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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of Anti Urolithiatic and Anti Anemic Activities of Leaves Extract of *Kalanchoe pinnata* and *Aerva lanata* by *In Vitro* and *In Vivo* Models

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ABSTRACT
Natural products with medicinal value are gradually gaining importance in clinical research due to their well-known property of no side effects as compared to drugs. Medicinal plants have been a source to control many diseases and anemia is no exception. In traditional systems of medicine including Ayurveda, many plants are claimed to be useful for anemia. The aim of the present investigation is to carry out phytochemical extraction and in vitro anti urolithic studies on the leaf ethanolic extracts of Kalanchoe pinnata and Aerva lanata. The in vitro anti-urolithic activity was studied as percentage inhibition of stones by aggregation assay method for ethanolic extracts by increasing concentrations taking cystone tablets as standard. The extract was screened for the anti-urolithiatic activity using aggregation method, the maximum inhibition rate shown at 1000µg/ml for both the Kalanchoe pinnata and Aerva lanata ethanolic extracts. The results indicated that the inhibition of growth of crystals increased with increase in concentration of the extract. This study also investigating anti-anemic potential against phenyl hydrazine induced anemia in rats. Phenyl hydrazine significantly decrease the RBCs, hemoglobin and hematocrit. Results are shown that the decrease in RBCs, hemoglobin and hematocrit significantly improved by the treatment with Kalanchoe pinnata & Aerva lanata extracts.
Keywords: *Kalanchoe pinnata, Aerva lanata, anti-urolithiatic activity, anti-anemic potential.*

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INTRODUCTION

The traditional systems of treatment such as Ayurveda, Unani, Siddha, western herbal medicine, traditional Chinese medicine and homeopathy use herbs for the treatment. Many researchers have prescribed about the importance of herbal medicine in the treatment of various diseases and because of the accessibility and cost effectiveness herbal treatment is still in practice by large number of practitioners. Over two billion people around the world suffer from anemia. Majority of populations are using dietary supplements and herbal medicines for the management of the anemic conditions. Anemia, a common public health problem, is characterized as decrease in erythrocyte mass or hemoglobin concentration in the blood leading to reduction in its oxygen carrying capacity. More than two billion people around the world suffer from anemia. Anemia is more common health problem in the developing countries. Dietary changes and iron supplementation are commonly preferred for the management of anemia. The formation of stones in urinary tract is worldwide, sparing no geographical, cultural or racial groups. Recurrence rates are estimated at about 10% year, totaling 50% over a 5-10 years period and 75% over 20 years. Urolithiasis is quite common in developing and under developed countries. Where the recurrence of endemic bladder stone is quite common due to the dietary proteins being mainly derived from plant sources. Urinary stone disease continues to occupy an important place in everyday urological practice. The average life time risk of stone formation has been reported in the range of 5-10%. A predominance of men over women can be observed with an incidence peak between the fourth and fifth decade of life a kidney stone is a hard, crystalline mineral material formed within the kidney or urinary tract. Kidney stones are a common cause of blood in the urine and often severe pain in the abdomen, flank, or groin. Kidney stones are sometimes called renal calculi. The condition of having kidney stones is termed nephrolithiasis. Urolithiasis is a complex process many remedies have been employed during the ages these stones are found in all parts of the urinary tract and kidney. Epidemiological studies revealed that nephrolithiasis is more common in men (12%) than in women (6%) and is more prevalent between the ages of 20-40 in both sexes. Urinary calculi are much more likely to be found in individuals who have sedentary occupation. Blackcock reported that

the incidence of urinary calculi was higher in administrative and sedentary personnel of the royal navy than in manual workers.

