In vitro Evaluation of Anti-urolithic and Anti-hemolytic activity of *Aerva lanata* Ethanolic extract

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ABSTRACT *Aerva lanata Linn, belonging to Amaranthaceae family is a medicinally important plant. Different plant parts are used in various ailments in folk medicine. The current study aimed at investigating the in vitro anti-urolithic and hemolytic activities of A. lanata. Therefore, its ethanolic extract has been investigated for possible anti-urolithic and anti-hemolytic effects. Aerva lanata Ethanolic extract (ALE) exhibited potent anti-hemolytic action in a dose dependent way and at higher concentrations also showed (2.58%) of haemolysis. In addition ALE showed anti-urolithic activity (63.13%). Hence the present study revealed that ethanolic extract of Aerva lanata might be used as adjuvant therapy for hemolytic anaemia as well as renal related disorders.*

Keywords: *Aerva lanata, anti-hemolytic, anti-urolithic.*

INTRODUCTION

Natural products derived from plants such as flavonoids, terpenes, alkaloids, anthraquinones, saponins, tannins, steroids, lactones and volatile oils received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and chemo-preventive effects.[Chevalier 2000] The active compounds of plants are used in folk, traditional and alternative medicine to treat various diseases [Nakachi et al 2000; Nwafor et al 2000; Houghton et al 2005; Howes et al 2003] and these have antioxidant, anti-inflammatory, anti-diarrheal, anti-microbial, anti-parasitic, antiviral activities, etc[Shahidi et al1992 ; Cowan 1999; Hammer et al 1999; Taylor 199 ; McGaw 2000; Elekwa et al 2003]. Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risks are evaluated.

Aerva lanata is an herb, found throughout tropical India as a common weed in fields and wasteland [Krishnamurthi 2003]. The plant is useful for curing diabetes. It is anthelmintic, demulcent and helpful in lithiasis, cough, sore throat and wounds [Pullaiah et al 2003]. The plant has been reported to possess anti-inflammatory [Vertichelvan et al 2000], diuretic [Udupihille et al 1998] and nephroprotective actions in rats [Shirwaikar et al 2004]. Recent times usage of plant/herbal medicine in treating several diseases is gaining momentum because of less side effects, easy access, natural origin and low cost. The occurrence of Calcium Oxalate stones is increasing in modern era. Though there are various methods in treatment there occurs a need for antilithic medicine from nature. The human body is abundantly made of erythrocytes which possess vital characteristics are extensively exploited in drug delivery. The erythrocyte membrane implicates in haemolysis during oxidative damage. Thus, the inhibition of *in-vitro* calcium-oxalate crystal formation and hemolytic activity by *Aerva lanata* was investigated.

MATERIALS AND METHODS

PLANT EXTRACT

Ethanolic extract of *Aerva lanata* was obtained as a gift sample from R. Rajendran, CEO, Green Chem Herbal formulations, Bangalore.

ANTIUROLITHIC ACTIVITY

It was done by procedure mentioned by [Agarwal et al 2000]. Calcium oxalate crystals (CaOx) were prepared by mixing calcium and sodium oxalate at 5mmol/L. Both solutions were equilibrated at 60°C in a water bath for 1hr and then cooled to 37°C overnight. The crystals were harvested by centrifugation and

then evaporated at 37°C. CaOx crystals were used at a final buffered with Tris-HCl-0.05mol/L at pH6.5. Experiment was conducted at 37°C in the absence or presence of *A. lanata* extract. The percent aggregation inhibition was calculated by comparing the turbidity at 620nm in the presence of standard at different concentration of (10-100mg/ml) with that obtained in the control using following formula

% inhibition=1-Turbidity sample ÷ Turbidity control×100

HEMOLYTIC ACTIVITY

It was done by procedure mentioned by [Saisha Vinjamuri et al 2015]

Preparation of red blood cells suspension: About five ml of the blood was collected from a healthy individual in a tube containing heparin. The blood was centrifuged at 1500 rpm for 3 min. The supernatant was collected and plasma was discarded. The pellet was washed for 3 times using 0.75% NaCl and centrifuged at 1500 rpm for 5 min. The cells were resuspended in normal saline to 0.05%**.** To 0.5 ml of cell suspension, 0.5 of different concentrations of plant extract (40, 60, 80, 100, 125, 250, 500 and 1000 µg/ml) in phosphate buffer saline (pH 7.2) was added. The mixture was incubated at 37^0C for 30 min and centrifuged at 1500 rpm for 10 min. The free hemoglobin in the supernatant was measured using UV/Vis Spectrophotometer at 540 nm. The phosphate buffer saline and distilled water were used as minimal and maximal hemolytic control [Gaurav Kumar et al 2001]

