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Evaluation of anti- Urolithiatic activity of *Aerva Lanata* in experimental animals

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Abstract
Objective: *Aerva lanata* has been used in traditional health systems to treat various disorders including as astringent, cooling, vermiuge, supparative, diuretic and lithontriptic. However, urolithiatic potential of leaves is not scientifically validated till date. The aim of present study is to evaluate urolithiatic effect of ethanol extracts of *Aerva lanata* leaves.
Methods: Ethylene glycol (EG) is used to induce urolithiasis in rats by adding \(0.75\%\) EG to their drinking water for several weeks, causing hyperoxalluria and the formation of calcium oxalate (CaOx) stones.
Results: Nearly equivalent to the standard drug, Cystone (70.58% inhibition), indicating a highly promising therapeutic potential. Comparable to the parent ethanolic leaf extract (66.15% inhibition), suggesting that the fractionation process successfully concentrated the anti-nucleation compounds within this specific fraction.
Conclusion: this finding strongly indicates that the chloroform fraction contains the key bioactive compounds responsible for the anti-urolithiatic activity of *Aerva lanata* leaves by effectively preventing the critical first step of stone formation.

Keywords: *Aerva lanata*, urolithiatic, ethylene glycol, ethanol

Introduction

Urolithiasis is a consequence of complex physical processes. The major factors are supersaturation of urine with the offending salt and crystallization [1]. Crystals retained in kidney can become nucleus for stone formation. This process is synonymously known as Urolithiasis, Nephrolithiasis, Kidney stones or Renal calculi [2].

There are several options available in the management of ureteral stones. Treatment selection depends on stone size, location and composition, efficacy of each modality and associated morbidity, equipment available, physician skill, patient health and preference and finally costs [1, 3].

In many cases, the management of urolithiasis is combined surgical and medical approach using percutaneous nephrolithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL) and antibiotics. These treatments are relatively costly, painful and require expert hands and availability of appropriate equipments. This has given rise to stimulation in the search for investigating natural resources showing antiurolithiatic activity [4, 6].

In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid of this painful disorder. Ayurveda, an indigenous system of Indian medicine, offers vast scope for the successful treatment of urolithiasis. Plants and other natural substances have been used as the rich source of medicine. All ancient civilizations have documented medicinal uses of plant in their own ethno-botanical texts. The list of drugs obtained from plant source is fairly extensive [7, 8].

Many remedies have been employed during ages to treat urolithiasis. Most of the remedies were taken from plants and proved to be useful, though the rationale behind their use is not scientifically established except for a few plants and some proprietary composite herbal drugs. In the indigenous system of medicine, several plants are reported to be useful in the treatment of urinary stones. Many of these plants have been screened by various researchers till date; and many more are yet to be diagnosed [9, 10].

Material and Methods

In the present study, the leaves of *Aerva lanata* was collected from local area of Alwar district, Rajasthan, with the help of field botanist. Ethylene glycol was procured from Sigma Chemical Co., USA. Pure drug Cystone was obtained from Aventis Pharma Ltd., Goa.

Methods: Preparation of extract: Dried course powder of the leaves was extracted with alcohol (90%) and water by using soxhlet apparatus separately until the extraction solvents becomes colorless.

Preliminary Phytochemical Screening

Extract of *Aerva lanata* leaves will be subjected to preliminary quantitative phytochemical investigation for the detection of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, gums and mucilages, fats & fixed oils using the following standard methods [11, 13].

Animals: The ethical clearance obtained via the institutional Animal Ethics Committee before the experiment. For acute toxicity study and for pharmacological activity evaluation Albino rats, weighing 150-200gm, were used for study, the animal was fasted whole night before the experiment starts for various extracts. Animal were kept in a constant humidity (55%), temp at (22± 20C), and exposed to dark and light {12hr) every day the bedding materials of the cages were changed [14].

In vivo anti-urolithic study

Acute oral toxicity

The acute oral toxicity studies of extracts were carried out as per the guidelines of Organization for Economic Co-operation Development (OECD) guidelines [15].

Experimental protocol
For the study, 30 male Wistar albino rats (100-200 g) were used which were supplied by animal house of College [16].

Ethylene glycol induced Urolithic study
Animal will be randomly divided into 5 groups of 6 each and assigned as below.

