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

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ABSTRACT

Urolithiasis commonly known as stone formation is one of the most painful urological disorder. The present study was undertaken to evaluate the in vitro anti-urolithiatic activity of *Merremia tridentata* by calcium oxalate 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 oxalate crystals. The ethyl acetate extract was more effective when compared to other extracts with a high rate of dissolution. The study revealed that *Merremia tridentata* possess potent anti-urolthiatic activity.

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

INTRODUCTION

Lithiasis is a common disease characterized by the formation of calculi. 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. The reoccurrence rate is high in male when compared to female. This is because testosterone has an enhancing capacity and oestrogen has an inhibiting capacity on stone formation.

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. The primary constituent of stone is the calcium oxalate.

Eventhough there exist advancement in the field of medicine, no satisfactory drug has been yet developed for the complete cure of Urolithiasis. Drug treatment, Extracorporeal shock wave lithotropsy, Percutaneous lithotomy, Laparoscopic surgery, Open surgery, etc. are the common treatments. Due to various side effects in these treatments such as drug addiction, excessive pain, retention of stone fragments, nausea, hemmorhage, etc. majority of population utilizes medicinal plants for their health care. Phytotherapy uses herbal drugs that are reported to be effective with least side effects. Owing to its cost effectiveness and better tolerability, phytotherapy proves to be a very beneficial and widely accepted modality for the prevention and treatment of renal calculi.

Merremia tridentata (L.) Hallier f. is a perennial weak stemed 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 already 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 (Calcium oxalate dissolution 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 such as 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

Sodium oxalate, Calcium chloride dehydrate, 0.1 M Tris buffer, con. HCl, 1 N H_2SO_4, Pottassium permanganate, Ammonium solution.

Synthesis Of Calcium Oxalate By Homogenous Precipitation

In a beaker containing 100 ml distilled water, dissolve 1.47 g of calcium chloride dehydrate and in another beaker containing 100 ml 2 N H_2SO_4, dissolve 1.34 g sodium oxalate.

Both the contents were mixed together in a beaker and stir constantly. The constant stirring precipitates out calcium oxalate. The formed calcium oxalate was washed with ammonium solution to remove the traces of H_2SO_4 and then wash with distilled water. Then it was allowed to dry at a temperature of 60^0C in an oven for ca 5 hour.

Preparation of 0.02 M $Kmno_4$ Solution

In a beaker containing 100 ml of distilled water, dissolve 0.32 g $KMnO_4$ and boil it for 30 minute. After cooling, the excess of MnO_4 was removed by the filtration process.

Estimation of Calcium Oxalate Dissolution

Blank: 1 mg calcium oxalate + 1 ml distilled water
Control: 1 mg calcium oxalate + 1 ml standard drug (Cystone®)
Group 1: 1 mg calcium oxalate + 1 ml extract in distilled water
Group 2: 1 mg calcium oxalate + 1 ml extract in ethanol
Group 3: 1 mg calcium oxalate + 1 ml extract in chloroform
Group 4: 1mg calcium oxalate + 1 ml extract in ethyl acetate
Group 5: 1 mg calcium oxalate + 1 ml extract in lime juice
Group 6: 1 mg calcium oxalate + 1 ml 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 100 ml 0.1 M 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 and 0.060-0.080 ml of 0.02 M $KMnO_4$ 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.

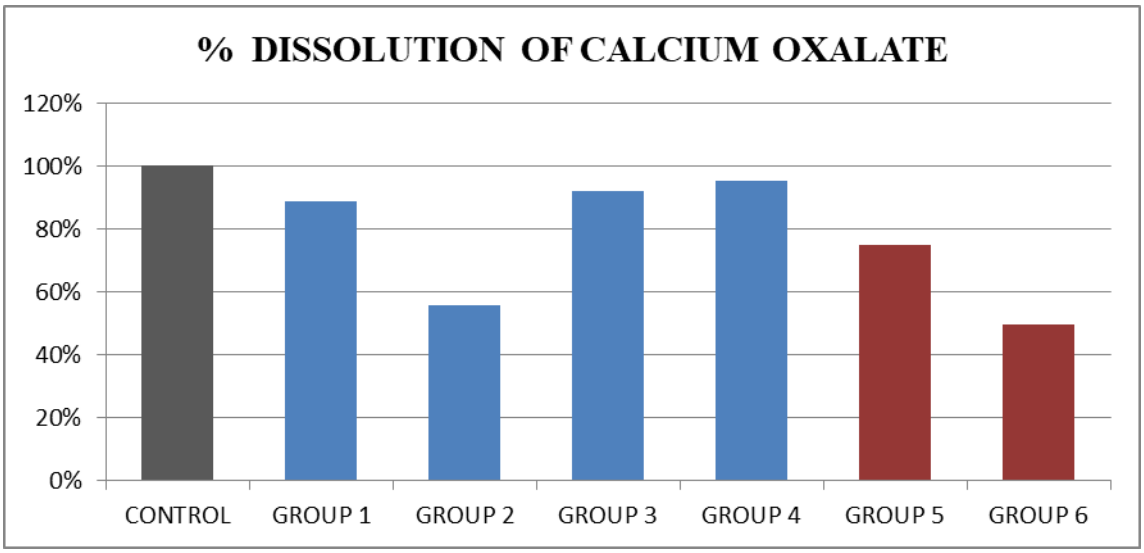
$$\% \text{ dissolution} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

RESULT

The study evaluates the anti-urolithiatic activity of six extracts and out of the six extracts, ethyl acetate extract has the greater capability to dissolve calcium oxalate, which is the foremost element of kidney stone. It shows the highest percentage of dissolution (95.14%), followed by chloroform extract (91.99%), distilled water extract (88.83%), lime juice extract (75%), ethanol extract (55.82%) and tender coconut water extract (49.75%).

Table 1: Spectrophotometric Measurement And Percentage Dissolution Of Calcium Oxalate.

GROUPS	% DISSOLUTION
Control	100
Group 1	88.83
Group 2	55.82
Group 3	91.99
Group 4	95.14
Group 5	75
Group 6	49.75



Graph 2.

DISCUSSION

Phytotherapy uses herbal drugs that are reported to be effective in the treatment of Urolithaisis with least side effects. Herbs also have the efficacy to improve the renal functions and to regulate the oxalate metabolism which helps to reduce the reoccurrence of renal calculi.

In Ayurvedic system of medicines *Merremia tridentata* have a vital position in the treatment of body pains, piles and toothache. Its extract are utilized to cure swellings, rheumatic infections, stiffness of joints and urinary infections (Aron *et al*, 2013). *Merremia tridentata* was reported for its anti-diabetic, anti-inflammatory, anti-arthritic, wound healing, analgesic and anti-microbial activitiy (Neyanila *et al*, 2013). Phytochemically the plant has been reported to contain flavanoids, quinone, tannins, phenolic acids, saponins, vaniliic acid and syrigic acid (Nair G.G., 1986), The presence of phytochemicals are considered as the responsible factor for the anti urolithiatic potential 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 oxalate

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 oxalate when performed in presence of standard drug, Cystone®. Concluding that the extracts of *Merremia tridentata* are beneficial in the treatment of kidney stones. 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.

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