Percent (%) hemolytic activity was calculated using the formula:

% hemolysis = At – An/Ac-An x 100

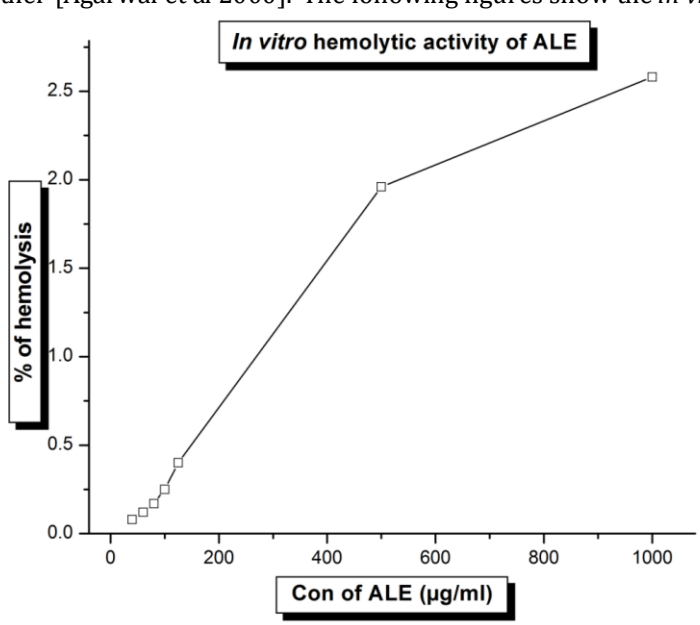
At – Absorbance of test sample

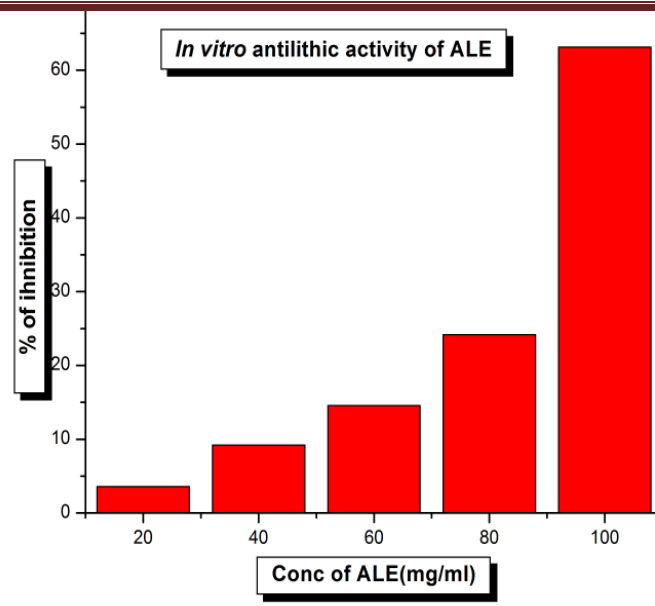
An – Absorbance of control (saline)

Ac – Absorbance of control (Distilled water)

RESULTS & DISCUSSION

Medicinal herbs are indispensable part of traditional medicine practiced all over the world due to their natural origin, low costs, easy access without side effects and ancestral experience [Cragg et al 2001]. The association of plants in human health is from ages. Phytochemicals produced by plants for their protection acts antagonist to human diseases. Haemolysis is the RBCs breakdown and release into surroundings. They are the main free radical targets. In this study even at high concentration of the plant extract less toxicity was observed by less percent of hemolysis. So plant posses less toxicity towards erythrocyte membrane which favors for further research. The incubation of solutions of calcium chloride and sodium oxalate resulted in formation of calcium oxalate crystals. The rate of nucleation was calculated by comparing the induction time in the presence of extract with control. At 620 nm after 30 minutes of reaction the optical density of each solution was monitored. It was observed that turbidity of plant extracts was lower than in control. This show less amount of calcium oxalate crystals were formed in presence of plant extracts. The increase in percentage inhibition of calcium oxalate crystals was shown with increase in the concentration of plant extracts. Thus, percentage inhibition and concentration were directly proportional to each other [Agarwal et al 2000]. The following figures show the *in vitro* results of ALE.





CONCLUSION

The results of the assay showed that the plant extract have both anti-urolithiatic and anti-hemolytic potential, thus further *in vivo* studies should be undertaken to confirm these results. As reported earlier the presence of some of the phytochemicals may be considered responsible for this inhibitory action.

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