



UROLITHIATIC ACTIVITY OF LAUNEA PROCUMBENS AGAINST CALCIUM OXALATE AND CALCIUM PHOSPHATE STONES

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ABSTRACT

Launea procumbens commonly known as Pathari belonging to family asteraceae has been investigated for its anti-urolithiatic activity by constructing an *in-vitro* model. Various crude extracts of *Launea procumbens* were evaluated for their potential to dissolve experimentally prepared kidney stones of Calcium oxalate, Calcium phosphate and the results were compared with cystone as a standard

polyherbal drug. The detailed phytochemical screening was done to identify active phytoconstituents and photo-micrography was carried out to understand dissolution of stones by extracts. Chloroform, alcoholic and acetone fractions of plant leaves showed highest dissolution of Calcium oxalate stones as compared to others (63,49,40% resp.) while ethyl acetate, pet ether, acetone fractions of plant leaves were more effective in dissolving Calcium Phosphate (80,30,25% resp.) compared to reference standard-formulation Cystone.

KEYWORDS: Bioactive, Alkaloids, Terpenoids, Urolithiasis, Kidney Stone.

INTRODUCTION

Launea procumbens (Family- Asteraceae) is a plant commonly found in the West India and has been reported to decrease renal calculi. Urinary tract and Kidney stone ailments have affected human beings since ancient time. Ancient Vedic literature describes stones as Ashmari. It occurs both in men and women but the risk is generally higher in men and is becoming more common in young women.^[1] They are a major problem and an important cause of renal failure in India. This is an entity which has high morbidity and low mortality

but having serious consequences and significant socio-economic impact. Kidney stone is a multi-factorial disorder resulting from the combined effect of epidemiological, biochemical and genetic risk factors. Calcium- containing stones, especially calcium oxalate and calcium phosphate are the most commonly occurring ones to an extent of 75-90%. Medical conditions that are associated with increased risk of kidney stone formation include hyperparathyroidism, hyperthyroidism, gout, cystic fibrosis^[2] Symptoms for urolithiasis include abnormal urine colour, blood in urine, fever, nausea and vomiting.^[3] The modern treatments of renal stone include lithotripsy, shock wave therapy, surgical techniques, local calculus disruption using high-power laser and medicines. These are much painful, expensive and have many side effects even post therapy the recurrence rate of renal calculi is very high. So there is no suitable medical therapy is available for such stone disorder, it is very important to show an interest in indigenous system of medicine and traditional herbal remedies which are regarded as quite safe with no side effects. Furthermore it should be cost effective, readily accessible and quite affordable.



Fig-1 : Launea procumbens plant

Kidney stones and urinary disorders are most painful conditions and have been affected people for several centuries. A large population of India suffers from urinary tract and Kidney stones, formed due to deposition of Calcium, phosphate and oxalates. These stones may persist for indefinite time, lead to secondary complications causing serious consequences to patients life. It is very painful and proper cure is needed to get rid of the problem.^[4] Depending on where they are located, kidney stones are known as Urinary calculi, Urinary tract stone disease, renal calculi, Nephrolithiasis, Ureterolithiasis and Urolithiasis. Appropriate and timely treatment of kidney stones can prevent severe complications such as partial or complete failure kidney functions. Stone in the urinary tract are common disorders because urine is a chemical solution that contains a large number of chemical substances.

These substances can crystallize easily and then grow in size to form stones.^[5] Though treatment of kidney stone is revolutionized by the development of non-invasive methods of stone disruption but the patients always try to refrain from surgical procedures. Moreover, it also carries the factors like high cost, availability, side effects etc: the recurrence rate is approximately 50-80%.^[6]

As no suitable medical therapy is available for such stone disorders, it is imperative to search for some new or less known medicinal plants, which are potential source for new bioactive compounds of therapeutic value. In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects.^[7] As per the indigenous system of medicine, the leaves of *Launea procumbens* are reported to be useful in the treatment of a wide range of ailments including urinary stones.^[8,9] However, so far no scientific study has been reported regarding the in vitro anti-urolithiatic activity of various extracts of *L. Procumbens* leaves against Calcium stones. The present study was carried out to evaluate the in vitro anti-urolithiatic activity of *L. Procumbens* leaves extracts against Calcium oxalate and Calcium phosphate stones.

