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Antiurolithiatic Activity On Aerva Lanata

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Abstract

Aerva lanata plant belongs to the family *Amaranthaceae. Aerva lanata* is also referred as the Mountain knotgrass.The leaves of *Aerva lanata* is one of the indigenous medicinal plants used in the treatment of diabetes mellitus, kidney stones and its associated problems in Africa. The present study was undertaken to evaluate the in vitro antiurolithiatic activity of the medicinal plant *Aerva lanata* (mountain knotgrass). The present study indicates the physicochemical & phytochemical studies of Aerva lanata. Ethyl acetate extract showed its maximum efficiency in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the Ethyl acetate extract of *Aerva lanata* shows better results than Methanolic extract and it was quite promising for further studies in this regard.

Key Words: Aerva lanata, Physicochemical, Phytochemical, In-vitro pharmacological activity.

INTRODUCTION:

Urolithiasis is a process of forming stones in the kidney, bladder, and/or urethra (urinary tract). Reduced urine production or increased excretion of substances that might cause stones, such as calcium, oxalate, urate, cystine, xanthine, and phosphate, are linked to the formation of stones. The kidney's pelvis, where urine collects, is where the stones develop, and they may between microscopic and staghorn stones the size of the renal pelvis.
The pain associated with kidney stones typically comes on suddenly, is excruciatingly painful, colicky (intermittent), does not ease with movement, and radiates from the back, down the flank, and into the groyne. Vomiting and nausea are frequent.
The *Amaranthaceae* family includes the medicinal plant *Aerva lanata*, which is found all throughout India's plains. *Aerva lanata*, a perennial herbaceous plant that can reach heights of up to two metres (30 cm to two metres), is found throughout the warmer Indian plains, including the states of Telangana, Andhra Pradesh,

and Karnataka and Tamil Nadu, Sri Lanka, the Arabian regions, Egypt, Africa, Java, and the Philippines are additional nations where this plant may be found (Baladrin and Kloeke, 1988; Kareru). Tropical Africa, South Africa, Madagascar, Saudi Arabia, and tropical Asia are the natural habitats of the *Aerva lanata*. The species, which favours dryer locations than *Aerva javanica*, may be found in open woods on mountain slopes, on disturbed and waste land, abandoned cultivations, and coastal scrub at elevations ranging from level to 900 m (3,000 ft). In bare stretches of earth and arable fields, it is a typical weed.*Aerva lanata*contains chemical constituents such as Carbohydrates, Tannins, Saponins, Alkaloids, Flavonoids and other compounds such as methyl grevillate, lupeol, lupeol acetate benzoic acid, β-sitosteryl acetate and tannic acid. The various parts of the plant such as leaves, stem, flowers are widely used as aerial parts. It has anti-oxidant strength. It also decreases blood sugar levels. It is useful in treating asthma. It treats diarrhoea. It helps in kidney stone treatment It helps to get rid of intestinal worms.

METHODOLOGY:

Plant material:

The whole plant *Aerva lanata* was collected in the month of January 2013 from the local area of Tirupati, Andhra Pradesh, India. The plant material was identified and authenticated by Dr.K. Madhava cheety, Asst. Professor, Department of Botany, SV University, Tirupati. The voucher specimen (0919) of the plant was deposited at the college for further reference.

Physicochemical Standards:

Materials methods:

Such as total ash, acid insoluble ash, water soluble ash, extractive values were determined separately for air dried powdered leaves of this plant as per the official method.

I. Determination of total ash:

About 2 to 3 grams (accurately weighed) ground leaf powder was taken in a silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (450 degrees centigrade) until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to the air-dried powder. The procedure was repeated five times to get constant weight.

II. Determination of water-soluble ash:

The total ash was boiled with 25 ml of water for 5 minutes and was filtered through an ash less filter paper (Whatman No 41). It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and the water insoluble matter was weighed. The water-soluble ash was calculated by subtracting the water insoluble matter from the total ash.

III. Determination of acid insoluble ash:

The total ash obtained was boiled for 5 minutes with 10% w/v dilute hydrochloric acid and filtered through an ash less filter paper (Whatman No. 41). The filter paper was ignited in the silica crucible, cooled and acid insoluble ash was weighed.

