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In-vitro antiurolithiatic activity of alcoholic and hydroalcoholic extracts of *Kalanchoe pinnata* leaves

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Abstract

Objective: The objective of this research was to find the efficiency of alcoholic and hydroalcoholic extracts of *Kalanchoe pinnata* leaves in dissolution of calcium oxalate crystals by using *in-vitro* dissolution model.

Methods: *In-vitro* dissolution model was prepared by using semipermeable membranes obtained from eggs that served as dissolution bags for the investigation. Dissolution bags containing calcium oxalate and different extracts were suspended in conical flasks containing TRIS buffer. Percentage dissolution of calcium oxalate by different extracts was evaluated by titrimetry.

Results: Hydroalcoholic extract of leaves of *Kalanchoe pinnata* showed more dissolution of calcium oxalate as compared to alcoholic extract. Although the dissolution of calcium oxalate by hydroalcoholic extract was less than that of standard drug.

Conclusion: Results obtained from this research work indicated promising effects of *Kalanchoe pinnata* leaves in dissolution of calcium oxalate. Extracts of *Kalanchoe pinnata* leaves can be used for the effective treatment of urolithiasis.

Keywords: *Kalanchoe pinnata*, Antiurolithiatic Activity, Calcium Oxalate, Hydroalcoholic Extract, Alcoholic Extract, Cystone

Lithiasis is characterized by the formation of calculi. Two major types of lithiasis are known i.e. nephrolithiasis and urolithiasis. In urolithiasis, formation of calculi occurs in urinary bladder, ureter or any part of urinary tract other than the kidneys whereas in nephrolithiasis, calculi formation occurs in kidneys. When calcium ions present in the body reacts or binds with oxalic acid/oxalate which are present in oxalate rich foods, they precipitate as calcium oxalate crystals in the body and lead to hypocalcaemia (due to low availability of free calcium ions) and urolithiasis. Generally kidney stones are comprised of high concentration of calcium oxalate with low concentrations of calcium phosphate, calcium carbonate etc. The major steps involved in the formation of stones are nucleation, crystal growth, crystal aggregation and crystal retention. Three general pathways for kidney stone formation are seen: (1) stones fixed to the surface of a renal papilla at sites of interstitial apatite plaque (termed as Randall's plaque), (2) stones attached to plugs protruding from the openings of ducts of Bellini and (3) stones forming in free solution in the renal collection system. [1, 2]

Several medicinal plants extracts have been reported for antiurolithiatic activity. Plants whose antirolithiatic activity is reported till date are *Herniaria hirsute*, *Amni visnaga*, *Tribulus terrestris, Bergenia ligulata*, *Dolichos biflorus*, *Dolichos biflorus*, *Vediuppu chunnam*, *Raphanus sativus*, *Achyranthus aspera*, *Quercus salicina*, *Phyllanthus niruri*, *Cynodon dactylon* [3]. *Kalanchoe pinnata* is one of the many medicinal plants used traditionally for the treatment of lithiasis.

Kalanchoe pinnata is a succulent perennial plant that grows 3-5 feet tall. Commonly known as 'air plant,' it has tall hollow stems, fleshy dark green leaves that are distinctively scalloped and trimmed in red, and bell-like pendulous flowers. It is also known by other synonyms such as patharchata, patharchuri, panfuti (in hindi) and miracle plant. It belongs to family Crassulaceae. The main plant chemicals found in Kalanchoe include: arachidic acid, astragalin, behenic acid, beta amyrin, benzenoids, beta-sitosterol, bryophollenone, bryophollone, bryophyllin, bryophyllin A-C, bryophyllol, bryophynol, bryotoxin C, bufadienolides, caffeic acid, campesterol, cardenolides, cinnamic acid, clerosterol, clionasterol, codisterol, coumaric acid, epigallocatechin, ferulic acid, flavonoids, friedelin, glutinol, hentriacontane, isofucosterol, kaempferol, oxalic acid, oxaloacetate, palmitic acid, patuletin, peposterol, phosphoenolpyruvate, protocatechuic acid, pseudotaraxasterol, pyruvate, quercetin, steroids, stigmasterol, succinic acid, syringic acid, taraxerol, and triacontane [4]. The aim of the present study was to find the efficiency of alcoholic and hydroalcoholic extracts of *Kalanchoe pinnata* leaves against lithiasis.

Calcium chloride dihydrate, sodium oxalate, potassium permanganate, sulfuric acid and ethanol were taken from B.R. Nahata College of pharmacy's store. Cystone was purchased from Red Cross Medical Store, Mandsaur, Madhya Pradesh, India. Fresh leaves were collected in the

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month of November, 2016. Plant material was identified and authenticated (Plant authentication no: Brncp / K / 002 / 2016 / *Kalanchoe pinnata* / Abu Sufiyan Chhipa) from Deptartment of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur.

Fresh leaves of *Kalanchoe pinnata* were taken and cut into small pieces and were put into separate Soxhlet apparatus containing ethanol and hydroalcoholic solvents (300 ml each) and extraction was carried out for 5 hrs. After 5 hrs extracts were cooled and filtered using Whatmann filter paper. Extract solutions were further concentrated on water bath. The ratio of water and ethanol for making hydroalcoholic solvent was 1:1.

4.4 gm of calcium chloride dehydrate was dissolved in 300 ml of distilled water and 4 gm of sodium oxalate was dissolved in 300 ml of 2N H_2SO_4 for the synthesis of calcium oxalate. Both solutions were mixed in a beaker to precipitate out calcium oxalate with continuous stirring. Calcium oxalate produced was treated with ammonia solution to remove traces of sulfuric acid followed by washing with distilled water and drying at 60 °C for 2 hrs [5].

