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EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF *CASCABELA THEVETIA*

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ABSTRACT

This experiment was conducted to evaluate the anti-urolithiatic potential of *Cascabela thevetia* leaves on experimentally produced calcium oxalate crystals. Maceration process was used for the extraction process in which the resultant extract was then heated on water bath to make semisolid and then refrigerated in air tight container for further use. Methanolic fraction of the leaves of *C. thevetia* was found to have anti-urolithiatic property but is lower when compared with the standard (cystone). It has shown efficiency in the dissolution of calcium oxalate crystals. *In-vitro* studies have shown a positive response to the anti-urolithiatic activity and further *in-vivo* studies are warranted for further investigations.

Keywords: *Cascabela thevetia*, anti-urolithiatic activity, calcium oxalate crystals.

INTRODUCTION

Indian traditional system encloses a lot of variable and valuable medicinal plants but lacking with scientific data which needs to be studied, evaluated and developed based on their biological and chemical significance. Formation of stone due to dietary and life style is the oldest and painful disorder of urology, nephrolithiasis is referred to as a formation of kidney stones and urolithiasis is referred to formation of calculi in the bladder, ureter and urinarytract (Kirtikar and Basu, 2005; Pethiyagoda and Pethiyagoda, 2016).

Pathogenesis of Stone formation involves many physiochemical events like supersaturation, crystal nucleation, aggregation and retention within UT. Promoters include Low urine volume, low pH, Ca, Na, oxalate, and urate. (Basavaraj *et al.*, 2007) About 80% of the urinary calculi is calcium oxalate crystals alone or with calcium phosphate. Studies interpret that likely 12% of males and 55% females suffer from kidney stone once in their whole life span. It's incidence is about 1 in 1000 adults PA. General cause for this urolithiasis is not known but family history, obesity, increased weight and Body Mass Index might be listed (Melissa and Cadnapaphornchai, 2008).

To strive to stop recurrence of hyper calciuria and hyper oxaluria, numerous therapies involving thiazide diuretics and alkali-citrate are introduced but scientific affirmation for their efficacy is less assured (Bashir and Gilani, 2009). Stone removal by endoscopy and ESWL did not inhibit the new stone formation but have upraised the treatment of urolithiasis. These procedures are generally expensive and not affordable by poor patients and also reoccurrence is also

common. So, there is need to find out herbal drugs which can dissolve the stone and show satisfactory results at an affordable cost (Prasad *et al.*, 2007).

Many plants with Phyto-constituents such as flavonoids, saponins and triterpenoids are reported to have anti-urolithiatic activity (Nagal and Singla, 2013) *Cascabela thevetia* is a small ornamental shrub belonging to family Apocynaceae known commonly as Yellow oleander with good pharmacological importance. Due to phyto constituents it shows various activities anti-fungal, anti-bacterial, anti-oxidant, antihelminthic, anti-spermatogenic, anti-inflammatory and infertility. There is no previous evidence of anti-urolithiatic activity, So, we have made an attempt to find out the anti-urolithiatic activity if any. (Chunendrakumar *et al.*, 2017)

MATERIALS AND METHODS

Plant material collection

The leaves of *Cascabela thevetia* were gathered from neighborhoods Karimnagar-505001, Telangana, India. They were verified by Botanical Survey of India, Hyderabad. (BSI/DRC/2020-2021/Tech./Identification/50). The leaves of *Cascabela thevetia* were gathered, washed, dried at room temperature and grounded into powder. The powder was exposed to different examinations for which materials and techniques introduced beneath.

Extraction process

Maceration extraction was performed for the dried leaves of *C.thevetia*. Resultant extract was then heated on water bath to make semisolid and then refrigerated in air tight container for further use.

