

# In-Vitro anti-Urolithiatic Activity of Theriophonum Minutum and Remusatia Vivipara



S.Anbuselvi, Roshini Esther. A, Punithavathy . R

**Abstract:** *Kidney stone is a very common disease worldwide threat. The calcium oxalate is the important compound of calculi formation in the urinary tract. Traditional studies on use of herbal based medicinal plants for treating severe disease is satisfying its bad to advanced world . In this regard, the study was analyzed with an objective to carry out the role of Theriophonum minutum and Remusatia vivipara leaves extract in different solvents to reduce stone formation and to find out the concentration of medicinal plant extract exhibit dissolution rate of kidney stones In dissolution studies, the extract of Remusatia vivipara has greater ability to dissolve artificial calcium stones and kidney stones rather than the standard drug. Calcium oxalate crystal formation was stimulated by the sodium oxalate solutions. The effect of different extract concentration was applied to measure the dissolution rate, turbidity, nucleation aggregation assay . The extract of Theriophonum minutum and Remusatia vivipara reflected the blocking action in both of nucleation and aggregation to better level*

**Keywords :** Aggregation, Anti-urolithiatic activity, Dissolution, Remusatia vivipara, Theriophonum minutum,

## I. INTRODUCTION

Many people continues to use plant based traditional herbs in normal health problems. In this century, most of the findings have been focused on scientific sources of ancient herbal drugs of plant as asource<sup>1</sup>. This lead to the design of drug or latest use of plant based products for treatments<sup>2</sup>. This herbal derived drugs is mainly called as green medicine which is good and more affordable than the chemical dangerous medicine with side effects. Urolithiasis is indicated by the kidney stone or stone in urinary tracts<sup>3</sup>

The genus Theriophonum (Araceae), represented by seasonally tuberous perennials is well grown in India and Sri Lanka. Theriophonum minutum is a wild edible plant show naturally variability and contains many higher nutritive values compare to conventional foods resources. Many research papers reveals that Theriophonum minutum has xplored in terms of its phytochemical profile and its pharmacological

activity, although it has been reported for its excellent nutritive values. It explores many medicinal properties of Theriophonum minutum to base on its phytochemical profile<sup>4</sup>. Remusatia vivipara is a epiphytic herb, an underground tuber around 2–4 cm in diameter and colored vivid red. The plant also shows the medicinal bioactive compounds and helps to know inhibitor of stone formation and its dissolution activity of P. niruri extracts<sup>5</sup>

Kidney stones are mainly calcium present in form of calcium oxalate (monohydrate or dehydrate) form. The most of stones (75-90%) cases are belonged to calcium oxalate type<sup>6,7</sup>. The formation of oxalate stone is a several stages - nucleation, growth of crystals, crystal gathering and crystal retention<sup>8,9,10</sup>. This formation is only possible in urine of supersaturated state. This mainly depends on pH, ionic state, solute availability and complexity with salts in urine<sup>11</sup>.

Urolithiasis, a condition of stone in the forms of calculi in within the urinary tract, is common and well known diseases<sup>12</sup>. This is a dangerous, debilitating problem, men are three times more easily affected than women in the 20-40ages. The reason is due to many factors and is highly related to life pattern, food habits<sup>13</sup>

The dissolution of stone based on the size and its occurrence. The size of calculi is greater than 5mm difficult to pass through tract. It can be diagnosis by ESWL, URS or percutaneous nephrolithotomy<sup>14</sup>

The patho physiology and diagnosis of calculi, there is no specific medicine used. The distraction of calculi by endoscopy and extracorporeal shock wave lithotripsy which are expensive<sup>15</sup> Treatments for urolithiasis is based on the type of stone. Urine sample can be strained and stones collected for evaluation. Water drinking of about six to eight glasses a day raises a urine flow. The dehydrated peoples have severe nausea and vomiting may need electrolytes. Proper hydration is an preventive measure. It can be substituted by drinking ginger ale, lemon-lime soda, and fruit juice to raise fluid level<sup>16</sup>. The main target of this study helps to analyze the anti-urolithic activity of Theriophonum minutum and Remusatia vivipara plant extracts in different solvents.

