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EVALUATION OF *IN-VITRO* STUDIES OF ANTI-UROLITHIATIC ACTIVITY BY ETHANOLIC EXTRACT OF *AMARANTHUS SPINOSUS*

P. Sailaja[1]*, Y. Prapurnachandra[2], K. Naga Jaya Varshini[3], M. Kavya[3], M. Dharani[3], T. Kaniha[3] and M. Suman[3]

[1,2]Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A.P., India.

[3]Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A.P., India.

***Corresponding Author: P. Sailaja**

Associate Professor, Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A.P., India.

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ABSTRACT

This study evaluates the anti-urolithiatic activity of ethonolic extract. Urolithiasis occurs when stones exit the renal pelvis and enter the urinary collection system, including the ureters, bladder, and urethra. Amaranth spinosus belongs to the family Amaranthaceae, is the most widely grown leaf vegetable in Kerala and Tamil Nadu. The succulent stem and leaves are high in iron, calcium, vitamins A, and C. Both leaf and grain types play an important role in combating malnutrition among the poor. The present study was aimed to investigate the effect of Ethanolic extract prepared from Amaranthus spinosus leaves on the nucleation and aggregation of CaOx crystals. Nucleation is the most critical step in the process of stone formation, which begins with the combination of stone salts in the solution into loose clusters that may increase in size by adding new components. Based on the results obtained in this study, Amaranthus spinosus and showed maximum inhibition and the significantly highest inhibition was shown at 1000µg/mL, 79.96±1.48.

KEYWORDS: Amaranth, urolithiatic activity, nucleation, renal stones.

INTRODUCTION

Herbal medicine (HM) is the pivot of complementary and alternative medicine, which has recently grown in popularity around the world and is gradually being integrated into mainstream healthcare systems and are comparatively more tolerable than pharmaceutical drugs especially in long time use for the management of chronic ailments.[1] Kidney stones cause the most serious urinary tract infection. Kidney stones were associated with an increased risk of chronic renal diseases, renal failure at the end of the stage, cardiac disease, diabetes and hypertension. Renal stone drugs are produced by renal calculus due to excessive high intake of drugs.[2] Stone prevention focuses on identifying and ameliorating the risk factors for crystal formation.[3] Cystone to decrease urine supersaturation and to prevent new stone formation and growth of existing stones via a short-term.[4] Cystone decreases urine supersaturation and to prevent new stone formation and growth of existing stones via a short-term.[5] Amaranth.[7-14] is the most common leaf vegetable grown in Kerala and Tamil Nadu. Leaves and succulent stem are good sources of iron (38.5 mg/100g), calcium (350-400 mg/100g), vitamin A and vitamin C. Both leaf and grain types play a vital role to combat malnutrition of poor people. The plant is a constituent of an herbal drug LEUCOSOL-H which is found to be effective in Leucorrhoea.[6] Drug therapy has developed in response to population health care needs. There are many crucial areas in medicine such as liver diseases, arthritis, old age-related problems, certain viral infections and cancer where conventional medicine is devoid of satisfactory treatment. These are among the promising areas of research and development of medicines from the vast highly potential plant resources. Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney problems.

Medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effect. The present study was aimed to investigate the effect of Ethanolic extract prepared from Amaranthus spinosus leaves on the nucleation and aggregation of CaOx crystals. Nucleation is the most critical step in the process of stone formation, which begins with the combination of stone salts in the solution into loose clusters that may increase in size by adding new components. Based on the results obtained in this study, Amaranthus spinosus and showed maximum inhibition and the significantly highest inhibition was

shown at 1000µg/mL, 79.96±1.48. The aggregation was monitored in the presence and absence of ethanolic extracts and was compared with the standard drug. the results of this in vitro study showed that all extract had inhibitory effect on CaOx crystal aggregation while the maximum inhibition was recorded by, showed a better inhibition on crystal aggregation which was significantly higher than the standard drug.

MATERIALS AND METHODS

Materials

Cystone, Distilled water, Absolute ethanol and hexane, 100% DMSO, Sodium oxalate, Tris buffer, Calcium chloride, Potassium permanganate (KMnO4), Sulphuric acid (H2SO4) and Allopurinol.

Titrimetric Method

Experimental Procedure

The experimental kidney stones of calcium oxalate (CaOx) were prepared in the laboratory by taking an equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H2SO4. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60 °C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in the semi-permeable membrane of the egg.This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tri's buffer.

The first group served as blank containing only1 mg of calcium oxalate. The second group served as a positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs, i.e. allopurinol. The 3rd group along with 1 mg of calcium oxalate contain methanolic extract. The conical flasks of all groups were kept in an incubator preheated to37 °C for 2 h. Remove the contents of semi-permeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube and titrated with 0.9494 N KMnO4 till a light pink color end point obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment, in the beginning, to know the total quantity of dissolved calcium oxalate by various solvent extracts.