IV. Extractive Values:

a) Determination of alcohol soluble extractive:

5 grams of the powder was macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during 6 hrs and allowing it to stand for 18 hrs. It was filtered rapidly taking precautions against loss of alcohol, and 25ml of the filtrate was evaporated to dryness in a tarred bottomed shallow dish at 105 degrees centigrade and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried powder.

b) Determination of water-soluble extractive:

About 5 gms of the powder was added to 50 ml of water at 80 degrees centigrade and to it 2 grams of kieselguhr was added and filtered. 5 ml of the filtrate was transferred to a tarred evaporating dish, the solvent was evaporated on a water bath, drying was continued for half an hour, finally it was dried in a hot air oven for two hours and weighed. The percentage of water-soluble extractive was calculated with reference to air dried drug.

c) Determination of loss on drying:

For the determination of loss on drying the following method was followed. About 1-2 gm of the powdered leaf was accurately weighed in a glass stoppered weighing bottle which is previously dried for 30 min in the drier. Then, the sample was gently shaken side wise for even distribution and dried in an oven at 100 degrees centigrade to 105 degrees centigrade by removing the stopper. It was cooled in a desiccator and again weighed. The loss on drying was calculated with the reference to the amount of air-dried powder.

A. Phytochemical Investigationof the *Aerva lanata*:

The phytochemical investigation of the plant involves the following

- Extraction of the plant material
- Fluorescence analysis
- Identification of the phytoconstituents
- The collected leaves of the plant were dried in the shade. Then the shade dried leaves were powdered to get a coarse powder. The coarse powder was subjected to a continuous percolation by using Soxhlet apparatus. Different solvents were used according to the polarity.

Materials Required:

Shade dried leaf powder of *Aerva lanata* was extracted by using different solvents.

- Ethyl acetate
- Methanol

Ethyl acetate extract of *Aerva lanata*:(EEAL)

About 650 grams of the dry powder extracted first with 2 litres of ethyl acetate at. Suitable temperature (depends on B.P) by continuous hot percolation method using Soxhlet apparatus. After completion extraction, the ethyl acetate extract was filtered and concentrated to dry mass by vacuum distillation. A green colour residue was obtained.

Methanol extract of *Aerva lanata*:(MEAL)

About 650 grams of the dry powder extracted first with 2 litres of methanol., at suitable temperature (depends on B.P) by continuous hot percolation method using Soxhlet apparatus. After completion extraction, the methanolic extract was filtered and concentrated to dry mass by vacuum distillation. A dark green colour residue was obtained.

Fluorescence analysis:

Fluorescence analysis of the drug was observed in day and UV light (365& 254 nm) by using powder and various extracts of the drug.

Analysis of drug powder:

The drug powder was treated separately with different solvents. The solvents used were 1N sodium hydroxide (aqueous), 1N sodium hydroxide (alcoholic), 1N hydrochloric acid, 50% nitric acid and methanol. Then they were subjected to fluorescence analysis in day and UV light.

Analysis of extracts:

The ethyl acetate, methanol, aqueous extracts were subjected to fluorescence analysis in visible and UV light.

IDENTICATION TESTS:

1) Test for alkaloids:

To the extract dilute hydrochloric acid will be added and filtered. The filtrate will be treated with various alkaloidal reagents.

a) Mayer's test:

The filtrate will be treated with Mayer's reagent: appearance of cream colour indicates the presence of alkaloids.

b) Dragendroff's test:

The filtrate will be treated with Dragendroff's reagent which leads to the appearance of reddish-brown precipitate indicates the presence of alkaloids.

2)Test for carbohydrates and reducing sugar:

The small quantities of the filtrate will be dissolved in 4ml of distilled water and filtered.

a) Molisch's test:

A small portion of the filtrate will be treated with Molisch's reagent and sulphuric acid. Formation of a violet ring indicates the presence of carbohydrates.

2)Fehling's test:

The extract will be treated with Fehling's reagent A and B. The appearance of reddish-brown colour precipitate indicates the presence of reducing sugar

3) Test for steroids:

Liebermann Burchard's test:

The extract will be treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green colour indicates the presence of steroids.

4) Test for proteins:

a) Biuret test:

The extract will be treated with copper sulphate solution, followed by addition of sodium hydroxide solution; appearance of violet colour indicates the presence of proteins.

b) Million's test:

The extract will be treated with Millon's reagent; appearance of pink colour indicates the presence of proteins.