## II. MATERIALS AND METHODS

### COLLECTION OF PLANTS

Plants Theriophonum minutum and Remusatia vivipara of Kerala origin were screened and authenticated by Prof. P Jayaraman, IHSBARC, Chennai. Kidney stones was collected from Tamilnadu Government Hospital, Thoothukudi. Tamil Nadu

Manuscript published on 30 September 2019

\* Correspondence Author

Anbuselvi, S\* Department of Industrial Biotechnology, BIHER, Chennai-73. drsanbuselvi@gmail.com

Roshini Esther. Department of Industrial Biotechnology, BIHER, Chennai-73. Punithavathy . R d Author Name, Department of Industrial Biotechnology, BIHER, Chennai-73.

© The Authors. Published by Blue Eyes Intelligence Engineering and Sciences Publication (BEIESP). This is an open access article under the CC-BY-NC-ND license <http://creativecommons.org/licenses/by-nc-nd/4.0/>

## EXTRACTION

Fresh leaves of *Theriophonum minutum* and *Remusatia vivipara* were cutted into small pieces taken in a conical flask. The different solvents such as ethanol, methanol, chloroform, petroleum ether and distilled water were added to the conical flask and heated to optimize the extraction. It was cooled and filtered through Whatmann filter paper and the solvent extracts were stored.

## PREPARATION OF EXPERIMENTAL KIDNEY STONE

1.47 g of  $\text{CaCl}_2$  in 100ml distilled water and 1.34gm of sodium oxalate in 100ml of 2N sulphuric acid.

All groups were sealed in membrane, thread tied at one side and were dipped in a 0.1 M Tris buffer. A supporting stick was placed at other side and covered with aluminum foil. All samples were treated to oxalate protocol and analyzed at 620nm.

## FORMATIONS OF CALCIUM PHOSPHATE BY HOMOGENOUS PRECIPITATION

Calcium chloride dehydrate (1.47 g%), disodium hydrogen phosphate (1.42%) in 2N  $\text{H}_2\text{SO}_4$ . Both were dissolved to coagulate out calcium phosphate. The calcium phosphate was eluted out. The concentration of calcium phosphate was taken at 630 nm. The nucleation assay and aggregation assay were also analysed.

## INVITRO-ANTI LITHIATIC TEST BY TURBIDITY METHOD

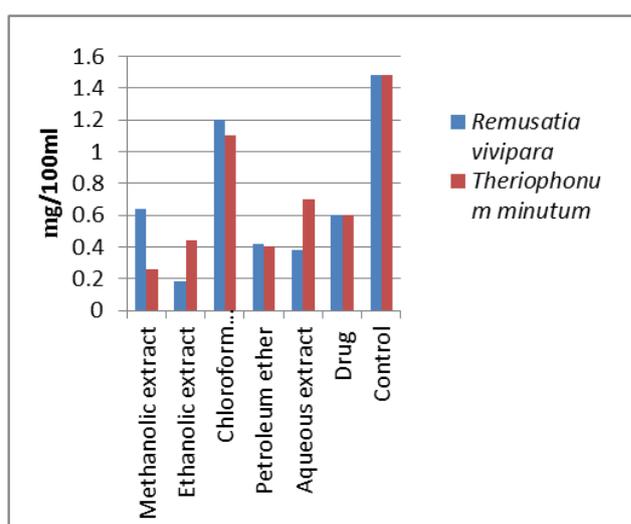
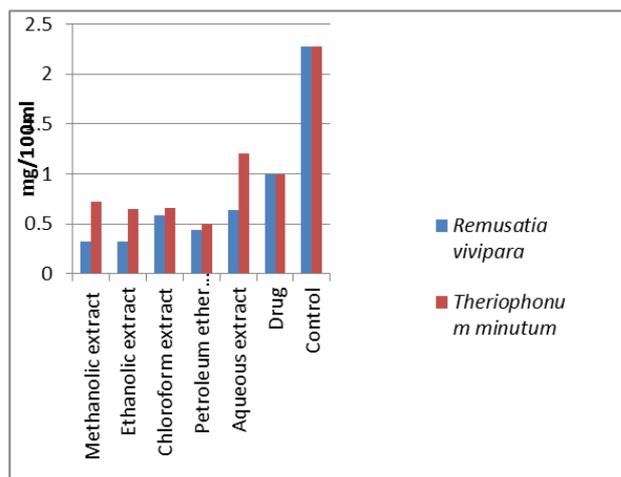
All the samples were tested for inhibition activity of calcium oxalate with and without inhibitors (Drugs and extract). The Coagulation of calcium oxalate was at 37°C and pH 6.8 has been in terms of turbidity at 620nm.