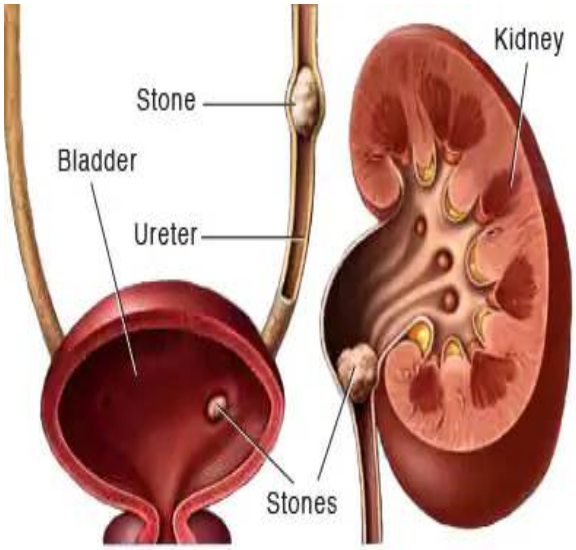

Figure 1: Kidney stones

Anaemia is a common nutritional deficiency disorder and global public health problem which affects both developing and developed countries with major consequences for human health and their social and economic development (WHO 2005). According to WHO (2004) reports, one third of the global populations (over 2 billion) are anaemic due to imbalance in their nutritious food intake. Iron is required for various cellular functions, including but not limited to enzymatic processes, DNA synthesis, oxygen transport and mitochondrial energy generation. As such, the symptoms of IDA can vary over a wide range. Shortness of breath, fatigue, palpitations, tachycardia and angina can result from reduced blood oxygen levels. This resultant hypoxemia can subsequently cause a compensatory decrease in intestinal blood flow, leading to motility disorder, malabsorption, nausea, weight loss and abdominal pain. Central hypoxia can cause headaches, vertigo and lethargy as well as cognitive impairment with several studies showing an improvement in cognitive functions once anaemia has normalised. It is well known that IDA significantly affects quality of life (QoL) with recent evidence demonstrating that treating IDA improves QoL, regardless of the underlying cause for anaemia.

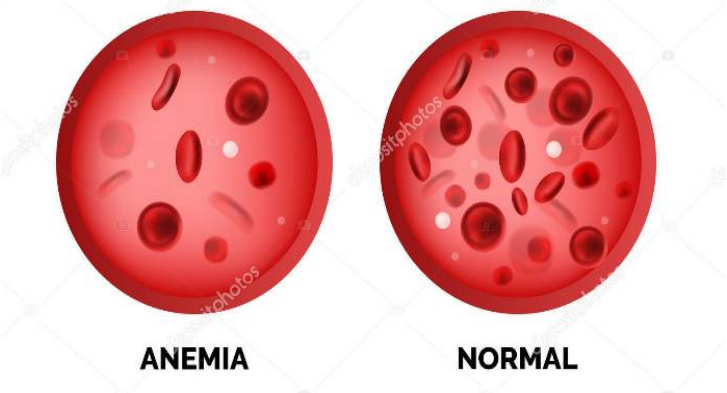

Figure 2: Anemia condition

The plant grows all over India in hot and moist areas, especially in Bengal. It is a succulent perennial plant that grows 1-1.5 m in height and the stem is hollow four-angled and usually branched. Leaves are opposite, decussate, succulent, 10-20cm long. The lower leaves are simple, whereas, the upper ones 3-7 foliate and are long-petioled. They are fleshy dark green that are distinctively scalloped and trimmed in red. Leaf blade pinnately compound with 3-5 leaflets, 10-30 cm; petiolules 2-4cm; leaflet blades oblong to elliptic, 6-8 x 3-5 cm, margin crenate with each notch bearing adormant bud competent to develop into a healthy plantlet apex obtuse 8. The leaves are furnished with rooting vegetative buds. Inflorescences terminal paniculate 10-40 cm. Flowers are many bell-like pendulous. Calyx tubular, 2-4 cm; Corolla reddish to purple, 5 cm, base sparsely ciliate; lobes ovate-lanceolate; stamens inserted basally on corolla; nectarscales oblong; follicles included in calyx and corolla tube. The fruit-pod with four septa and numerous, ellipsoid, smooth

striate seeds within. The plant flowers in Nov-Mar and fruits in April. It is astringent, sour in taste, sweet in the post digestive effect and has hot potency.

