



Original Research Article

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In-vitro Anti-Urolithiatic Activity of Aerial parts of *Aerva lanata* (L.) Juss

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Abstract

Urolithiasis is a complex process that occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Data from *in-vitro*, *in- vivo* and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. Medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. Various plant species of *Aerva lanata* (L.) have been reported to possess antiurolithiatic property. In this study aqueous, chloroform, benzene extracts of *Aerva lanata* (L.) and standard for dissolving kidney stones-calcium oxalate by an *in-vitro* model. To check their potential to dissolve experimentally prepared kidney stones-calcium oxalate by an *in-vitro* model for *Aerva lanata* (L.) and cystone as a standard compound collected from market. Phenolic compound isolated from the benzene and aqueous, flavanoids and steroids from aqueous fraction of the leaf. Aqueous fractions showed highest dissolution of stones as compare to others. Aqueous fraction was more effective in dissolving calcium oxalate (54.5±0.022%). Phenolic and flavanoids fractions of *Aerva lanata* (L.) were found to be more effective when compared to reference standard-formulation Cystone.

Keywords: *Aerva lanata* (L.), Antiurolithiatic, Calcium Oxalate.

Introduction

Aerva lanata (mountain knotgrass) is a woody, prostrate or succulent, perennial herb in the Amaranthaceae family of the genus *Aerva*, native to Asia, Africa, and Australia. The plant sometimes flowers in the first year.

A. lanata is a common weed which grows wild everywhere in the plains of India. The root has a camphor-like aroma. The dried flowers which look like soft spikes, are sold under the commercial names as Buikallan or Boor. It is one of the plants included in Dasapushpam, the ten sacred flowers of Kerala.

Uses

This plant is used for food for people and animals. The whole plant, especially the leaves, is edible. The leaves are put into soup or eaten as a spinach or as a vegetable. The plant provides grazing for stock, game and chickens. The plant is used as a traditional medicine for snakebites.

The plant is also used as a talisman against evil spirits, a good-luck talisman for hunters, and a talisman for the well-being of widows.

Aerva lanata (L.) Juss. Schultz is herbal plant; its extract exhibits significant therapeutic effects

such as antihyperglycaemic effect, Antiurolithic effect, anthelmintic effect, antihyperlipidemic effect, hepatoprotective activity, anti oxidant, and anti microbial activity etc. The *A. lanata* comprises the alkaloids, flavonoids, phenol, tannin, proteins, amino acids and carbohydrates respectively. The presence of minerals is responsible for conducting many activities in the body. The scientific validation is essential for acceptance of medicinal plant therapeutic effects against disorders.

Plant description:

Aerva lanata belongs to the family of Amaranthaceae. It is one of the important medicinal plants have ever grown throughout the plains of India. *Aerva lanata* is found to be an erect or prostrate herbaceous weed that is common throughout the hotter parts of India especially all over the plains, this extends up to an altitude of 3000m. It is also be present in Sri Lanka, Arabia, Egypt, tropical Africa, Java and Philippines. In India, it spreads in the states of Tamil Nadu, Andra Pradesh and Karnataka. *Aerva lanata* had been used in the Indian folk medicine for the treatment of diabetes mellitus, urinary calculi, hematemesis, bronchitis, nasal bleeding, cough, scorpion stings, fractures, spermatorrhea, to clear uterus after delivery and also to prevent lactation. Botanical name - *Aerva lanata* Family - Amaranthaceae Habitat - Herb Ayurvedic name - paashaanabheda, Gorakshayanjaa, Aadaanpaahi, Shatkabhedi Bengali name - Chaya Hindi name - kapurijadi, Gorakhabumdi . Medicinal properties: The plant *Aerva lanata* is diuretic and used in lithiasis. The roots are demulcent, diuretic, and useful in strangury, cure kidney stones .The root has a camphor like aroma and medicinally important. Decoctions of the flowers are used to cure stones. The roots are also used in the treatment of headache. The plant is regarded as a demulcent on the Malabar Coast. It has valued for cough in Ceylon and also as a vermifuge for children. The Meena tribals of the Sawaimadhapur district of Rajasthan orally used to give the juice of the roots of *Aerva lanata* to patients who are suffering from liver congestion, jaundice, biliousness and dyspepsia. They also give decoction of the whole plant to cure pneumonia, typhoid and other prolonged