Nucleation Assay

The stone formation initiates with the occurrence of nuclei. The inhibitory activity of the extracts (250, 500, 750, and 1000 µg/mL) on the nucleation of CaOx crystals was determined by a spectrophotometric assay as described by Bawari et al. with slight modifications. Solution of calcium chloride (CaCl2) and sodium oxalate (Na2C2O4) were prepared at the final concentrations of 5 mmol/L and 7.5 mmol/L, respectively, in a buffer containing Tris (0.05 mol/L) and NaCl (0.15 mol/L) at pH 6.5. 1 ml of each concentration was mixed with 1 ml CaCl2 solution followed by the addition of 1 ml Na2C2O4 solution.

Final mixtures were incubated for 30 min at 37°C. The optical density (OD) of the mixtures was measured at 620 nm with a UV-visible spectrophotometer (UV-1800 240 V, Japan). Percentage inhibition of nucleation was calculated using the following formula.

% inhibition = [1-ODTest/OD control] ×100, (1)

Where % Inhibition is percentage of inhibition, OD Test is optical density with plant extract/standard drug, and OD Control is optical density without plant extract/standard drug. CaOx crystallization was observed under a light microscope in the presence and absence of extracts.

Aggregation Assay

When the crystals in solutions stick together, they form large particle aggregates. The inhibition of aggregation in the presence of the extract was CaCl2 and Na2C2O4 solutions (50mmol/l each) were mixed together, heated to 60°C in a water bath for 1 h and then incubated overnight at 37°C to prepare seed CaOx crystals. After drying, CaOx crystal solution (0.8 mg/ml) was prepared in a 0.05 mol/l Tris-HCl and 0.15mol/l NaCl buffer (pH 6.5). 1 ml of extract (200, 400, 600, 800, and 1000 µg/mL) was added to 3 ml CaOx solution, vortexed, and then incubated at 37°C for 30 min. Optical density of the final mixtures was read at 620 nm wavelength and percentage inhibition of aggregation was calculated as described. CaOx crystal aggregation was also observed under the light microscope in the presence and absence of extracts.

RESULTS

Phytochemical Screening

Table 1: Phytochemical Screening of Amaranthus spinosus leaves extract.

Bioactive Constituents	Ethanolic Extract of Amaranthus spinosus L leaves
Alkaloids	+
Saponins	–
Terpenoids	–
Phloba tannins	–
Flavonoids	+
Steroids	+
Glycosides	+

Carbohydrates	+
Proteins	+

Table 2: Percentage dissolution of calcium oxalate by Amaranthus spinosus leaves extract.

S. No.	% Dissolution of Calcium oxalate	
1	Groups	Amaranthus spinosus
2	Blank	0
3	Positive Control	85
4	Ethanolic extract	96.12

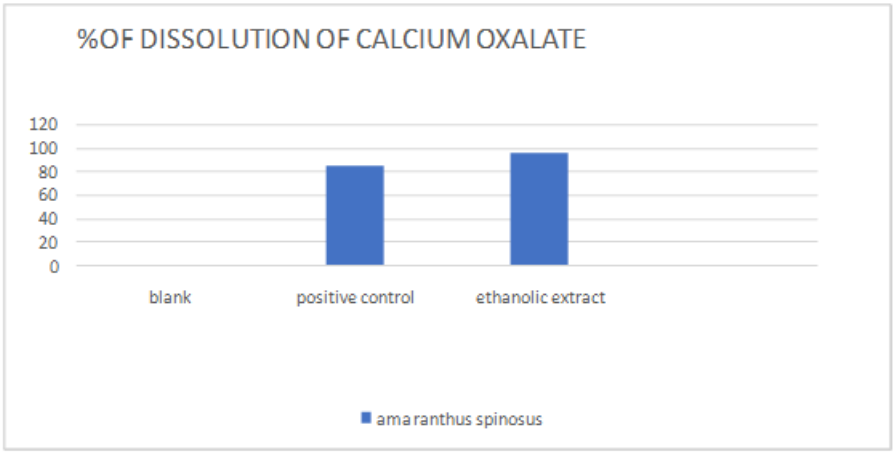

Figure 1: Percentage dissolution of Calcium oxalate.

Table: 3 Nucleation Assay.