MATERIAL AND METHOD

Collection of Plant material and preparation of extracts

The leaves of *L. procumbens* were collected from the local area of place Sangola (District Solapur) of Maharashtra state in the month of July-August and shade dried. The plant was authenticated by botanists. The dried leaves were coarsely powdered (passed through sieve no. 40). The 5g. of powder was extracted by using 100 ml of various organic solvents such as acetone, ethyl acetate, petroleum ether, chloroform, ethyl alcohol and distilled water. The crude extract obtained was evaporated at 45°C, then dried and stored in airtight container (yield 29.82 %, w/w).^[10]

Chemicals

The solvents used for extraction were acetone, ethyl acetate, petroleum ether, chloroform, ethyl alcohol purchased from Merck Ltd., Mumbai, India.

Phytochemical Analysis

The extracts were screened for the presence of active phytochemical constituents by following standard procedures.^{[11],[12]}

1) Test for Phenols

To 1 ml of extract 1% ferric chloride solution was added. Appearance of green colour indicated the presence of phenols.

2) Test for Flavonoids

To the 1ml of alcoholic extract, 3-5 drops of 2% lead acetate solution was added. Development of orange or yellow colour indicated the presence of flavonoids.

3) Test for Alkaloids

Hagers Test- one drop of Hagers reagent was added into 1ml of extract, yellow precipitate is formed indicated the presence of alkaloids.

4) Test for Triterpenoid Saponins

Froth formation test- 1ml of extract was shaken with water in a test tube, froth developed indicated the presence of terpenoids and saponins.

Evaluation of Anti-urolithiatic Activity for *L.procumbens*^[13,14]

Step-1 Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous precipitation

Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H₂SO₄ were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of (2N H₂SO₄), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipitates freed from traces of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60°C for 4 hours. Calcium phosphate(AR) was purchased from Merck and used directly for the study.

Step-2 Preparation of Semi-permeable membrane from farm eggs

The semi-permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the contents squeezed out

completely from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Step-3: Estimation of Calcium oxalate/phosphate by Titrimetry

Weighed exactly 1mg of the calcium oxalate and 10mg of the extract/compound/ standard and packed it together in semi permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate). Placed the conical flask of all groups in an incubator, preheated to 37°C for 2 hours, for about 7-8 hours. Removed the contents of semi-permeable membrane from each group into a test tube. Added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N KMNO₄ till a light pink colour end point obtained. 1ml of 0.9494 N KMNO₄ equivalent to 0.1898mg of Calcium. The amount of undissolved calcium oxalate/phosphate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate/phosphate actually test substance (s) could dissolve.

RESULTS AND DISCUSSION

The detailed phytochemical analysis revealed that Chloroform and alcoholic, ethyl acetate, pet ether extracts are found to be rich in saponins, flavonoids, terpenoids, alkaloids and phenols.

Table-1: Phytochemical analysis of *L. procumbens* extracts

Extract	Saponin	Flavonoid	Terpenoid	Alkaloid	Phenol	Terpenoid
P.ether	+	+	+	+	+	+
CHCl ₃	+	+	+	+	+	+
EtOH	+	+	+	+	+	+
EtOAc	+	+	+	+	+	+
Acetone	-	-	+	+	+	+
Aqueous	-	+	+	-	-	+