5)Test for tannins:

The extract will be treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

6) Test for phenolic compounds:

a) The extract will be treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenolic compounds.

b) The extract will be treated with 10% sodium chloride solution; appearance of cream colour indicates the presence of phenolic compounds.

7) Test for flavonoids:

a) 5ml of extract will be hydrolysed with 10%sulphuric acid and cooled. Then, it will be extracting with diethyl ether and divided in to three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution will be added to the first, second and third test tubes respectively. In each test tube. Development of yellow colour demonstrated the presence of flavonoids

b) Shinoda's test the extract will be dissolved in alcohol, to which few magnesium turnings will beaded followed by concentrated HCL drop wise and heated, and appearance of magenta colour shows the presence of flavonoids.

8) Test for gums and mucilage:

The extract was treated with 25 ml of absolute alcohol, and filtered. The filtrate will examine for its swelling properties.

9) Test for glycosides:

When a pinch the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of conc. Sulphuric acid, formation of ring at the junction of two liquids indicates the presence of glycosides.

10) Test for saponins:

Foam test:

About 1 ml of the extract was diluted to 20 ml of with distilled water and shaken well in a test tube. The formation of foam in the upper part of test tube indicates the presence of saponins.

11) Test for Triterpenoids:

The substance was warmed with tin and thionyl chloride. Pink colour indicates the presence of triterpenoids.

IN-VITRO PHARMACOLOGICAL ACTIVITY:

Drugs and Chemicals:

Cystone (Himalaya), Tri's buffer, Di-Sodium oxalate, Calcium chloride, Sodium chloride, Methanol (Merck Pvt. Ltd. Mumbai) and Ethyl acetate other chemicals were procured from suppliers.

Groups:

Group I -control

Group II-Test Samples

¬ Ethyl Acetate Extract of *Aerva Lanata*

¬ Methanol Extract of *Aerva Lanata*

Group III- Standard Drug (Cystone)

Procedure for Anti- Urolithic Activity of *Aerva lanata*:

Calcium chloride was added to distilled water to create the calcium oxalate crystals in a lab setting. It was given time to react with the sodium oxalate and 2N sulphuric acid combination. In the presence of distilled water, the two solutions were given enough time to react. At the end of the process, precipitate calcium oxalate was produced. The resulting precipitate was collected, cleaned of contaminants with distilled water, and dried at 60°C. By combining 10 mg of the plant extract with 1 mg of calcium oxalate and packing it in an egg's semi-permeable membrane, the percentage of dissolution was calculated. The egg membrane was put into a sterile beaker containing 100 ml of 0.1M Tri's buffer. Four separate classes made up the experiment. The first class was empty and contained just one milligram of calcium oxalate. The second class is the positive control, which consists of 10 mg of Cystone (standard drug) and 1 mg of calcium oxalate. The final class had 1 mg of calcium oxalate together with 10 mg of *Aerva lanata* extract, whereas the third class contained 1 mg of calcium oxalate. All classes beakers underwent a two-hour incubation at 37° C. Following the incubation time, the contents of the semipermeable membrane were taken out and put into a clean tube. It was diluted further with 2 ml of 1 N sulphuric acid before being titrated against KMnO4.

Titration was carried out until the hue became pink. Finally, the starting concentration measured at the start of the operation was subtracted from the undissolved calcium oxalate. The difference gives an idea about the amount of dissolution of calcium oxalate crystals by the ethyl acetate and Methanol extracts of *Aerva lanata.*

RESULTS:

Fluorescence analysis for different extracts of *Aerva lanata*

TYPES OF SAMPLES	UV LIGHT 365nm	SHORT UV LIGHT254nm	VISIBLE LIGHT
Powder sample	Dark green	Dark green	Light green
Alcoholic sample	Purple with black	Purple with black	Greenish black
Ethyl acetate sample	Purple	Purple	Greenish black

***Aervalanata* powder on treatment with different chemical Reagents**

S.NO	POWDER+REAGENT	OBSERVATION
1	Powder+conc HNO3	Reddish Brown
2	Powder+conc.H2SO4	Black Colour
3	Powder+1N HCL	Light Brown
4	Powder+dil HNO3	Brown colour
5	Powder + 5% Ferric chloride	Greenish Brown
6	Powder+10% NaoH	Light Green
7	Powder + Iodine Solution	Brown colour
8	Powder + conc. Hcl	Light Green

