

In vitro Urolithiasis Activity of *Tridax procumbens* Methanolic Extract on Calcium Oxalate Crystals Prepared by Precipitation Method

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ABSTRACT

Background: The main purpose of present to reduce the kidney stone with herbal drugs due increase in incidence rate of kidney stones significantly with change in life style from the last two decades. **Aim and Methodology:** The present study aimed to evaluate *in vitro* urolithiasis activity of *Tridax procumbens* on experimentally prepared calcium oxalate crystals. Calcium oxalate crystals were prepared by homogenous ppt method by using calcium chloride and sodium oxalate. The crude extract was prepared by the simple maceration with methanol 1:3 ratio and the solvent was evaporated by rotary evaporator and two doses of extract selected i.e 10mg and 20 mg and compared against standard cytosine all were assayed against calcium oxalate crystals which were incubated in semi-permeable membrane with sulphuric acid. **Results and Conclusion:** The results were 68.02% (10 mg), 72.41% (20 mg) and 90% and we conclude that the *Tridax procumbens* showed significant effect of urolithiasis.

Keywords: *Tridax procumbens*, Calcium oxalate crystals, PPT, Cytosine.

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INTRODUCTION

Stone formation in the human body is the oldest and most painful urologic illness, and it is brought on by food and lifestyle changes. The term "lithiasis," which refers to the formation of stones or calculi, affects about 12% of the world's population. Urolithiasis is the medical term for the development of calculi in the urinary bladder, ureter, or anywhere else in the urinary tract. Nephrolithiasis is the medical term for the development of stones in the kidney.^[1]

The word "urolithiasis" comes from the Greek words "Lithos" for stones and "Urone" for urine. The frequency and incidence of urolithiasis, one of the most serious urinary tract illnesses, are rising globally. About 12% of the world's population suffers from these urologic illnesses, and males are more likely than females to develop them. It frequently recur after one year, five years (25%–31.5%), ten years (49%–52%), and twenty years (72%). The process of kidney stone production involves several steps and is influenced by epidemiological, biochemical, and genetic

risk factors. Crystal nucleation, aggregation, and retention in the urinary tract are the first three phytochemical processes that contribute to kidney stone formation. Supersaturation will occur prior to the crystallisation process. The supersaturation process has an impact on elements including the PH of the urine, ionic strength, solute concentration, and factors that have to do with skin tone.^[2] In the course of their lifetime, one in ten people, according to the National Institute of Health of the United States, may acquire urinary stones. Nevertheless, according to Abeygunasekara 2011, there are no such data documenting Sri Lanka. A high fluoride intake, however, may encourage the development of kidney stones, according to certain research. Urolithiasis prevalence increases by 4.6 times when fluoride levels in drinking water are raised from 3.5 to 4.9 ppm. Fluoride levels more than 3 ppm are found in water wells in Sri Lanka's dry zone cities of Anuradhapura, Polonnaruwa, and Ampara.^[3]

Depending on the type, location, patient characteristics, and size of the urinary tract stones, different urolithiasis treatments are available. It is possible to cure minor calculi by drinking a lot of water throughout the day and using alpha-blockers to flush out the small stones (Medical Expulsion Therapy). Extracorporeal Shock Wave Lithotripsy (ESWL), which reduces large stones to minute bits, is a therapy option for large stones. This treatment



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can harm the urinary system and is quite pricey. They do not stop the growth of new stones either.^[4]

The use of medicinal herbs for the treatment of urolithiasis is not only easy, less likely to cause negative effects, but also affordable. The World Health Organization (WHO) estimates that 70% of global people use traditional medicines to treat a range of illnesses.^[5]

Numerous medicinal plants have been used for millennia in folk and ayurveda medicine as part of traditional health care systems.^[6]

According to reports, various medicinal plants are used in traditional and ayurvedic medicine to treat urolithiasis. In studies conducted by Niharika M., Himabindu J., and Ramanjaneyalu K., these plants significantly increased both *in vitro* and *in vivo* anti-urolithiatic activity.^[7] Therefore, this study was conducted based on the medicinal plants that were chosen for the evaluation of *in vitro* anti-urolithiatic that have been reported to have anti-urolithiatic property according to the ayurvedic medicinal system in Sri Lanka. In this study, calcium oxalate was prepared by mixing calcium chloride dihydrate and sodium oxalate in laboratory conditions by homogenous precipitation method. The study was conducted as an experimental study or as the initial phase in the drug development process, despite the fact that there were variations between naturally occurring kidney stones and experimentally manufactured stones.^[8] The trial can move forward utilising stones removed from people who were impacted by kidney stones if any meaningful findings are found.

MATERIALS AND METHODS

Our garden was used to grow *Tridax procumbens*, and the fresh leaves were collected, shade-dried, and ground before being macerated with methanol.