500 mg Cystone tablet was kept in ethanol to remove its outer color coating. Resultant 400 mg tablet was crushed and dispersed in 100 ml distilled water.

Semi permeable membrane of eggs lies between outer calcified shell and inner contents of the eggs. Eggs were punctured by a glass rod and entire contents of eggs were removed. Empty shells were kept in 2M HCl overnight to achieve decalcification of outer shell. Shells treated with HCl were washed with distilled water and semi permeable membranes were removed from decalcified shells carefully. Semi permeable membranes were washed with distilled water followed by treatment with ammonia solution to neutralize the traces of acid and stored in refrigerator in the moistened condition at pH 7-7.4 [6, 7].

To prepare the stock solution of calcium oxalate 100mg of calcium oxalate was suspended in 100ml of distilled water. To prepare of 1N H_2SO_4 solution 2.75 ml of concentrated sulfuric acid was dissolved in 100 ml distilled water to obtain 1N sulfuric acid.

To prepare solution of potassium permanganate solution 0.3 gm of $KMnO_4$ was dissolved in 100 ml of water and filtered to obtain 0.9494N $KMnO_4$ solution.

Experimental groups (n=6) are Group 1 (Negative control): 1ml of calcium oxalate (1 mg/ml) + 1 ml of distilled water; Group 2 (Standard solution): 1 ml of calcium oxalate (1 mg/ml) + 1 ml of cystone solution (4 mg/ml); Group 3 (Test solution 1): 1 ml of calcium oxalate (1 mg/ml) + 1ml of ethanolic extract (10 mg/ml); Group 4 (Test solution 2): 1ml of calcium oxalate (1 mg/ml) + 1 ml of hydroalcoholic extract (10 mg/ml).

All groups were packed in semi permeable membranes. Opening of membranes was tied with a thread and suspended in conical flasks containing 150 ml of 0.1m Tris buffer each. Mouths of conical flasks were covered with aluminium foil. Conical flask of each group was placed in an incubator (preheated at 37 ºC for 2 hrs) for 24 hrs. Contents of semi permeable membrane of each group were emptied in separate test tubes and 2 ml of 1N sulfuric acid was added to all test tubes and titrated with 0.9494N $KMnO_4$ solution till a light pink colour end point is obtained.

1 ml of 0.9494N $KMnO_4$ is equivalent to 0.1898 mg of calcium. Percentage dissolution of calcium oxalate by various groups is shown in (Table 1). *In vitro* antiurolithiatic activity of ethanolic and hydroalcoholic extracts of *Kalanchoe pinnata* leaves was evaluated by comparing the two extracts with standard solution of cystone. Percentage dissolution of calcium oxalate is shown in table 1.

Table 1: Dissolution studies of calcium oxalate by standard cystone, ethanolic and hydroalcoholic extracts

Group	Vol. of $KMnO_4$ (ml)	Wt. of calcium estimated	Wt. of calcium reduced	Percentage dissolution
Negative	2.7±0.4	0.5124	---	---
Standard (cystone)	1.1±0.05	0.2087	0.3037	59.2
Ethanolic extract	1.9±0.1	0.3606	0.1518	29.6
Hydroalcoholic extract	1.6±0.2	0.3036	0.2088	40

Values are mean ± SEM, n=6

Hydroalcoholic extract of *Kalanchoe pinnata* leaves showed more dissolution of calcium oxalate as compared to ethanolic extract of the same. Also cystone was found to be more effective than the two extracts (Figure 1).

The present studies provide useful information about the antiurolithiatic activity of different extracts (ethanolic and hydroalcoholic) of *Kalanchoe pinnata* leaves. Hydroalcoholic extract showed maximum dissolution of calcium oxalate as compared to ethanolic extract. The present investigation will be supportive as additional information to the scientific evidences regarding in vitro studies.

Since the correct mechanism of antiurolithiatic activity of the extracts is not completely known till date, correlation between *in vitro* and *in vivo* should be further studied to determine the phytochemical constituents responsible for the antiurolithiatic activity.

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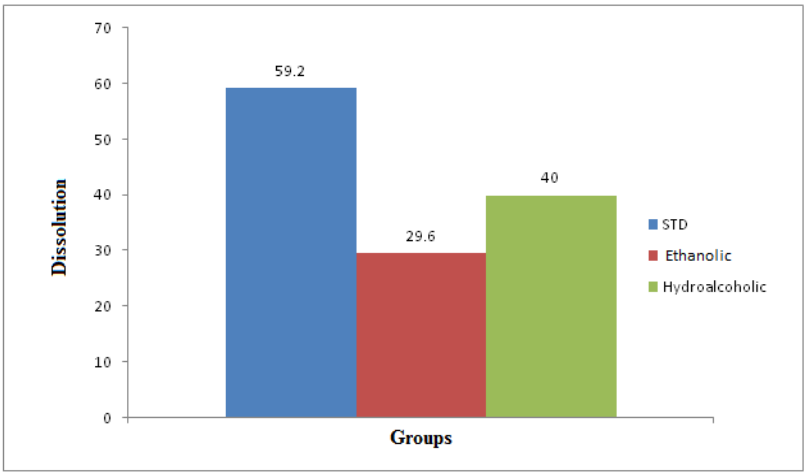

Figure 1: Dissolution of calcium oxalate

Results are given in mean of triplicate. Error bars was omitted for the simple presentation of graph.

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