq. extract add few drops of α naphthol sol in alc. shake and add conc. H2SO4	Violet ring observed at junction of two liq.	Carbohydrates was present
Test for starch: Iodine test-3 ml test sol and few drops of dil. iodine sol.	Blue colour observed and disappears on boiling.	Starch was present.
Test for protein: Biuret test-3 ml test sol. add 4% NaOH and few drops 1% CuSO4 sol.	Violet colour appeared	Protein was present.
Test for cardiac glycosides: Baljet's test-add sodium picrate in test sol.	Yellow colour not observed.	Cardiac glycosides was absent.
Test for anthraquinone glycosides: Borntrager's test-3 ml extract+dil. H2SO4 boil and filter. To cold filtrate, add equal vol. chloroform, shake well. Add ammonia.	No observation	Anthraquinone glycoside was absent
Test for Saponin glycosides: Foam test-Shake the dry powder vigorously with water.	Persistent foam observed	Saponin glycosides was present
Test for Coumarin glycosides: Alcoholic extract when made alkaline.	Blue fluorescence not observed	Coumarin glycoside was absent.
Test for flavonoids: Shinoda test-to powder add 5 ml. 95% ethanol, few drops conc. HCL and 0.5 gm magnesium turnings.	Pink colour not observed.	Flavonoid was absent.
Test for tannins and phenolic comp: To 2-3 ml aqs. Extract add few drops of 5% FeCl3 sol.	Deep blue colour was observed.	Tannin and phenolic comp was present.
Test for organic acids: Aqs. Drug extract with Dil. NH4OH sol. Take 2 ml above sol add few drops of 5%CaCl2 sol.	No any observation	Organic acids were absent.
Test for inorganic elements: A)Test for potassium: 2-3 ml test sol, add few drops of sodium cobalt nitrate sol.	Yellow ppt of potassium cobalt nitrate was observed.	Potassium was present.
B)Test for iron: 5 ml test sol add few drops 2% potassium ferrocyanide.	Dark blue colour observed.	Iron was present[9].

Shilapushpa (Didymocarpus pedicellate), is known for its antilithiatic property, which prevents the formation of urinary stones. As a lithotriptic, Shilapushpa helps dissolve kidney stones. The herb is also known for its antimicrobial properties [11].

Cystone tablets are an Ayurvedic treatment for stones, traditionally practiced in India. Many studies and long experience attest to the safety of this compound. It is also claimed that cystone decreases urinary supersaturation or micro pulverizer and expels kidney stones, but existing studies have been limited by small patient numbers, weak methodology, and poor study design, including lack of proper controls [12].

Preparation of calcium oxalate

1.47 gm of calcium chloride dehydrate was dissolved in 100 ml distilled water and 1.34 gm of sodium oxalate was dissolved in 100 ml of 2N H_2SO_4. Both were mixed equally in a beaker to precipitate out calcium oxalate with stirring. The resultant calcium oxalate was freed from traces of sulfuric acid by ammonia solution; washed with distilled water and dried at a temperature 60 °C for 2h [13].

Preparation of egg membrane

The outer calcified shell was removed chemically by placing the eggs in a beaker consisting 4 ml concentrated HCL or H_2SO_4 in 200 ml distilled water for few hours, which caused complete decalcification. Further, washed with distilled water and carefully with a sharp pointer a hole was made on the top and the contents was squeezed out completely from the decalcified egg. Then it was washed thoroughly with distilled water and placed in ammonia solution. Then rinsed with distilled water and stored in the refrigerator at a pH of 7-7.4 [13].

Fig. 4: Egg dipped in acid solution

Fig. 5: Prepared egg membrane

Preparation of phosphate buffer

- Preparation of Potassium dihydrogen phosphate (0.2M) solution:

Potassium dihydrogen phosphate (13.609) was dissolved in water and made the volume with water to 500 ml.

- Preparation of Sodium hydroxide (0.2M) solution:

Sodium hydroxide (4 gm) was dissolved in 500 ml water.

- Preparation of Phosphate buffer:

In a volumetric flask, potassium dihydrogen phosphate (156.25 ml, 0.2M) was taken and to this solution, sodium hydroxide (70 ml, 0.2M) was added and made the volume with water [14].

Procedure

Incubation

All groups of samples were packed together in egg semi-permeable membrane and tied with thread at one end and suspended in conical flask containing 100 ml phosphate buffer (pH-6.8), each. At another end, the thread was tied with a stick placed on the mouth of conical flask and covered with aluminum foil. All groups were kept in an incubator, pre-heated to 36 °C-38 °C for 2 h, kept for 7-8 h. The entire content of each group were removed from the semipermeable membrane and were used for further test [15].