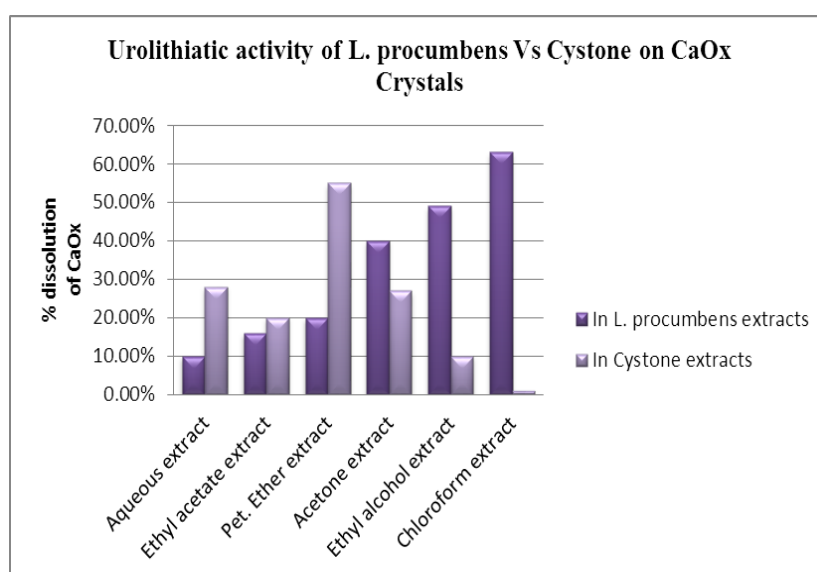
(+) = present, (-) = absent

Qualitative chemical tests indicated the presence of phenolic compounds, flavonoids, phenols, terpenoids and saponin in different extracts of *Launea procumbens*. On the basis of this fraction we performed *in vitro* anti-urolithiatic activity by comparing different extracts of *Launea procumbens* using cystone as standard against Calcium oxalate and Calcium phosphate stones. Table-2 represents percentage dissolution of Calcium oxalate crystals by *L. procumbens* extracts in comparison with Cystone.

Table-2: % Dissolution of Calcium oxalate

Extract	L. procumbens extracts	Cystone extracts
Aq. extract	10.00%	28%
EtOAc extract	16%	20%
P.Ether extract	20%	55%
Acetone extract	40%	27%
EtOH extract	49%	10%
CHCl ₃ extract	63%	1%

Fig.-2 represents graphical representation of percentage dissolution of Calcium oxalate crystals by L.procumbens extracts in comparison with Cystone.

**Fig 2: % Graphical representation of dissolution of Calcium oxalate**

The graphical representation shows % Dissolution of calcium oxalate by *in vitro* anti-urolithiatic activity of fractions of *Launea procumbens*. drug.Chloroform ,alcoholic and acetone extracts at 10 mg concentration produced higher dissolution of Calcium oxalate as compared to other fractions and the Standard.Standard shows higher dissolution as compared to others for aqueous,ethyl acetate and petroleum ether extracts.

Table-3: % Dissolution of Calcium phosphate

Extract	L. procumbens extracts	Cystone extracts
Aq. Extract	19.00%	22%
EtOAc extract	80%	56%
P.Ether extract	30%	29%
Acetone extract	25%	5%
EtOH extract	20%	32%
CHCl ₃ extract	20%	20%

Table-3 represents percentage dissolution of Calcium phosphate crystals by *L. procumbens* extracts in comparison with Cystone.