Preparation of calcium oxalate crystals by homogeneous precipitation method

Two separate beakers with solutions of Calcium chloride dihydrate (4.41g) in dist. water in one beaker and Sodium oxalate (4.02g) in 2N sulphuric acid in another beaker were mixed together with stirring until precipitate of Calcium oxalate(CaC_2O_4) is formed. Ammonia solution and dist. Water was used to wash excess amount of H_2SO_4 and dried at 60°C for 4 hours. (Jha *et al.*, 2016)

Preparation of semi-permeable membranes from farm eggs

A small hole on the tip of the eggs is made, emptied, washed with distilled water and decalcified overnight using 2M HCl. Membranes after washing with distilled water neutralized using ammonia solution in moistened condition. Later rinsing was done with distilled water and refrigerated at pH - 7-7.4 for further use (Jha *et al.*, 2016)

Evaluation of anti-urolithiatic activity by the titrimetric method

Calcium oxalate crystals 5mg, extracts with varying concentration (10-40mg) and standard were sutured in

semipermeable membranes and placed in separate conical flasks with 100ml of 0.1M of tris buffer solution and later incubated for 7hrs at 37°C . Contents were transferred from semipermeable membrane to test tube and 1N sulphuric acid was added and resultant mixture was titrated against standard KMnO_4 solution till light pink color was obtained. Whole procedure is done in triplicate to get exact results. Here calcium oxalate crystals sample is taken as control. The activity was evaluated by calculating individual dissolution percentages of calcium oxalate crystals (Jha *et al.*, 2016).

Phytochemical analysis:

Referring sofowora and co-workers, chemical tests were performed and phytochemicals were analyzed (Sofowora, 1993).

RESULTS

Following phyto constituents were found after preliminary screening Preliminary Phytochemical screening of *Cascabela thevetia*, revealed the presence of following phyto constituents.

Table 1: Results of preliminary Phyto-chemical screening of *Cascabela thevetia*

S. No.	Phyto constituents	Observation	
		Methanol	Aqueous
A	Alkaloids	+++	---
B	Carbohydrates	---	---
C	Proteins & amino acids	---	---
D	Steroids	---	+++
E	Phenols	+++	---
F	Tannins	+++	---
G	Flavonoids	+++	+++
H	Glycosides	+++	+++
I	Saponins	+++	+++

Anti-urolithiatic activity of leaves of *Cascabela thevetia*:

The dissolution percentage by the extract of *Cascabela thevetia* at 10 mg, 20 mg, 30 mg and 40 mg concentrations were 59.8 (± 0.322) %, 67.2 (± 0.222) %, 71.8 (± 0.213) % and 79.3 (± 0.229) % respectively. Dissolution percentage by the standard drug cystone at 10 mg, 20 mg, 30 mg and 40 mg were found to be as 62.5 (± 0.522) %, 71.1 (± 0.084) %, 80.9 (± 0.220) % and 85.9 (± 0.583) % respectively. Dissolution percentage for the control test was 20.1 (± 0.144) % Phytochemical analysis of the Methanolic and aqueous extracts of the *Cascabela thevetia* shows the positive results for the presence of alkaloids, terpenoids. The research findings revealed that even though cystone polyherbal drug has high dissolution ability, methanolic extracts of *Cascabela thevetia* leaves also have considerable anti- urolithiatic activity.

Table 2: Dissolution percentage of the extract of *Cascabela thevetia*

Concentration	Methanolic Extract	Standard
10mg	59.8(± 0.322)%	62.5(± 0.522)%
20mg	67.2(± 0.222)%	71.1(± 0.084)%
30mg	71.8(± 0.213)%	80.9(± 0.220)%
40mg	79.3(± 0.229)%	85.9(± 0.583)%

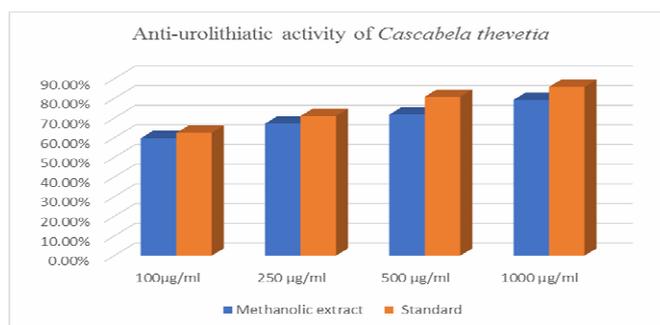


Fig. 1 : Anti-urolithiatic potential of methanolic extract of leaves of *Cascabela thevetia*

CONCLUSION

An attempt was made to evaluate the anti-urolithiatic potential of *Cascabela thevetia* leaves on experimentally produced calcium oxalate crystals. Methanolic fraction of the leaves of *C. thevetia* was found to have anti-urolithiatic property but is lower when compared with the standard (cystone). It has shown efficiency in the dissolution of calcium oxalate crystals. Further *in-vivo* studies are warranted for further investigations.

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