## III. RESULTS AND DISCUSSION

The experimental kidney stones were prepared artificially. In vitro kidney stones contained calcium oxalate and calcium phosphate.

### a. Analysis of calcium oxalate by using dissolution method

The calcium concentration was considered as control and its concentration 1.48mg/100ml. The standard drug Cystone showed 0.60mg/100ml after treatment with oxalate crystals. *Remusatia vivipara* and *Theriophonum minutum* were subjected to different solvent extracts. These extracts were used to reduce oxalate crystals. Ethanolic extract of *Remusatia vivipara* showed maximum dissolution rate of 87.09% and its remaining calcium oxalate concentration found to 0.18 mg/100ml (Fig 1). The petroleum ether extract of *Remusatia vivipara* reflected gradual dissolution rate and its calcium oxalate concentration were found to be 0.42 mg/100 ml. The maximum calcium oxalate reduction was observed in methanolic extract of *Theriophonum minutum* when compared with *Remusatia vivipara* and its dissolution rate of 75.80%. The less amount of calcium oxalate reduction was observed in methanolic extract of *Remusatia vivipara* 0.64mg/100ml and its slow reduction rate of 47.58%. The standard drug show 50% of dissolution rate but methanolic *Theriophonum minutum* exhibit 75.8% of dissolution rate. The leaves of *Remusatia vivipara* and *Theriophonum minutum* were found in dissolution rate of 69.35% and 48.38% respectively.



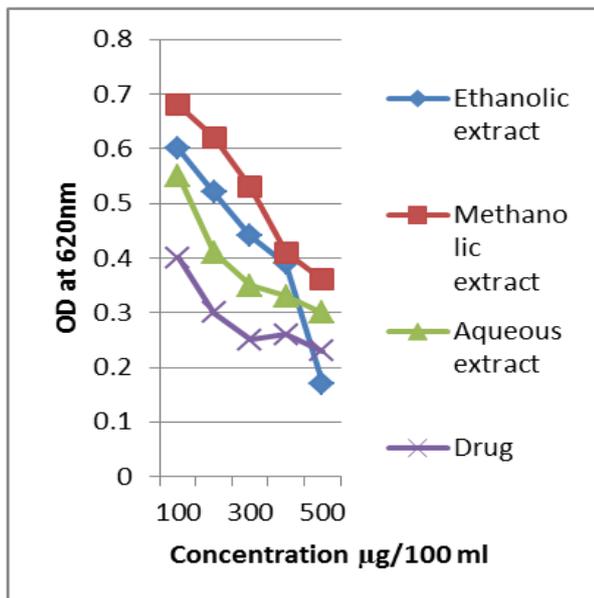
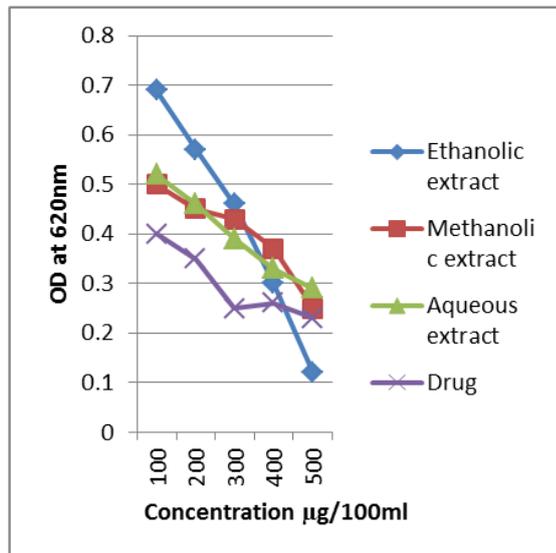
**Fig 1(a,b): Effect of leaf extracts of *R.vivipara* and *T.minutum* on Calcium oxalate crystals and Calcium phosphate using dissolution method.**