Table 1: Experimental design for anti- Urolithic activity

Groups	Animals
I	Normal control
II	Lithiatic control
III	750 mg/kg standard (Cystone)
IV	200 mg/kg hydro-ethanolic extract
V	400 mg/kg hydro-ethanolic extract

Urine collection and biochemical analysis: All the urine samples thus collected were analysed for volume, pH and specific gravity. Additionally, a drop of concentrated hydrochloric acid was added to all the samples after which they were stored at 4°C and further examined for calcium, oxalate, phosphate, magnesium, and citrate.

Blood collection and serum evaluation: On 30th day, blood collection was done through tail vein technique under thiopental sodium (TS) anesthesia in serum separating tubes and kept for 30 min following which the serum was separated by centrifuging the tubes at 10,000 rpm for 5 min and kept at 20ºC till further analysis. The serum samples were then subjected to analysis of calcium, urea, uric acid (UA), & creatinine [17].

Statistical Analysis
Data were represented as mean±standard deviation (SD), where, n=3 in case of *in vitro* studies and n=6 in case of *in vivo* studies. Statistical analysis was performed for analysing significant differences between groups by one-way analysis of variance (ANOVA) which was followed by Tukey's multiple comparison test using GraphPad Prism, version 6.01. The values were considered statistically significant when $p<0.05$.

Results and Discussion:

1. Organoleptic characters of crude drug

Table 2: Organoleptic characters of *Aerva lanata* leaf

S. No	characteristic	Result
1	Color	Green to grey-green
2	Odor	faint Characteristic
3	Taste	Slightly bitter
4	Size	L- 1.5 - 4 cm, W-0.5-2.5 cm
5	Shape	Circular-Ovate
6	Texture	Soft, Velvety

2. Ethanolic extraction of *Aerva lanata* leaves
The % yield of ether & ethanol extract of *A. lanata* leaves was found to be 0.8 %, 3.3 %, where as chloroform soluble extract, acetone soluble fraction and acetone insoluble fraction of ethanolic extract were found *35.7* %, 16.1%, and 42.8% respectively. These extracts and fractions were stored in airtight container for further studies.

Table 3: Percentage yield, consistency and color of different extracts / fraction of *Aerva lanata* leaf

S. no.	Extraction /fraction	Colour	Consistency	(% w/w) Yield
1	Petroleum ether extract	Light brown	Sticky mass	0.8%
2	Ethanol extract	Dark brown	Sticky mass	3.3%
3	Chloroform soluble extract	Dark brown	Sticky mass	35.68 % of ethanol extract
4	Acetone soluble fraction	Dark brown	Sticky mass	16.1 % of ethanol extract
5	Acetone insoluble fraction	Dark brown	Sticky mass	42.8 % of ethanol extract

3. Qualitative phytochemical investigation of *Abutilon Indicum*

Table 4: Qualitative chemical tests of different extracts of *Aerva lanata*

Tests	Petroleum ether Extract	Ethanol extract	Chloroform soluble ethanolic extract F1	Acetone soluble Ethanolic extract F2	Acetone insoluble Ethanolic extract F3
Steroid	+	+	-	-	+
Triterponoids	-	+	-	+	-
Glycosides	-	-	+	-	+
Carbohydrates	-	-	-	-	+
Alkaloids	-	+	+	+	+
Flavonoids	-	+	+	-	+
Tannins	-	+	-	-	+
Proteins and amino acid	-	-	-	-	+
Lipids	+	-	-	-	-
Steroid	-	-	-	-	-

Results and discussion
Petroleum ether extract leaves showed presence of steroid, chloroform soluble extract showed the presence of glycoside, carbohydrate, acetone soluble fraction showed the presence of triterponoids, glycoside, alkaloid and flavonoid where as ethanolic extract and acetone insoluble fractions leaves showed the presence of various phytochemical groups alkaloids, flavonoids, phytosterols, tannins and proteins.

4. Quantitative phytochemical analysis
The results of quantitative phytochemical analysis of the ethanol extract of *Aerva lanata* leaf is shown in below
Table. The total phenol and flavonoid content were 87.75 mg/g and 62.25 mg/g respectively

Table 5: Phytochemical analysis of ethanol extract of leaf of *A. lanata*

Total Phenol (mg/g)	Flavonoid (mg/g)
87.75 ± 1.54	62.25 ± 0.7

5. *In vivo* anti-urolithic activity by ethylene glycol (EG) induced urolithiasis model

Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hrs. In all the cases no death was observed within first 24 hrs. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern. Attention was also given to observation of tremors and convulsions.