Table 2: Grouping for incubation

Groups	Contents
Group 1 (Control)	1 mg calcium oxalate+1 ml distilled water
Group 2 (Standard)	1 mg calcium oxalate+10 mg cystone+1 ml distilled water
Group 3 (Potato powder)	1 mg calcium oxalate+10 mg powder+1 ml distilled water
Group 4 (Potato Ash)	1 mg calcium oxalate+10 mg Ash+1 ml distilled water

Table 3: Grouping for incubation

Set	Group	Content
Set-A	Group-1	1 mg calcium oxalate+1 ml distilled water
	Group-2	1 mg calcium oxalate+10 mg cystone+1 ml distilled water
	Group-3	1 mg calcium oxalate+10 mg powder+1 ml distilled water
	Group-4	1 mg calcium oxalate+10 mg Ash+1 ml distilled water
Set-B	Group-1	2 mg calcium oxalate+1 ml distilled water
	Group-2	2 mg calcium oxalate+10 mg cystone+1 ml distilled water
	Group-3	2 mg calcium oxalate+10 mg powder+1 ml distilled water
	Group-4	2 mg calcium oxalate+10 mg Ash+1 ml distilled water
Set-C	Group-1	3 mg calcium oxalate+1 ml distilled water
	Group-2	3 mg calcium oxalate+10 mg cystone+1 ml distilled water
	Group-3	3 mg calcium oxalate+10 mg powder+1 ml distilled water
	Group-4	3 mg calcium oxalate+10 mg Ash+1 ml distilled water

Chemical preparation

Preparation of 0.1N^{KMnO_4}

3.16 gm potassium permanganate dissolved in 1000 ml purified water, heat on water bath for 1 h.

Preparation of 0.1N Oxalic acid

6.3 gm of oxalic acid dissolved in 1000 ml distilled water.

Preparation of 0.02M^{KMnO_4}

0.32 gm of KMnO4 was dissolved in 100 ml of distilled water. If it was boiled for 30 min. After that, let it cool and then filter it.

Preparation of 1N^{H_2SO_4}

9 ml of concentrated sulfuric acid was diluted in 250 ml of distilled water [13].

Titration

Standardization of ^{KMnO_4}

Into a conical flask pipette out exactly 20 ml oxalic acid. Add 5 ml conc. Sulfuric acid and boil the contents of the flask up to 70°C. Titrate the contents of the flask against KMnO4 solution until a faint pink colour is obtained [16].

Procedure for titration

Contents of semi-permeable membrane from each group were removed into a conical flask. 2 ml of 1N sulfuric acid was added and titrated with 0.1069 N ^{KMnO_4} till a light pink colour, at the end was obtained [15].

Turbidity

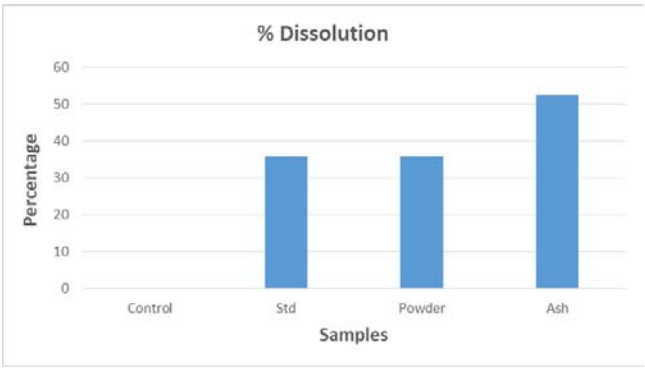
After incubation, the entire content of the semi-permeable membrane and was transferred into test tube individually 4 ml of 1N H2SO4 and 0.6 to 0.7 ml of 0.02 M KMnO4 were added and kept aside for 15-20 min. The change in color was observed. The change of color intensity was measured against 620 nm with the help of UV spectrometer [13].

Fig. 6: *In vitro* setup for each sample

RESULTS AND DISCUSSION

Titration

The amount of calcium oxalate that was dissolved was subtracted from the total quantity of calcium oxalate used in the experiment. This shows the actual quantity of calcium oxalate the test drug can dissolve in a dissolution study; the negative control shows zero dissolution. The standard group (Cystone) showed a dissolution of 35.71%. The Potato Ash and Powder showed dissolution of 52.43% and 35.71 %, respectively.