Figure 3: *Kalanchoe pinnata*

Aerva lanata is commonly called as Polphala of Amaranthaceae is a perennial shrub which is seen commonly in different waste parts of India. It is also known as Gorakha Ganga, belonging to the family amaranthaceous, in the genus Aerva and the species *lanata*. They are originated in India, Africa, as well as Australia. It is familiar in the name of knot grass. They are branching shrub. It is mainly used for urinary disorder. The plant has different name in different language astmabayda in Sanskrit, gorakhbuti in Hindi, cherula in Malayalam, pindi-kura in Telugu. Other than urinary disorder many pharmacological uses are identified like urolithiasis, diuretic activity, antimicrobial activity etc. It is one of the plants included in dasapushpam, the sacred flowers of Kerala.

Figure 4: *Aerva lanata*

MATERIAL AND METHODS

Ethical approval

This experiment was approved by the Institutional Animal Ethical Committee (IAEC) (I/IAEC/AGI/001/2022 WR ♀♂), School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal, Hyderabad, Telangana.

Study area

The investigation was conducted at Pharmacology Laboratory, Department of Pharmacology, School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal, Telangana.

Collection of plant material

A large quantity of fresh leaves of plants were collected in November 2022 from their natural habitat in the Yadadri Bhuvanagiri, Telangana. Their ethnopharmacological use was revealed through the interactions and knowledge of local communities and traditional health practitioners (THP). The identification of plant leaves was certified by a taxonomist in the Department of Pharmacognosy, the School of Pharmacy, under the Anurag Group of Institutions. A voucher specimen of leaves was deposited at the herbarium of the

college for future references. Information gathered included vernacular names and parts used in the preparation of herbal anti-urolithiatic and anti-anemic remidies.

Drying and processing of leaves

The collected leaves were soaked in freshwater for about 15 to 20 min to wash out the dirt and mud particles over their surface. After washing, they are shade dry, drying them at a room temperature of 37^{o}C for 2-3 weeks. The dried leaves were then milled to powder using an electric mill. The powdered plant material is kept in the dark in a plastic container at room temperature.

Figure 5: Dried *Kalanchoe pinnata* leaves

Figure 6: Dried *Aerva lanata* leaves

Preparation of extract

The collected powder is then subjected to the extraction process by a Soxhlet extraction method using 90% ethanol as a solvent at room temperature for 20-24 h. The ethanolic extract was then concentrated by simple distillation to dry. The collected extract was stored in desiccators and used for further pharmacological screening.

Experimental design

***In vitro* anti-urolithiatic activity**

Aggregation assay:

The calcium oxalate monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60°C in a water bath for 1 h and then cooled to 37°C overnight. The crystals were harvested by centrifugation and then evaporated at 37°C. Calcium oxalate (CaOX) crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37°C in the absence or presence of the plant extract after stopping the stirring. The cystone tablets are used as standard drug solution . The percentage aggregation inhibition rate (Ir) was then calculated by comparing the turbidity in the presence of the extract with that obtained in the control using following formula

Ir = (1−Turbiditysample/Turbidity control) × 100

***In vivo* anti anemic activity:**

Phenyl hydrazine induced anemia in rats:

A total of 42 male albino rats were used for this experiment. All the test animals were randomly divided into five groups (6 rats per group);

Group I: Vehicle control- received only normal saline orally once in a day during the entire study period

Group II: Phenyl hydrazine control(disease induced) - received PHZ i.p. 40 mg/kg body wt., once daily for 3 consecutive days.

