World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

EVALUATION OF IN VITRO ANTI-UROLITHIATIC POTENTIAL OF *MERREMIA TRIDENTATA* (L.) HALLIER F. BY CALCIUM PHOSPHATE DISSOLUTION ASSAY

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Article Received on 23/11/2020 **Article Revised on 13/12/2020** **Article Accepted on 03/01/2021**

ABSTRACT

Urolithiasis is as old as the human civilization. Ancient scientist identified the disease Urolithiasis and named it as 'Ashmari' which means a structure showing similarity to stone. Urolithiasis is commonly known as calculi formation. The present study was undertaken to evaluate the in vitro anti-urolithiatic activity of *Merremia tridentata* by calcium phosphate dissolution assay. The distilled water extract, ethanol extract, ethyl acetate extract, chloroform extract, lime juice extract and tender coconut water extract were taken for the evaluation. All the six extracts shown their effectiveness in the dissolution of calcium phosphate crystals. The tender coconut water extract was observed more effective than other extracts with a high rate of calcium phosphate dissolution. The study revealed that *Merremia tridentata* possess potent anti-urolthiatic activity.

KEYWORDS: *Merremia tridentata*, Urolithiasis, calcium phosphate, anti-urolithiatic activity.

INTRODUCTION

The term Urolithiasis is derived from the Greek word Ouron and Lithos which means urine and stone respectively. Lithiasis is a common disease characterized by the calculi formation. It is mainly of two types- Urolithiasis and Nephrolithiasis. Urolithiasis is the formation of stone in urinary tract, urinary bladder or ureter while Nephrolithiasis is the occurrence of stone within the kidney. About 12 % of the global population is suffering from this disease. Epidemiological studies revealed that Urolithiasis is more common in men than in women. This is because testosterone has an enhancing capacity and oestrogen has an inhibiting capacity on stone formation. The disease is more prevalent between the age of 20-40 in both sexes.

The kidney stone formation is a complex process which includes physiochemical events such as supersaturation, nucleation, growth, aggregation and retention with in the kidney. Based on the mineral composition, stones are broadly divided in to four groups- Calcium stones, Uric acid stones or urate stones, Struvite stones or magnesium ammonium phosphate stones and Cystine stones. Calcium stones alone contributes to 90 % of the total renal calculi. Calcium stones are again divided in to calcium oxalate and calcium phosphate stones, of which calcium oxalale contributes to 75 % and calcium phosphate contributes to 15 % of the stone formation.

Eventhough there exist advancement in the field of medicine, no satisfactory drug has been yet developed for the complete cure of Urolithiasis. The overuse of synthetic drugs, which results in higher incidence of adverse drug reactions and the painfull surgical treatment methods has motivated human to return to nature for safe remedies.

Merremia tridentata (L.) Hallier f. is a perennial herb belonging to the family Convolvulaceae and is distributed in the tropical parts of the world. It is commonly known as 'Prasarani', 'Thiruppan pallu', 'Thrippan pallu', 'Savulikodi', 'Mudiyarkunthal'. The plant is reported to possess anti-diabetic, anti-inflammatory, anti-arthritic and anti-microbial activities.

MATERIALS AND METHODS

Plant Material

The whole plant of *Merremia tridentata* (L.) Hallier f. was collected in the month of May 2019 from Kulanada village, Pathanamthitta district of Kerala, India. The plant was authenticated by experts.

Invitro Anti-Urolithiatic Assay

Preparation of Plant Extract

The fresh and sterilized whole plant is grinded properly. After grinding, weigh the content obtained and immerse

it in solvents such as distilled water, ethanol, chloroform and ethyl acetate in the ratio 1 (content): 3 (solvent), taken in seperate screw cap bottles and kept them undisturbed for about four weeks. After the time period, the content is filtered out to obtain the crude extract. The extracts obtained using the two natural solvents lime juice and tender coconut water are prepared freshly by grinding the plant along with the solvents in the ratio 1 (plant material): 3 (solvent). After grinding, filter it through a double folded cheese cloth to obtain the natural extract.