fevers. Traditionally, leaves of *Aerva lanata* are used as sap for eye-complaints; an infusion is given to cure diarrhoea and kidney stone; and the root is used in snake bite treatment. The decoction of leaf is used as gargle for treating sore throat and it is also used in various complex treatments against guineaworm. In addition to the traditional uses, the plant is reported for a number of pharmacological activities viz., anthelmintic, demulcent, anti-inflammatory, diuretic, expectorant, hepatoprotective and nephroprotective, anti-hyperglycemic, anti-microbial, Antiurolithiatic, hypoglycemic, anti hyperlipidemic, anti-parasitic and anti -helmintic activities.

Kidney stones are hard, solid particles that form in the urinary tract. In many cases, the stones are very small and can pass out of the body without any problems. However, if a stone (even a small one) blocks the flow of urine, excruciating pain may result, and prompt medical treatment may be needed. Recurrent stone formation is a common part of the medical care of patients with stone disease. Calcium- containing stones, especially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10- 15%, uric acid 3-10% and cystine 0.5-1% .¹

In most of the cases the commonly occurring stones are calcium oxalate or magnesium ammonium phosphate type. Helps in spontaneous passage of calculi by increasing urine volume, pH and anti-calcifying activity. Balance the Inhibitor and promoter of the crystallization in urine and affects the crystal nucleation, aggregation and growth (Crystallization inhibition activity). Relieves the binding mucin of calculi (lithotriptic activity) Improved renal function². Herbs and herbal drugs have efficient pharmacological action and potent effects on body. Also, the overuse of synthetic drugs, which results in higher incidence of adverse drug reactions, has motivated humans to return to nature for safe remedies. The concept of 'Traditional' medicines for the developing countries. The problem of urinary stones or calculi is a very ancient one.

The incidence of urolithiasis is very common in Northern India compared to southern state.³

The study have been undertaken to evaluate *Aerva lanata* (L.) extracts and cystone as a standard for their possible potential to dissolve experimental kidney stone using a modified *in vitro* model to isolate the chemical constituent responsible for the activity.

Materials and Methods

Plant Material

Aerva lanata (L.) leaf samples were collected. The plant was identified and the specimen was dried in shade and stored in air tight container at 25⁰C for further study.

Extraction and Isolation

The leaves are pulverised and about 60 gms of powder was extracted with chloroform, benzene and aqueous in soxhlet⁴. All extracts were concentrated on a water bath and residue was dried in a desiccator.

Evaluation for Anti-urolithiatic Activity⁵

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 60⁰C for 4 hours.

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.

Estimation of Calcium oxalate by Titrimetry:

Weighed exactly 1mg of the calcium oxalate and 10mg of the extract/compound/standard and packed it together in semi evaluation. 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 37⁰C 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 4 Calcium.

The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve.

Results and Discussion

Qualitative chemical tests indicated the presence of phenolic compounds, flavnoids, steroids and Saponin in extract of *Aerva lanata* (L.). On basis of this fraction we performed *in vitro* Anti-Urolithiatic Activity by comparing different extracts of *Aerva lanata* (L.) with standard. % Dissolution of Calcium oxalate table is given below

Sr. No.	Group	% Dissolution Calcium Oxalate
1	Blank	0 %
2	Chloroform extract	40± 0.024
3	Aqueous extract	54.5±0.022
4	Benzene	43±0.032
5	Standard (Cystone)	46.5±0.038

The results shows % Dissolution of calcium oxalate by *in vitro* Anti-Urolithiatic Activity of extracted fraction of *Aerva lanata* (L.) drug. An aqueous extract at 10mg concentration produced higher dissolution of calcium oxalate as compare to other fraction. Aqueous shows higher dissolution as compare to others.

Conclusion

This study evaluate that antiurolithiatic activity of extract of *Aerva lanata* (L.) and isolated phenolic compound, steroidal 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 aqueous fraction show highest dissolution of calcium oxalate in comparison to other fractions. This study has given primary evidence for *Aerva lanata* (L.) as plant which possess Antiurolithotriptic property. This *in vitro* study has given lead data, and shown that phenolics and steroids form aqueous fraction is quite promising for further work in this regard.

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