The therapeutic dose was calculated for the purpose of anti-diabetic investigations. The LD_{50} value determined by the method as per guidelines of Organization for Economic Co-operation Development was found to more than 2000 mg/kg b.w. by oral route.

5.1 Ethylene glycol induced urolithiasis model

EG is employed for development of kidney stones as it enhances the activity of enzymes glycolic acid oxidase (GAO) and lactate dehydrogenase (LDH) which are involved in oxalate synthesis causing the oxidation of glycolate to glyoxylate by GAO which is continued by the oxidation of glyoxylate to oxalate catalysed by LDH

a. Analysis of urinary parameters

A significant reduction of 5.07±0.61 ml in the urine volume was seen in the rats of diseased group when compared to normal group with a urine volume of 11.27±0.63 ml ($p<0.0001$). However, the volume was significantly raised to 7.85±0.83 ml which was towards the normal by treatment with ethanolic extract of aerial parts in comparison to diseased group ($p<0.0001$). The pH was however, not significantly increased when treatment was done with the best active fraction I (on the basis of the results of *in vitro* studies) derived from the same extract (6.22±0.59 ml) as compared to the diseased group. A significant decrease in the urinary pH of 5.17±0.23 was observed in the lithiatic group as compared to the normal rats with pH of 6.5±0.46 ($p<0.01$). It was significantly restored towards normal by administration of extract (6.4±0.6, $p<0.01$) and fraction (6.32±0.73, $p<0.05$), respectively as compared to diseased group [18].

Effect of AL Extract & Fraction on Physical Parameters of Urine

Group	Volume (ml)	pH
Normal	9.5±0.25	6.7±0.46
Lithiatic	4.1±0.61	5.17±0.23
Standard (Cystone)	8.45±0.82	6.62±0.69
Ethanol Extract	7.35±0.52	6.5±0.60
Chloroform soluble EE	6.6±0.83	6.45±0.73

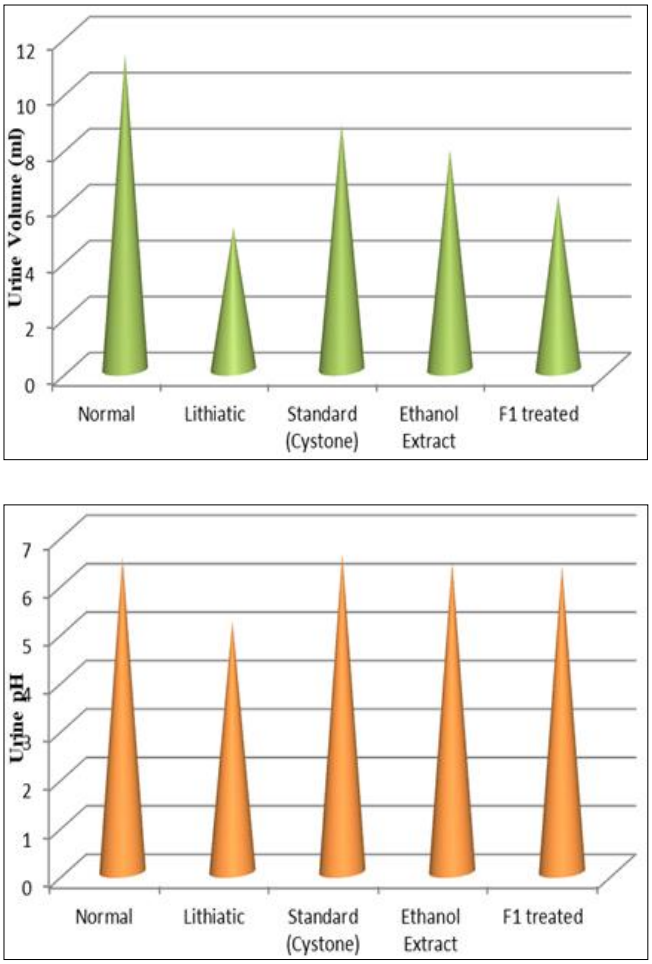

Fig 6.3: Effect of AL Extract & Fraction on Physical Parameters of Urine

b. Analysis of Urinary Promoters and Inhibitors

A significant rise of 10.1±1.12 mg/24 h in urinary calcium was observed in the lithiatic control group when compared to normal group with urine calcium of 5.15±0.85 mg/24 h ($p<0.001$). It was significantly lowered to 7.15±1.32mg/24 h and 8.21±1.48 mg/24 h by administration of ethanol extract of leaf at a dose of 200 mg/kg and the fraction I at a dose of 32 mg/kg, respectively in comparison to the lithiatic group. Likewise, a significant hyper-phosphaturia was witnessed in lithiatic group (11.3±1.08 mg/24 h) at $p<0.001$ when compared with normal rats (5.52±0.25 mg/24 h). Administration of extract significantly decreased the raised urinary phosphorus levels towards normal (7.88±1.28 mg/24 h) as compared to diseased group ($p<0.05$).