Reagents Used

Methanol, Sodium oxalate, Tris buffer, Calciumchloride, Potassiumpermanganate (KMNO₄) Conc. Sulphuric acid (H₂SO₄), Mayer's reagent, Wagner's reagent.

Extraction Process

Utilizing 400 mL of methanol and 400 g of powdered seeds in a soxhlet device at 64°C temperature, all the components were extracted into the solvent to produce the methanolic extract. At a temperature of 45°C, a rotary evaporator was used to evaporate and concentrate the extract. For additional examination, dried extract was kept in a light- and airtight container and kept in the refrigerator at 4°C.^[9]

Preparation of calcium oxalate crystals by homogeneous precipitation method

In separate beakers, calcium oxalate precipitate was created by stirring together calcium chloride dihydrate (4.41g) and sodium oxalate (4.02g) solutions that had been previously dissolved in distilled water and 2N sulphuric acid, respectively. By washing with ammonia solution and distilled water, respectively, excess sulfuric acid was eliminated. For four hours, it was dried at 60°C.^[10]

Preparation of semi-permeable membranes from farm eggs

A glass rod was used to pierce the egg's apex and remove the entire contents. Empty egg shells were properly cleaned with distilled water before being placed in a beaker with 2M HCl for an overnight process that completely decalcified the shells. Membranes were then thoroughly cleaned with distilled water before being submerged for a period in an ammonia solution to neutralise any remaining acid residues. After that, they were cleaned with distilled water and kept in a refrigerator with a pH of 7–7.4.^[11]

Evaluation of anti-urolithiatic activity by the titrimetric method

Nine semi-permeable membranes in total were made, and each membrane was filled with precisely 5 mg of calcium oxalate crystals, along with four different extract concentrations (10 mg, 20 mg, standard, and positive control).^[12] The membranes were then properly sutured. The negative control sample was made up of just one sample of calcium oxalate crystals. They were left to float in their respective conical flasks, each of which contained 100 mL of tris buffer solution (0.1M). All of the conical flasks underwent a 7 hr incubation period at 37°C.^[13] After that, 2 mL of 1N sulfuric acid was added to the contents of the semi-permeable membrane in the test tube. The resultant combination was titrated against the reference KMnO₄ solution until the light pink colour was seen. To obtain the precise results, the entire process was carried out three times. To assess the activity, the calcium



Egg decalcification.



Experimentation processes.

oxalate crystal dissolution percentages were computed for each sample.^[14]

Phytochemical analysis

In order to determine the constituent compounds mentioned by Sofowara and colleagues, chemical tests on the plant extract were performed using established methods. *Tridax procumbens* was prepared as ethanolic and aqueous extracts. On these plant extracts, a phytochemical analysis was then done.^[15]

RESULTS AND DISCUSSION

The plant extract was analysed for various tests like test for alkaloids, sponins, triterpenoids etc the presented phytochemicals were represented in Table 1.

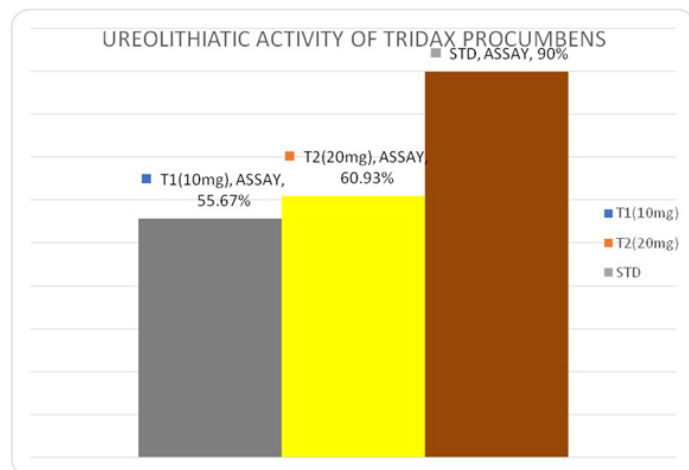


Table 1: Preliminary phytochemical screening.

Test name	<i>Tridax procumbens</i>
Carbohydrates	+
Glycosides	-
Saponins	+
flavonoids	+
Proteins	-
Tannins	+
Alkaloids	-
Oils	+
terpenoids	+
phlobatannins	-

From the above table (+) indicates presence; (-) indicates absence.

Table 2: Effect of *Tridax procumbens* on percentage dissolution of calcium oxalate crystals.

Sl. No.	Drug	Percentage (%)
1	Control	-
2	<i>Tridax procumbens</i> (T1)	55.67%
3	<i>Tridax procumbens</i> (T2)	60.93%
4	Standard	90%

The Dissolution percentage by the extract of *Tridax procumbens* at 10mg; 20mg; concentrations were 55.67%, 60.93% respectively.