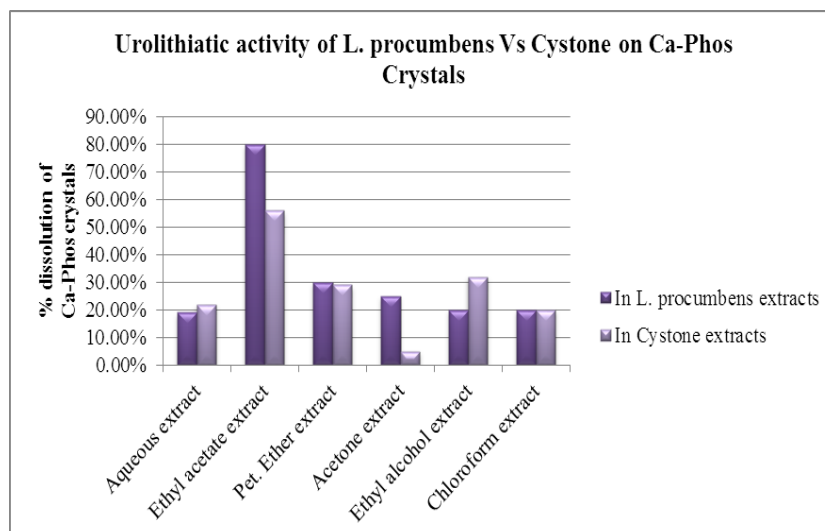


Fig-3: % Graphical representation of dissolution of Calcium phosphate

Fig.-3 represents graphical representation of percentage dissolution of Calcium phosphate crystals by *L. procumbens* extracts in comparison with Cystone. The graphical representation shows % Dissolution of calcium phosphate by *in vitro* Anti-Urolithiatic Activity of fractions of *Launea procumbens* crude drug. Ethyl acetate, petroleum ether and acetone extracts at 10 mg concentration produced higher dissolution of calcium phosphate as compared to other fractions and the Standard drug shows higher dissolution as compared to others for aqueous, alcoholic and chloroform extracts.

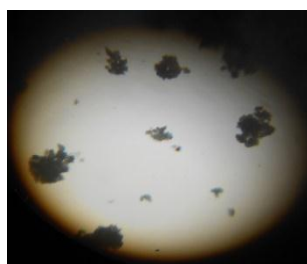


Image-a
CaOx crystals
(Without extracts)(Day 1)



Image-b
CaOx crystals –dissolution
in progress (Day 3)



Image-c
CaOx crystals –reduced in
size (Day 7)

Photomicrography Study: The photomicrographical study for dissolution of CaOx crystals was conducted with the help of Labline 10x 10 compound microscope using Olympus Digital Camera. The images of the slides were recorded at first day i.e. before treating CaOx crystals

with extracts(Image-a), at the third day when dissolution was in progress(Image-b) and finally at the seventh day where crystals were reduced in size(Image-c)

This study evaluates antiurolithiatic activity of different extracts of *Launea procumbens* leaves and isolated compound. The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. From the study results it is observed that chloroform ,alcoholic and acetone fractions of plant leaves showed highest dissolution of calcium oxalate in comparison to other fractions and standard drug cystone (63,49,40% resp.) while ethyl acetate, pet ether, acetone fractions of plant leaves were found to be more effective in dissolving calcium Phosphate (80,30,25 % resp.) This study has given primary evidence for *Launea procumbens* as plant which possess lithotriptic property. The present *in vitro* study has given lead data, and shown that various active components present in these extracts are quite promising for further work in this regard.Medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. The plant based therapeutics are easily available, cost effective with no or minimum side effects and hence rural and remote area residing people trust it as primary health care system. Further strong scientific explorations and experimental proofs are needed to support these preliminary findings. In future, there is a strong need to carry out number of *in vitro*, *in vivo* testings and clinical trials to examine efficacy and safety of such plant based therapeutics. An additional work can be carried out to isolate, purify and characterize active compounds and to identify their possible mechanism of action.

CONCLUSION

The detailed phytochemical analysis revealed that the crude extracts of *Launea procumbens* leaves are rich in bioactive compounds like terpenoids, alkaloids, flavonoids, phenols and saponins.Evaluation of anti-urolithiatic activity has been performed for *Launea procumbens* using *in vitro* Antiurolithiatic Model for calculating % dissolution of kidney stone and results were compared with standard drug. Many extracts are found to be the most effective in urolithiatic action.Data from our preliminary findings of *in vitro* evaluation revealed that phytotherapeutic agents from *L.procumbens* could be useful as either alternative or an adjunct therapy in the management of Urolithiasis.

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