Table 6: Effect of *Aerva lanata* Fraction on Urinary Promoters and Inhibitors in Ethylene Glycol- Induced Urolithic Rats

Group	Calcium (mg/24 h)	Phosphorus (mg/24 h)	Magnesium (mg/24 h)
Normal	5.15±0.85	6.52±0.25	2.92±0.46
Lithiatic	10.1±1.12	11.3±1.08	0.85±0.36
Standard (Cystone)	6.32±1.2	7.42±1.15	1.64±0.44
Ethanol Extract	7.15±1.32	7.88±1.28	1.9±0.45
F1 treated	8.21±1.48	9.38±1.3	2.28±0.59

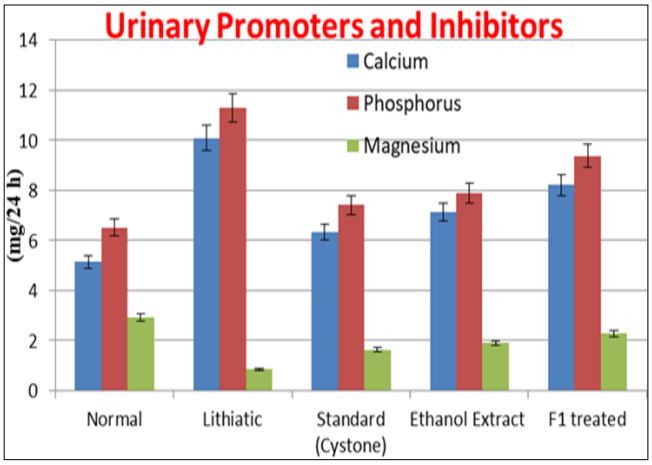

Fig 6.4: Effect of *A. lanata* fraction on urinary stone promoters and inhibitors

c. Analysis of serum parameters

The level of serum urea in diseased rats was significantly higher (158.72±4.26 mg/dl) as compared to normal rats (50.2±1.85 mg/dl) at p<0.001. Treatment with extract and fraction significantly brought down the abnormal rise in serum urea (78.25±4.25 mg/dl and 108.86±4.8 mg/dl, respectively) when compared to diseased group (p<0.001). Likewise, a significant and abnormal rise was observed in serum Uric Acid levels in diseased group (6.25±1.05 mg/dl) compared to normal group (1.52±0.76 mg/dl). It was significantly brought down towards normal by administration of extract (2.52±0.56 mg/dl, p<0.001) and fraction (3.3±0.74 mg/dl, p<0.001), respectively when compared to lithiatic control group. A significant increase in creatinine levels were seen in serum of diseased animals (1.42±0.08 mg/dl) compared to normal group (0.52±0.03 mg/dl) at p<0.001.

Table 7: Effect of *Aerva lanata* Fraction on Serum Nitrogenous Substances

Group	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Normal	50.20±1.85	1.52±0.76	0.52±0.03
Lithiatic	158.72±4.26	6.25±1.05	1.42±0.08
Standard treated	65.6±3.5	1.85±0.55	0.65±0.05
Extract treated	78.25±4.25	2.52±0.56	0.82±0.08
Fraction treated	108.86±4.8	3.3±0.74	1.02±0.06

Conclusion

In conclusion, the treatment effectively restored normal renal function, as evidenced by the significant reduction in the elevated serum levels of key nitrogenous waste products: urea, uric acid, and creatinine. The marked elevation of these parameters in the diseased group indicated severe kidney damage and an inability to filter waste effectively. The fact that both the extract and fraction brought these levels down towards the normal range confirms that they preserved glomerular filtration rate (GFR) and protected the functional integrity of the kidneys from the lithiatic insult [19].

This action, combined with the previously observed effects on urinary parameters, demonstrates that *Aerva lanata* not only prevents stone formation but also safeguards overall kidney health by mitigating the toxic effects of crystal deposition and oxidative stress. This underscores its comprehensive therapeutic potential in urolithiasis [20].

Conflict of interest

No conflicts of interest are mentioned by the researchers.

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