Preparation of Semi Permeable Membrane

The apex of the egg was punctured to remove the entire content from it. The empty eggs are washed with distilled water and placed in a beaker containing 8 ml con. HCl in 400 ml distilled water. This was kept for an overnight. It results in the complete decalcification of the semipermeable egg membrane. On the next day, the semipermeable membranes were removed from the egg shells carefully. The obtained semipermeable membranes are washed properly with distilled water and are placed in the ammonia solution. Later, rinse again with the distilled water and keep in the refrigerator at a p^H 7-7.4 in the moistened condition until the time of use.

Preparation of Positive Control

0.5 g of Cystone® tablet was placed in absolute ethanol for about an hour for removing the colour coating. The tablet was then crushed in to a powder form and dispersed in 100 ml distilled water and filtered out. The filtrate obtained was used as the positive control for the in vitro anti-urolithiatic assay.

Chemicals

Disodium hydrogen phosphate, Calcium chloride dihydrate, Sodium molybdate solution, Ammonium solution, Sodium meta bisulfite solution, Phenylene diamine, 0.1 M Tris buffer, 1 N H_2SO_4, 2 N H_2SO_4.

Synthesis of Calcium Phosphate By Homogenous Precipitation

Dissolve 1.47 g of calcium chloride dehydrate in 100 ml distilled water and dissolve 1.42 g of disodium hydrogen phosphate in 100 ml of 2 N H_2SO_4 seperately. Both the solutions are then mixed together in a beaker and stir the solution constantly. It results in the formation of calcium phosphate as precipitate. The precipitate was then washed with ammonium solution followed by distilled water to remove the traces of sulphuric acid. Later it was dried up at a temperature of 60^0C for about 2 hours.

Preparation of Reducing Solution

Dissolve 1 g of p-phenylene diamine in 100 ml of 3 % w/v of sodium meta-bisulfite solution.

Preparation of Molybdate-Sulphuric Acid Reagent

Add 5 % w/v of sodium molybdate solution and 13 ml con. H_2SO_4 in 80 ml distilled water and adjust the volume to 100 ml using distilled water.

Estimation of Calcium Phosphate Dissolution

Blank: 1 mg calcium phosphate + 1 ml distilled water
Control: 1 mg calcium phosphate + 1 ml standard drug (Cystone®)
Group 1: 1 mg calcium phosphate + 1 ml extract in distilled water
Group 2: 1 mg calcium phosphate + 1 ml extract in ethanol
Group 3: 1 mg calcium phosphate + 1 ml extract in chloroform
Group 4: 1 mg calcium phosphate + 1 ml extract in ethyl acetate
Group 5: 1 mg calcium phosphate + 1 ml extract in lime juice
Group 6: 1 mg calcium phosphate + 1ml extract in tender coconut water

Each of the six groups, blank and control were packed separately in eight different semipermeable membrane. The open end of the membrane was tied carefully with a thread and were suspended in conical flasks, each containing 100ml 0.1M Tris buffer. The end of the thread was tied on a stick placed on the mouth of the conical flask and was covered with aluminium foil. The whole set up was then kept in an incubator, preheated to 37^0C for 4 hour and kept for 3 days.

After 3 days of incubation, the entire content in each membrane was removed by gently piercing the semipermeable membrane and was transferred in to eight individual test tubes carefully.

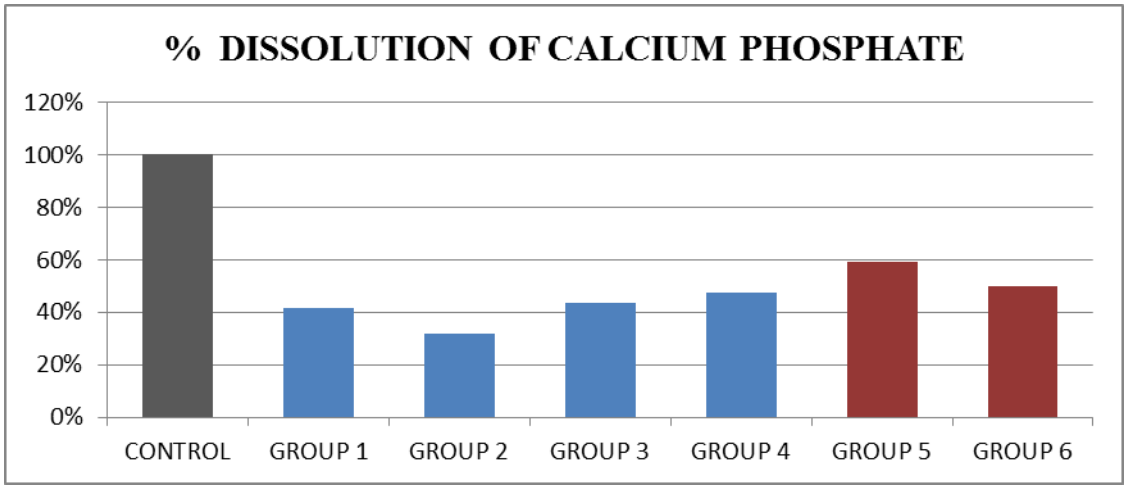
To each of the test tube, 4 ml of 1 N H_2SO_4, 3 ml Molybdate sulphuric acid reagent, 1 ml reducing solution were added and kept aside for 2 hour. The colour change can be observed from dark pink to colourless. The change in the intensity of the colour can be measured spectrophotometrically at 620 nm.