Group III: Standard Group - received standard drug Livogen XT oral (9mg/kg, twice daily)

Group IV: Test Group I - received phenyl hydrazine i.p 40 mg/Kg body wt/day and *Kalanchoe pinnata* extract orally 200 mg/kg/day for entire treatment.

Group V: Test Group II – received phenyl hydrazine i.p 40 mg/Kg body wt/day and *Kalanchoe pinnata* extract orally 350 mg/kg/day for
the entire treatment periods.

Group VI: Test Group III - received Phenyl hydrazine i.p 40 mg/Kg body wt/day and *Aerva lanata extract* orally 200 mg/kg/day for entire treatment.

Group VII: Test Group IV- received Phenyl hydrazine i.p 40 mg/Kg body wt/day and *Aerva lanata* extract orally 350 mg/kg/day for entire treatment.

On day 4, blood samples were withdrawn from the orbital plexus of rat eye in EDTA vials and evaluated for blood parameters using Sahli's hemoglobinometer for Hb and Neubars chamber for RBC count. Treatment with extracts continued for all groups except Group I and II, Group III for a further period of 10 days. Blood samples were collected on day 14 from all the rats used in this experiment and evaluated for hematological parameters such as RBC, Hb, and packed cell volume (PCV). And for further parameters like WBC Count and Platelet Count samples sent to Vani diagnostics.

Figure 7: Collection of blood samples from orbital plexus of rat eye

Figure 8: Blood samples of rats

RESULTS AND DISCUSSION

The present study was designed to evaluate the anti-urolithiatic and anti-anemic activities of *Kalanchoe pinnata* and *Aerva lanata* leaves extract. The literature review conducted on this plant indicated the presence of various active constituents like glycosides, flavonoids, saponins, terpenoids, carbohydrates, alkaloids, etc. since these compounds were soluble in ethanol and was used as solvent for extraction.

In vitro anti-urolithiatic activity:

Aggregation assay :

The ethanolic extract of *Kalanchoe pinnata* and *Aerva lanata* leaves showed the inhibition of formation of stones in the kidney. The percentage of inhibition rate results were shown below.

Table 1: Percentage of inhibition rate of *Kalanchoe pinnata* ethanolic extract (KPEE)

S. No.	Conc. (ug/ml)	KPEE (%)	Standard (Cystone) (%)
1	200	52.72±0.12	61.58±0.22
2	400	59.83±0.22	73.23±0.34
3	600	75.68±0.24	81.65±0.41
4	800	88.94±0.34	92.2±0.36
5	1000	91.47±0.54	96.2±0.32

Table 2: Percentage of inhibition rate of *Aerva lanata* ethanolic extract (ALEE)

S. No.	Conc. (ug/ml)	ALEE (%)	Standard (Cystone) (%)
1	200	43.7±0.43	61.58±0.32
2	400	49.47±0.36	73.23±0.54
3	600	58.59±0.42	81.65±0.66
4	800	71.46±0.56	92.2±0.70
5	1000	80.28±0.52	96.2±0.65