### b. Analysis of calcium phosphate by using dissolution method

The calcium phosphate crystal dissolution was found to be higher in methanolic and ethanolic extraction of *Remusatia vivipara* and petroleum extract of *Theriophonum minutum*. The drug exhibited 57.53% of dissolution rate. The leaves of *Remusatia vivipara* and *Theriophonum minutum* exhibited 72.60% and 52.73% of dissolution rate of calcium phosphate. The minimum calcium phosphate dissolution was observed in *Theriophonum minutum* methanolic extract.

### c. Nucleation assay

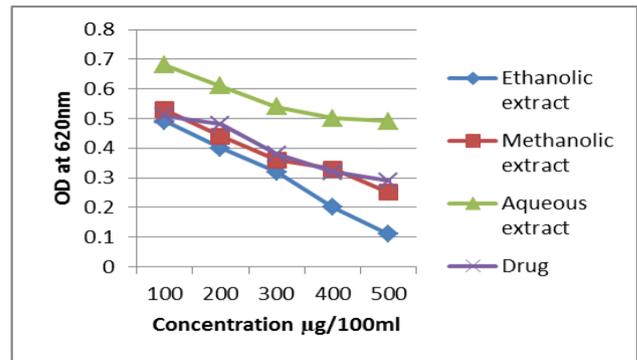
Different solvent extract of *Remusatia vivipara* and *Theriophonum minutum* showed Nucleation activity at regular intervals of time. The rapid inhibition was observed in ethanolic extracts of *Remusatia vivipara* 0.12mg in 500µg/100ml and *Theriophonum minutum* to be 0.17mg. The leaf extract of *Remusatia vivipara* and *Theriophonum minutum* showed good nucleation activity than standard drug even in crude form. The minimum nucleation activity was observed in aqueous extract (Fig 2).



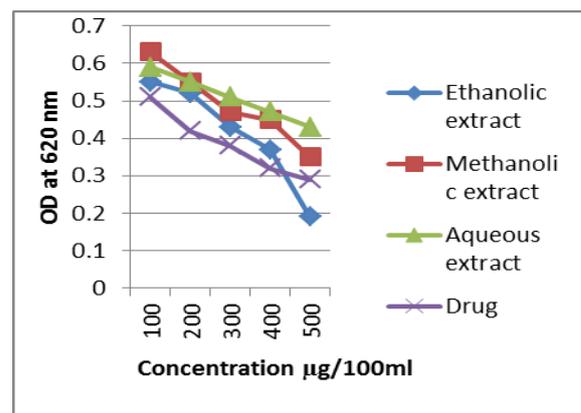
**Fig 2(a,b): Effect of different concentrations of leaf extracts of R.vivipara and T.minutum on Calcium oxalate crystallization. (Nucleation assay).**