Two different concentrations of plant extract and one std were performed dissolution on Calcium oxalate crystals and the results are represented in Table 2.

CONCLUSION

Urinary stone illness, which is a frequent and excruciating condition that affects people worldwide, is known to affect the human race. Ayurvedic and traditional medicine are the two that use *Tridax procumbens* herbs most frequently to treat urinary calculi. According to the material contained in the Sri Lankan ayurvedic medications, the *Tridax procumbens* plant was chosen. In comparison to the common polyherbal medication cystone, the methanolic extract of *Thiopyrum intermedium* possesses higher anti-urolithiatic activity. The primary evidence for the existence of anti-urolithiatic properties in the leaves of *Tridax procumbens* has been provided by this research work.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; **KMnO₄:** Potassium permanganate; **H₂SO₄:** Sulphuric acid.

SUMMARY

Tridax procumbens is commonly available herb it is showing the various activities like hepatoprotective anti-inflammatory etc. From the present study the plant consisting the phytochemicals like carbohydrates, saponins, flavonoids, tanins, oils and terpenoids and the plant showing urolithiatic activity significantly with standard drug.

REFERENCES

- Ram J, Moteriya P, Chanda S. An overview of some promising medicinal plants with *in vitro* anti-urolithiatic activity. *J Pharmacol*. 2015;5:23-8.
- Vijaya T, Kumar MS, Ramarao NV, Babu AN, Ramarao N. Urolithiasis and its causes—short review. *J Phytopharmacol*. 2013;2(1-3):1-6. doi: <https://doi.org/10.31254/phyto.2013.2130910.31254/phyto.2013.21309>.
- Pethiyagoda A, Pethiyagoda K. Descriptive evaluation of ureteric urolithiasis between genders. *Int J Sci Res Publications*. 2016;6:47-50.
- Atodariya U, Barad R, Upadhyay S, Upadhyay U. Anti-urolithiatic activity of *Dolichos biflorus* seeds. *J Pharmacogn Phytochem*. 2013;2(2):209-13.
- Niharika M, Himabindu J, Ramanjaneyalu K. Evaluation of *in vitro* anti-urolithiatic activity of *Tridax procumbens*. *Int J Sci Res*. 2018;7:93-8.
- Jha R, Ramani P, Patel D, Desai S, Meshram D. Phytochemical analysis and *in vitro* urolithiatic activity of leaves (DC) Baker. *J Med Plants Stud*. 2016;4(3):18-22.
- WWW [cited Aug 10 2021]. Available from: urologyspecialistsatlanta.com.
- Abeygunasekera AM. Urinary stone disease in Sri Lanka. *Ceylon Med J*. 2011;49(2):41-3. doi: <https://doi.org/10.4038/cmj.v49i2.325810.4038/cmj.v49i2.3258>.
- Perera ND, Perera JS. The role of extracorporeal shock wave lithotripsy in renal calculi. *Sri Lanka J Surg*. 2014;31(3):6-12. doi: [10.4038/sljs.v31i3.6423](https://doi.org/10.4038/sljs.v31i3.6423).
- Sharma D, Dey YN, Sikarwar I, Sijoria R, Wanjari MM, Jadhav AD. *In vitro* study of aqueous leaf extract of *Chenopodium album* for inhibition of calcium oxalate and brushite crystallization. *Egypt J Basic Appl Sci*. 2016;3(2):164-71.
- Akinyeye AJ, Solanke EO, Adebiji IO. Phytochemical and antimicrobial evaluation of leaf and seed of *Moringa oleifera* extracts. *Int J Res Health Sci*. 2014;4(6):2307-83.
- Bijauliya RK, Alok S, Jain SK, Singh V. K. and Singh, D. *Int J Pharm Sci Res*. 2017. Herbal and allopathic medicine for kidney, gallbladder, and urinary stones: a review;1935:8.
- Johri N, Cooper B, Robertson W, Choong S, Rickards D, Unwin R. An update and practical guide to renal stone management. *Nephron Clin Pract*. 2010;116(3):c159-71. doi: [10.1159/000317196](https://doi.org/10.1159/000317196), PMID 20606476.
- Kumar BN, Kumar GK, Srinivasa V, Syed B. A review on urolithiasis. *Int J Univers Pharm Life Sci*. 2012;2:269-80.
- Sujatha D, Singh K, Vohra M, Kumar KV, Sunitha S. Antilithiatic activity of phlorotannin rich extract of *Sorghassum wightii* on calcium oxalate urolithiasis *in vitro* and *in vivo* evaluation. *Int Braz J Urol*. 2015;41(3):511-20. doi: [10.1590/S1677-5538.IBJU.2014.0357](https://doi.org/10.1590/S1677-5538.IBJU.2014.0357), PMID 26200544.

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