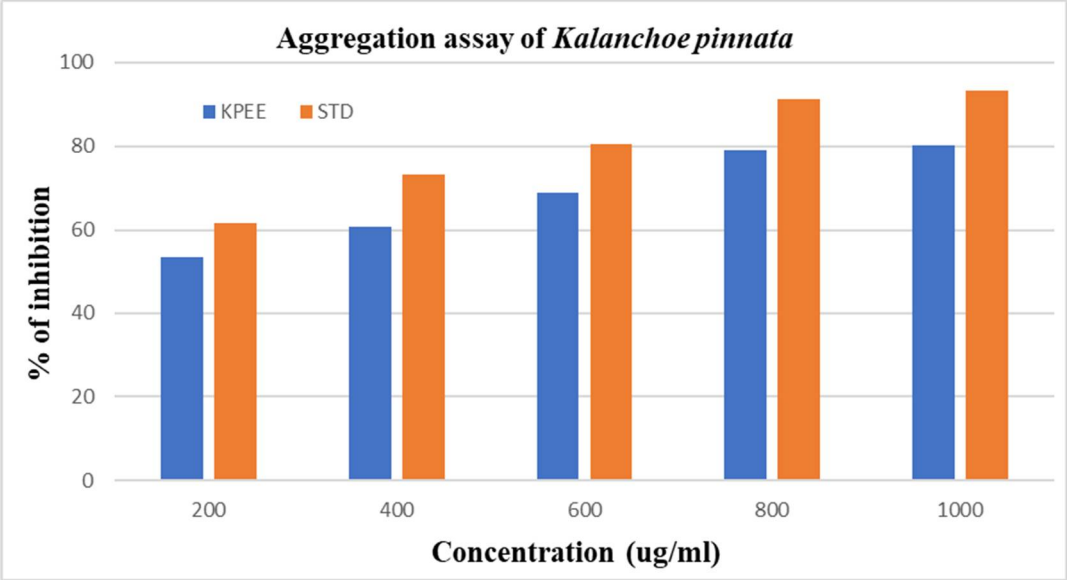

Figure 9: Aggregation assay of *Kalanchoe pinnata*

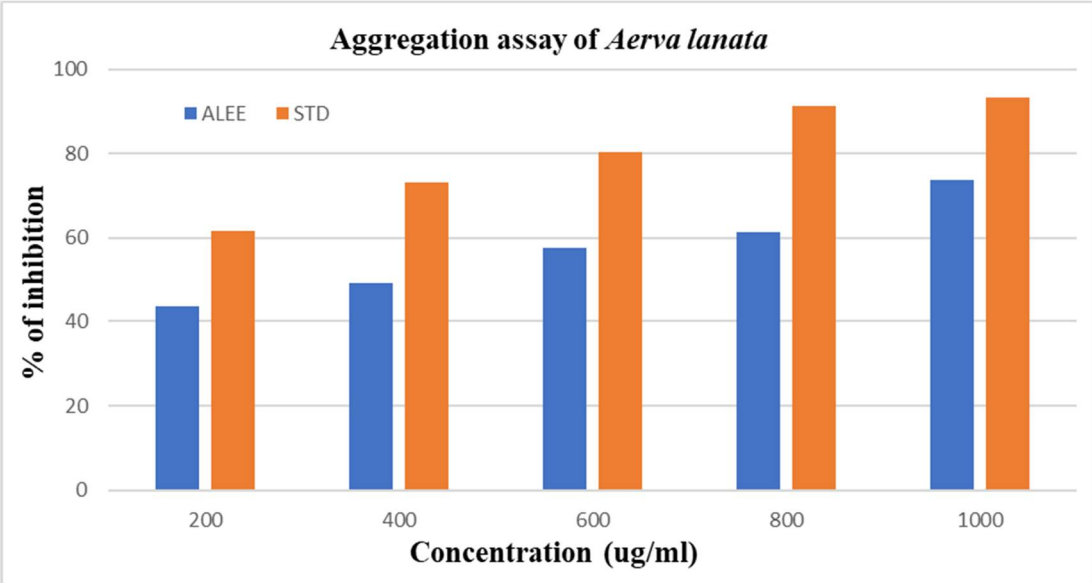

Figure 10: Aggregation assay of *Aerva lanata*

***In vivo* anti-anemic activity:**

Phenyl hydrazine induced anemia in rats:

The ethanolic extract of *Kalanchoe pinnata* and *Aerva lanata* leaves showed the increase in Hemoglobin value, RBC Count, WBC Count, Platelet count and HCT. The results were showed in the below table.