Graph 1: % Dissolution of calcium oxalate crystals

Table 4: Titration reading

	Reading (y) ml	Undissolved calcium oxalate (x) mg	Dissolved calcium oxalate (a) mg	% Dissolution
Control	0.7 ml	0.01491 mg	-	0.0
Standard	0.45 ml	0.009585 mg	0.005325 mg	35.71
Potato Powder	0.45 ml	0.009585 mg	0.005325	35.71
Potato Ash	0.333 ml	0.0070929 mg	0.0078171 mg	52.43

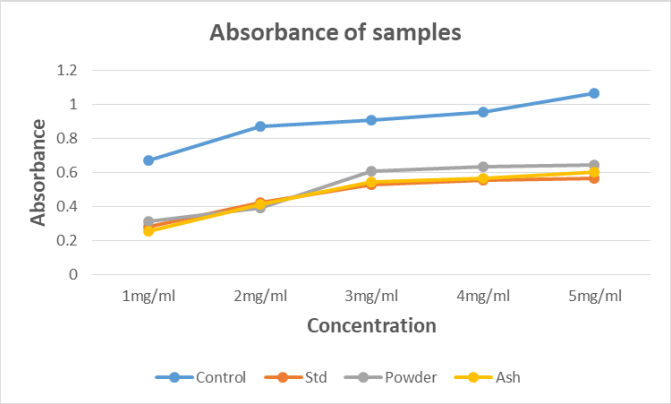
Turbidity

The turbidity of $KMnO_4$ in sample was observed in UV spectrometer at the wavelength of 620 nm. The absorbance observed are shown in the table. By plotting the graph of absorbance vs concentration of all the sample, the ability of the sample to show activity was determined.

The graph plotted shows that the sample has the ability to show the desired activity.

Table 5: Turbidity reading

	1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml	5 mg/ml
Control	0.669	0.869	0.9077	0.9569	1.066
Standard	0.2814	0.4207	0.5304	0.5547	0.565
Potato Powder	0.3135	0.3893	0.6046	0.6342	0.6457
Potato Ash	0.254	0.4092	0.5415	0.5648	0.601



Graph 2: UV absorbance of samples

CONCLUSION

From the present study, we can conclude that, the sample prepared from *Solanum tuberosum*, had the ability to act as an anti-urolithical drug. We can conclude that both potato ash as well as potato powder had ability to show activity.

The reason behind it could be both or any one of it; those are, the presence of saponin, as it is known for its anti-crystallization property and the presence of potassium, as it has the ability to dissolve kidney stone. *In vivo* study can be carried out for further investigation.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Smith DR. General urology. 9th ed. CA: Lange Medical Publication; 1978. p. 212-32.
2. Fowler C. The kidneys and ureters. In: Mann CV, Russel RCG, Williams NS. Editors, and Bailey Love's short practice of surgery. 22nd ed. London: ELBS, Chapman & Hall; 1995. p. 915-39.
3. Friedlander AM, Braude AI. Production of bladder stones by human t mycoplasmas. Nature. 1974;247(5435):67-9. doi: 10.1038/247067b0.
4. Robertson WG, Davison CS, Grunfeld JP, Kerr D, Ritz E, editors. Aetiological factors in stone formation. Oxford textbook of Clinical Nephrology. 1992;3:1846-82.
5. Wolf JS. Nephrolithiasis; 2004. Available from: https://www.emedicne.com/MED/topic1600htm. [Last accessed on 15 Nov 2005]
6. Brent J. Current management of ethylene glycol poisoning. Drugs. 2001;61(7):978-88.
7. Jarald EE, Kushwah P, Edwin S, Asghar S, Patni SA. Effect of unex on ethylene glycol-induced urolithiasis in rats. Indian J Pharmacol. 2011 July-Aug;43(4):466-8. doi: 10.4103/0253-7613.83124, PMID 21845008.
8. https://en.org/wiki/potato.wikipedia. [Last accessed on 15 May 2022]
9. Khandelwal KR. Practical pharmacognosy techniques and experiments. 12th ed. Nirali Prakashan; 2004. p. 149-56.
10. https://himalayawellness.in/products/cystone-tablets?variant=30300905865315. [Last accessed on 02 May 2022]
11. https://www.1mg.com/otc/himalaya-cystone-tablet-otc169306. [Last accessed on 02 May 2022]
12. Ramalingam SR, Sarkaraisamy P, Seeni P. *In vitro* effect of cystone against calcifying microorganisms isolated from human kidney stone. Int J Pharm Sci Res. doi: 10.13040/IJPSR.0975-8232.5(9).3952-56.
13. Phatak RS, Hendre AS. *In vitro* antiurolithiatic activity of Kalanchoe pinnata extract. Int J Pharmacogn Phytochem Res. 2015;7(2):275-9.
14. Gov India Minist Health Fam Welf Indian Pharmacopoeia. 8th ed. Jan 2018. The Indian Pharmacopeia Commission; 2018. p. 888.

15. Sasidharan H, Mallya SV, Suchitra P, Kumar KNS. *In vitro* evaluation of scoparia dulcis linn. for anti-urolithiatic activity. J Phytopharmacol. 2018;7(3):285-7. doi: 10.31254/phyto.2018.7309.

16. Government of Indian ministry of Indian Health and family welfare, Indian pharmacopoeia. Vol. II P to Z Controller Delhi publication; 1996.