**d. Aggregation assay**

Different solvent extract of Remusatia vivipara and Theriophonum minutum were subjected to Aggregation Assay. The Ethanolic extract of Remusatia vivipara showed maximum gradual degradation from 0.49 to 0.11 according to concentration of 100µg to 500µg. The aqueous extract showed mild degradation (Fig 3). The drug exhibited 0.29mg in 500µg. The ethanolic extract of Remusatia vivipara and Theriophonum minutum found to be more active in calcium oxalate dissolution when compared with standard drug. The ethanolic extract of Theriophonum minutum also showed less calcium oxalate dissolution

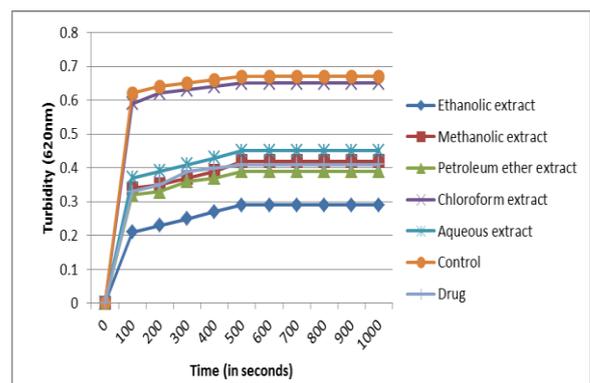


when compared with Remusatia vivipara.



**Fig 3(a,b): Effect of different concentrations of leaf extracts of R.vivipara and T.minutum on CaOx crystallization.**

The turbidity was developed in all treatments. The ethanolic extract of Remusatia vivipara exhibit good turbid form at 400 seconds and attain constant rate of formation after 500 seconds. The methanolic extract showed better turbidity activity than other extract. The aqueous extract of Remusatia vivipara and Theriophonum minutum were found to be trace amount of turbid formation(Fig 4)..



**Fig4 : Change in turbidity without and with R.vivipara extracts at 620 nm.**

**e.. Dissolution of kidney stones**

The calcium oxalate crystals dissolution procedures also subjected to kidney stones. The ethanolic extract of Remusatia vivipara and Theriophonum minutum showed rapid dissolution of calcium oxalate present in kidney stones when compared with artificial calcium oxalate crystals.

## In-Vitro anti-Urolithiatic Activity of *Theriophonum Minutum* and *Remusatia Vivipara*

The ethanolic extract of *Remusatia vivipara* showed better anti-urolithiatic activity than standard drug even in crude form against kidney stones (Table 1).

**Table 1: Dissolution of calcium oxalate by different solvent extracts of *R.vivipara* and *T.minutum***

S.N O	SAMPLE	Remusatia vivipara		Theriophonum minutum	
		Concentration (mg/ml)	% dissolution	Concentration (mg/ml)	% dissolution
1.	Ethanolic extract	0.02	98.38	0.05	95.96
2.	Methanolic extract	0.19	84.67	0.24	80.64
3.	Drug	0.16	87.09	0.16	87.09
4.	Control	1.24	-	1.24	-

### IV. CONCLUSION

Kidney stone accumulation is a multi-step process and calculi within renal tubules. The stages of supersaturation or crystallization steps can be arrested and lithiasis not occur. This result gives due to the herb extracts which inhibit crystallization and stop the stone formation. The results of this work were that from *Remusatia vivipara* and *Theriophonum minutum* plants stopped the crystal formation of CaOx in solution. The plant extract gave the limited crystals followed by little supersaturation and minimize the nature of the particles. The Ethanolic and Methanolic extract on both *Remusatia vivipara* and *Theriophonum minutum* have greater blocking effect on Calculi formation and be best in the cure of urolithiasis. This plant acts as an anti-urolithiatic agent in-vitro cases. This salient feature of the extract is to prevent urinary calculi formation by dissolving the elution of small particles from the kidney and relaxing the chance of clearance in urinary tract.