Table 3: Hematological findings of hemoglobin, RBC count, WBC count, platelet count and HCT percentage

Groups	Group 1	Group 2	Group 3
Parameters	Normal saline	Phenyl hydrazine induced	Standard (Livogen XT)
Hemoglobin (Gms)	12.8±0.26	9.0±0.17	14.2±0.22
RBCs count (M/C.mm)	7.32±0.50	2.88±0.32	9.72±0.43
WBC count (cells/C.mm)	14,530±0.95	9,540±0.83	33,830±0.60
Platelet count (M/C.mm)	7,44,000±0.43	5,74,000±0.45	10,86,00±0.52
HCT (%)	34.1±0.24	22.8±0.33	45.6±0.27

Groups	Group 4	Group 5	Group 6	Group 7

Parameters	KPEE (200mg)	KPEE (350mg)	ALEE (200mg)	ALEE (350mg)
Haemoglobin (Gms)	8.6±0.32	10.3±0.42	11.0±0.26	9.6±0.34
RBC count (M/C.mm)	6.39±0.22	8.21±0.31	7.87±0.31	8.76±0.54
WBC count (cells/C.mm)	15,390±0.43	17,910±0.26	22,870±0.46	29,480±0.52
Platelet count (M/C.mm)	7,23,000±0.62	8,70,000±0.50	9,86,000±0.51	7,89,000±0.56
HCT (%)	34.4±0.31	38.2±0.33	36.3±0.34	41.2±0.43

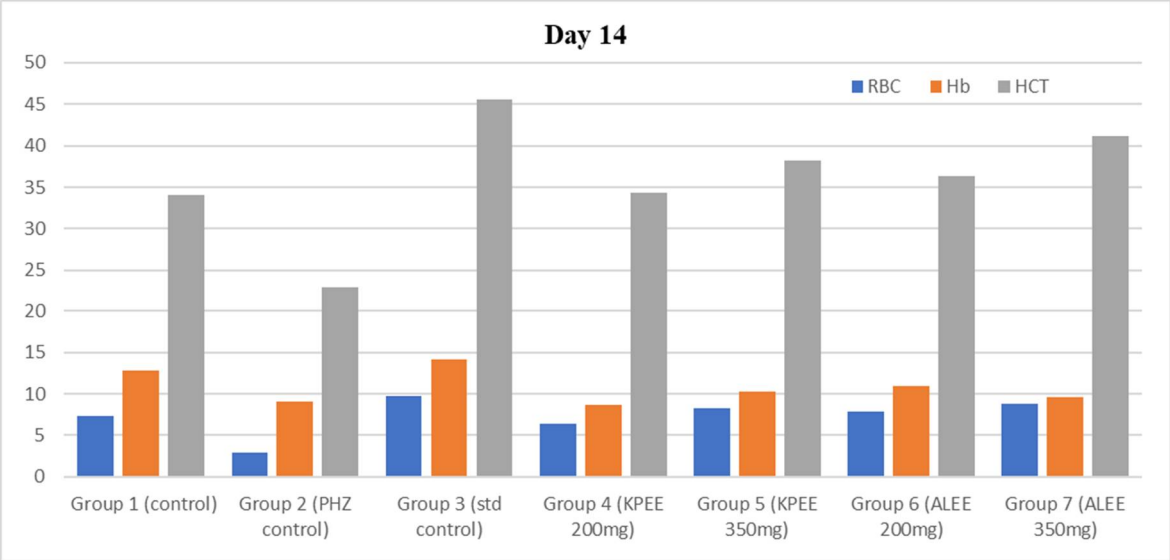

Figure 11: Hematological findings of hemoglobin, RBC count, WBC count, platelet count and HCT percentage

CONCLUSION

The ethanolic leaf extract of *Kalanchoe pinnata* and *Aerva lanata* exhibits anti-urolithiatic, and anti-anemic activity. It shows good anti-urolithiatic, and anti-anemic activity effects produced by of *Kalanchoe pinnata* and *Aerva lanata* leaves may be due to contents of tannins, sterols, flavonoids, alkaloids and carbohydrates.

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