9	Powder + dil. H2SO4	Pale brown

Physicochemical properties of *Aerva lanata*

S.NO	PARAMETERS	Values (%)
1	TOTAL ASH (%)	8.32%
2	WATER SOLUBLE ASH (%)	6.10%
3	ACID INSOLUBLE ASH	3.12%
4	WATER SOLUBLE EXTRACT (%w/w)	0.80%
5	ALCOHOL SOLUBLE EXTRACT (%w/w)	1.140%
6	LOSS ON DRYING	3.91%

Preliminary phytochemical screening for different extracts of *Aerva lanata*

S.NO	TYPE OF CONTITIUENTS	METHANOLIC EXTRACTS	ETHYL ACETATE EXTRACT
1	Carbohydrates	+	-
2	Proteins	+	-
3	Amino acids	-	+
4	Fats & oils	+	-
5	Saponins	+	+
6	Glycosides	-	+
7	Flavonoids	+	-
8	Alkaloids	+	+
9	Tannins	+	-
10	Phenolic compounds	-	+

(+) =Present (-) =Absent

Percentage of dissolution of Calcium oxalate crystals by *Aervalanata* extracts for Anti urolithic activity

S.NO	Category	Percentage of dissolution of Calcium oxalate crystals
1	BLANK (Control)	-
2	EEAL	87
3	MEAL	78
4	CYSTONE (Standard drug)	98

Ethyl acetate extract of *Aerva lanata* (**EEAL**)
Methanolic extract of *Aerva lanata* (**MEAL**)

WATER AND METHANOLIC EXTRACT OF *AERVA LANATA*

EGG FOR DECALCIFICATION WITH 10% ACETIC ACID

DECALCIFIED EGG

TITRATION WITH POTASSIUM PERMANGANATE FORDISSOLUTION OF CALCIUMOXALATE CRYSTALS

DISCUSSION:

The plant selected for the present study is *Aerva lanata* belongs to the family *Amaranthaceae*. Fluorescence analysis was performed with powder & plant extract by using visible light, long & short U.V light. The powder of *Aerva lanata* on treatment with various chemical reagents which gives a different colours. The results on various tests for physicochemical parameters such as ash values, extractive values, loss on drying will help in the correct identification of this plant for future work. Preliminary phytochemical screening for different extracts (methanol and Ethyl acetate) of *Aerva lanata* was performed. The results indicate that it contains alkaloids, flavonoids, glycosides, saponins, amino acids, phenolic compounds, proteins and carbohydrates.

In-vitro Pharmacological Studies:

The data on Invitro urolithiasis has been performed on the selected plant *Aerva lanata* by using the standard drug (Cystone). The work was performed by using Invitro antiurolithiatic model for calculating percentage dissolution of kidney stone.

This study evaluates the antiurolithiatic activity of ethyl acetate& methanolic extracts of *Aerva lanata*. The highest percentage i.e. 95% of calcium oxalate (CaOx) dissolution was observed for standard drug (Cystone). which had a percentage dissolution. From this study, it was observed that ethylacetate extract of *Aerva lanata* showed its highest dissolution of calcium oxalate than methanolic extract. This study has given primary evidence for *Aerva lanata* as the plant which possess lithotriptic property. This Invitro study has given lead data and shown that ethylacetate extract of *Aerva lanata* is quite promising for further studies in this regard.

Ethylacetate extract of *Aerva lanata* shows better dissolution of calcium oxalate than methanolic extract when compared to standard drug (Cystone).

CONCLUSION:

Alkaloids and flavonoids which were isolated from this plant may be responsible for its pharmacological activities. Finally, we conclude that Ethyl acetate extract of *Aerva lanata* shows better dissolution of calcium oxalate than methanolic extract when compared to standard drug (Cystone). The road ahead is to establish specific bioactive molecules, which might be responsible for these actions. Therefore. the cultivation, collection, and further pharmacological exploration of *Aerva lanata* are essential. The present study was carried out to evaluate Physicochemical, phytochemical and pharmacological activities of different extracts of *Aerva lanata*.

These properties of *Aerva lanata* justify its use as a better choice of natural drug in the treatment of Kidney stones. However, a detailed phytochemical and pharmacological evaluation should be necessary to deduce a definite conclusion.

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