### REFERENCES

- Bashir, S., A.H. Gilani, A.A. Siddiqui, S. Pervez, S.R. Khan, N.J. Sarfaraz and A.J. Shah., "Berberis vulgaris root bark extract prevents hyperoxaluria induced urolithiasis in rats". *Phytother.* 2010, Res., 24:pp 1250-1255.
- Daniel D, Salviya U, "A historical overview of natural products in drug discovery", *Jour. Metabolite* 2002,2(2):pp 302-336.
- Jain, S.K., "Notable foreign medicinal uses for some plants of Indian tradition". *Indian J. Tradit. Knowledge* 2006, 2pp 321-332.
- N.P. Yadav, V.K. Dixit, "Recent approaches in herbal drug standardization", *Int. J. Integ. Biol.* 2008,2,pp195-203.
- Ashwini Khubalkar, Amol Warokar, Radheshyam Lohiya, Milind Umekar "Phytochemical Studies on *Theriophonum minutum* Extracts and Evaluation of Its Anticancer Activity" *Journal of Natural Products and Resources*, 2018, 4(2):pp:185-187.

- Masao T, Osamu M, Kazuhiro Y, Ken-Ichi K, Shiro T, Akihiko O "Fibronectin as a potent inhibitor of calcium oxalate urolithiasis". *J Urol* ,2000,164pp:1718-1723.
- JBensatal A, Ouahrani MR "Inhibition of crystallization of calcium oxalate by the extraction of *Tamarix gallica* L". *Urol Res*,2008;36pp:283-287.
- Worcester EM, Gillen DL, Evan AP, Parks JH, Wright K, Trumbore L, et al. "Evidence that postprandial reduction of renal calcium reabsorption mediates hypercalciuria of patients with calcium nephrolithiasis." *Am J Physiol Renal Physiol.* 2007;292(1):pp66-75. [[PubMed](#)]
- Wesson JA, Worcester EM, Wiessner JH, Mandel NS, Kleinman JG "Control of calcium oxalate structure and cell adherence by urinary macromolecules". *Kidney Int* 1998,53:952-957.
- Kanu Priya Aggarwal, Shifa Narula, Monica Kakkar and Chandrdeep Tandon "Nephrolithiasis: Molecular Mechanism of Renal Stone Formation and the Critical Role Played by Modulators" *BioMed Research International*, Volume 2013, Article ID 292953, <http://dx.doi.org/10.1155/2013/292953>
- Selvam R, Kalaiselvi P, Govindaraj A, Murugan VM and Satishkumar AS "Effect of *A. lanata* leaf extract and VEDIUPPU chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria". *Pharmacol Res*, 2001 43: pp89-93.
- Basavaraj DR, Biyani CS, Browning AJ, Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. *EAUEBU Update Ser.* 2007;5:pp12-36
- Taylor EN, Stampfer MJ, Curhan GC. "Obesity, weight gain, and the risk of kidney stones". *JAMA.* 2005;293:pp455-462
- Coll DM, Varanelli MJ, Smith RC. "Relationship of spontaneous passage of ureteral calculi to stone size and location as revealed by unenhanced helical CT". *Am J Roentgenol.* 2002;178:pp101-103
- Prasad KVSRG, Sujatha D, Bharti K, "Herbal drugs in urolithiasis: a review". *Pharmacog Rev*; 2007,1(1)pp175-178.
- Wadood A, Ghufraan A, Jamal S.B, Naeem M, Khan A, et al. "Phytochemical analysis of medicinal plants occurring in local area of Mardan", *Anal. Biochem.* ,2013,2(4): pp144-146.

### AUTHORS PROFILE



**Dr. S. Anbuselvi** a medical biochemist, She is working as professor in BIHER, Chennai. She is having 19 years of teaching experience. She has published more than 50 papers in referred and science indexed journals.



**Roshini Esther** a Industrial Biotechnology student studied at BIHER Chennai She has interested in biopharmaceutical technology.



**Punithavathy** a Industrial Biotechnology student studied at BIHER Chennai She has interested in biopharmaceutical technology.