$$\% \text{ dissolution} = \frac{\text{Absorbance of control - Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

RESULS

Table 1: Spectrophotometric Measurement and Percentage Dissolution Of Calcium Phosphate.

GROUPS	SPECTROPHOTOMETRIC VALUE	% DISSOLUTION
Control	0.135	100
Group 1	0.079	41.48
Group 2	0.092	31.85
Group 3	0.076	43.70
Group 4	0.071	47.40
Group 5	0.055	59.25
Group 6	0.028	79.25



Graph 1.

The natural extracts has shown a high percentage of calcium phosphate dissolution when compared to other solvents. The dissolution by tender coconut water extract (79.25 %) is followed by lime juice extract (59.25 %), ethyl acetate extract (47.40 %), chloroform extract (43.40 %), distilled water extract (41.48 %) and ethanol extract (31.88 %).

DISCUSSION

Phytotherapy is an ancient treatment method, proved to be vital in the treatment of Urolithaisis with least side effects. Herbal drugs used in phytotherapy functions by increasing the urine volume, $_{P}^{H}$ and thereby allow the small calculi to pass through the urine. It also have the efficacy to improve the renal functions and helps to reduce the reoccurrence of renal calculi. The phytochemicals present the plants helps to dissolve the formed stones. Thus the phytochemicals present in the plants are considered as the responsible factor for the anti urolithiatic activity of the plant, which can be confirmed by the in vivo analysis.

Photo 1: Extracts prepared in different solvents.

Photo 2: Decalcification of egg membrane.

Photo 3: Semipermeable membrane.

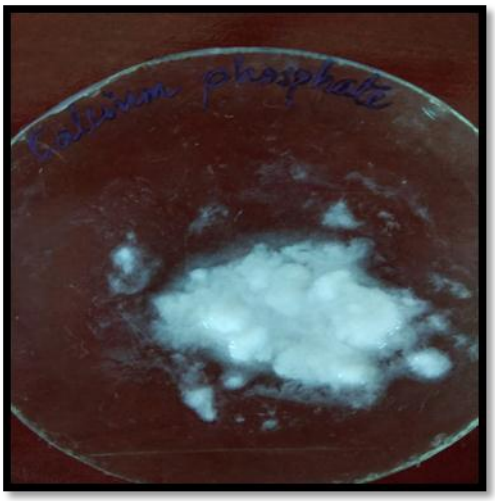


Photo 4: Calcium phosphate.

Photo 5: Control Cystone®.

Photo 6: Set up for invitro anti-urolithiatic assay and incubated condition.

CONCLUSION

From the present study it has been concluded that the extracts of *Merremia tridentata* produced significant in vitro anti-urolithiatic activity in the dissolution of calcium phosphate when performed in presence of standard drug, Cystone®. The tender coconut water extract and lime juice extract (natural solvent extracts) possess a greater efficiency in disintegrating the calcium phosphate crystals. This focuses in to the field of phytotherapy which uses herbal drugs for safer treatment of urolithiasis. This study has given the primary evidence for *Merremia tridentata*, the plant which possess anti-urolithiatic property. However, to develop a potent anti-

urolithiatic agent from this plant, the in vitro results should be confirmed by the in vivo analysis.

ACKNOWLEDGEMENT

We are indepted to the Department of Biotechnology of Bishop Moore College, especially to the Head of Department to provide proper facilities and suggestions in the successful completion of the work.

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