Dose (µg/ml)	Standard [Cystone]	Ethanolic Extract of Amaranthus spinosus[Leaves]
250µg/ml)	15.86±2.12	24.11±1.61
500 (µg/ml)	30.53±1.95	41.38±2.69
750(µg/ml)	43.90±0.62	65.17±2.78
1000(µg/ml)	74.18±2.57	79.96±1.48

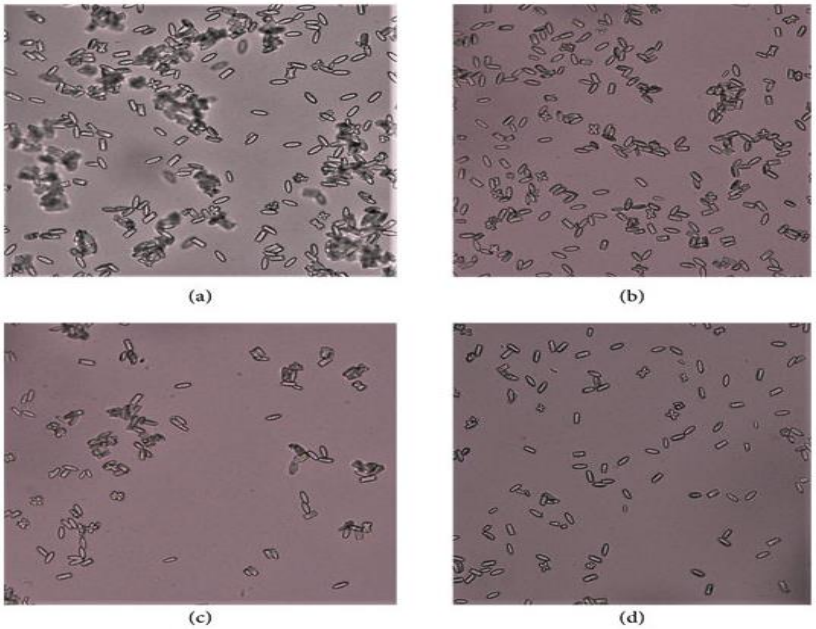

Figure 2: Representative micrographs of calcium oxalate crystals in the nucleation assay as observed under the light microscope (x40 objective) in the absence of the extract (a) and in the presence of ethanol extract of Amaranthus spinosus 250µg/mL (b), 500µg/mL (c), 750µg/mL (d), 1000µg/Ml.

Table: 4 Aggregation Assay.

Dose (µg/ml)	Standard (cystone)	Amaranthus spinosonus leaves of ethanolic Extract
250µg/ml)	14.46 ±1.02	23.27± 1.28
500 (µg/ml)	21.35 ± 1.24	27.24 ±1.02
750(µg/ml)	23.41 ± 1.40	27.89 ± 1.23
1000(µg/ml)	37.24 ±1.52	40.24 ± 1.12

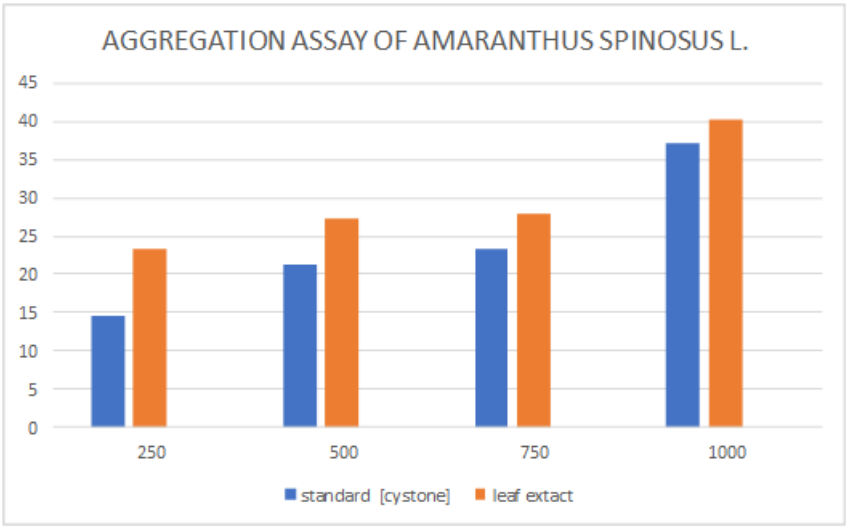

Figure 3: Aggregation assay of Amaranthus spinosus L.

CONCLUSION

In vitro, urolithiasis has been performed on the selected plant Amaranthus spinosus by using the standard drug. The work was performed by using in vitro model for calculating the percentage dissolution of kidney stones. Ethanolic extract of Amaranthus spinosus shows the highest dissolution of the standard drug. This study has given primary evidence for the Amaranthus spinosus plant which possesses anti-urolithiatic properties. The present study demonstrates a significant Antiurolithiatic selected medicinal plants having traditional claims in ayurvedic medicine against CaOx urolithiasis in vitro. Phytoconstituents such as saponins, tannins, and flavonoids, responsible for Anti-urolithic activity Amaranthus spinosus showed better inhibition at 1000µg/mL on CaOx crystal nucleation compared to other doses.

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