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AGERATUM CONYZOIDES L.: A PLANT WITH PROMISING ANTILITHIC ACTIVITY

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ABSTRACT*: - Ageratum conyzoides* L. has been used to treat various diseases including urinary stone diseases, since ancient time in India. The inhibition of *in-vitro* calcium-oxalate crystal formation by its extract was also investigated by two different nucleation assays. In these assays, the aim was to evaluate the effectiveness of different concentrations of the extract on calcium oxalate crystallization *in-vitro*. In both the assay % inhibition for calcium oxalate crystal formation was found directly proportional to the increase in concentration of the plant extract with maximum inhibition of 55.36% at 1000 mg/ml concentration (Atmani *et al.* assay). Thus *Ageratum conyzoides* L. was found to be a potent and promising antiurolithiatic agent, which is in accordance with its use in traditional medicine.

KEYWORDS: *Ageratum conyzoides* L., urolithiasis, *in-vitro*, calcium oxalate (CaOx), calcium oxalate monohydrate (COM).
ABBREVIATIONS: mmol: milimolar, OD: optical density

INTRODUCTION

Urolithiasis is one of the oldest and most wide spread diseases known to man. In India people living in different states utilize different plants for curing urolithiasis (Chitme *et al*. 2010). Urolithiasis is derived from the Greek words "ouron" (urine) and "lithos" (stone).It is considered as the third most common affliction of the urinary tract (Khan *et al*. 2012). The deposition or formation of stones in any part of the urinary system i.e the kidney, the ureters or the urinary bladder is called Urolithiasis. A stone is an aggregation of solute materials from urine such as calcium, oxalate, phosphate and uric acid which forms stone. In India two distinct 'stone belts' have been identified in Northwestern region. One stone belt starts from Amritsar in North and while passing through Delhi and Agra ends up in Uttar Pradesh. The other belt starts from Jamnagar in the West Coast and extends inward towards Jabalpur in Central India. The Bhopal district lies in the second stone belt region. In India, calcium oxalate is found to be the most predominant constituent of urolithiasis. Urine is normally supersaturated with most stone forming salt components, as well as contains chemicals that prevent or inhibit crystal development in urinary tract. However, the presence of certain molecules raise the level of super saturation of salts needed to initiate crystal nucleation or reduce the rate of crystal growth or aggregation and prevents stone formation (Nayak *et al.* 2011).

Though technological advancements have made dramatic improvement in the removal of urinary stones still some of the drawbacks of these methods exists which includes their being too costly for a common man and recurrence of stone formation along with a number of other side effects (Prasad *et al*. 2007). Hence search for new antilithiatic drugs from natural sources has assumed greater importance as herbal drugs are cost effective and cause least side effects. In ayurveda many plants having the property of disintegrating and dissolving the stone are referred to as "pashanbheda".

Ageratum conyzoides L. is a fast growing, aromatic, herbaceous weed of the rainy season. It is known since ancient times for its medicinal properties (Kambooj *et al.* 2008). It (leaf juice) is reported to be used as antilithic agent by the traditional communities in India (Tailor *et al.* 2012, Arora *et al.* 2005). Similar work by using different methods has been reported by some workers. Singh *et al.* (2005) and Ramana *et al.* (2007) reported that its roots, while Khan *et al.* (2011) reported that the hydroalcolohlic extract of its whole plant, are used for the treatment of renal calculi. Mukund (2011) and Joy *et al.* (2012) reported the use of aqueous and alcoholic extracts of its leaves against ethylene glycol induced urolithiasis in rats. Tailor *et al.* (2013) reported the antilithic activity of ethanolic and aqueous extracts of its leaves and roots on calcium oxalate and calcium phosphate stones in *in-vitro* assay by using semipermeable membrane of egg. Gindi *et al.* (2013) reported the antiurolithiatic potential of aqueous extract of its leaves against gentamicin induced urolithiasis in rats. Several ethnobotanical studies, have also reported its use in lithiasis. Sharma *et al.* (2011) reported the use of its leaves in the treatment of kidney stone and urinary tract troubles. Similar work has been reported by Ahmad *et al.* (2009); Pant *et al.* (2010) and Tiwari *et al.* (2012). Hossan *et al.* (2010) reported that the Murong tribe in Bangladesh uses its leaves and root in case of cloudy urination in women.

Ageratum conyzoides was studied for the presence of various chemical constituents (Usman *et al.* 2013, Hussiena *et al.* 2010, Amadi *et al.* 2012, Sarin *et al.* 2011, Kamboj *et al.* 2011, Oyewale *et al.* 1999, Ukwe *et*

al. 2010, Dash *et al.* 2011, Tailor *et al.* 2013, Sultana *et al.* 2012) .

Literature on traditional medicines show the use of fresh decoction of leaves of *Ageratum conyzoides* (leaf juice) in treating urinary stones but no such study by the *in-vitro* methods considered in this study has been undertaken. Thus the aim of the present study is to evaluate the effectiveness of aqueous extract of leaves of *Ageratum conyzoides* L. for its antiurolithiatic activity using two *in-vitro* nucleation assays. In nucleation assay the effectiveness of different concentrations (100-1000 mg/ml) of the extract on calcium oxalate crystallization *in-vitro* was studied.

MATERIALS AND METHODS:

All chemicals used were of high purity Merck grade. Sodium oxalate was obtained from Burgoyne reagents, while sodium chloride and calcium chloride dihydrate were procured from Sigma Aldrich. The leaves of *Ageratum conyzoides* L. were collected from Kolar road, Bhopal, Madhya Pradesh, during the month of October 2012 and the plant was identified with the help of regional Floras (Oommachan 1976) and taxonomists and finally confirmed with the herbarium of Botanical Survey of India (BSI), Allahabad, with voucher specimen No. 1212-88.01-355. Fresh plant, after collection was shade dried at room temperature and then grinded. The plant material (100 g leaf) was boiled in distilled water, filtered and then the filtrate was further concentrated after which it was dried at 30-40°C temperature for obtaining extract. The dried extract was stored in refrigerator for further use.

The two different nucleation assays utilized were methods proposed by N.A.M. Farook *et al.* (2004) and Atmani *et al.* (2000).

In method proposed by N.A.M. Farook *et al.* (2004) the study of crystallization without inhibitor and with it was undertaken. Crystalloid forming solutions and inhibitor solutions were prepared in distilled water. 1L each of 0.01 M solutions of calcium acetate and sodium oxalate were prepared while that of the different plant extracts and cystone (a marketed herbal formulation for urolithiasis) were prepared at a concentration of 200mg/ml. The extracts of various plants were compared with that of cystone for their antilithiatic activity. Crystallization was started by taking the inhibitor solution in a beaker and allowing the salt forming solutions to run into it dropwise.

The resulted mixture was boiled on a heating mantle (Elite scientific instruments co.), cooled and then centrifuged (Remi equipments, Bombay) after which the supernatant was rejected. The final weight of precipitate (ppt) was noted after being kept in hot air oven (Ambassador model no.). Whole experiment was carried out at room temperature. Percentage efficiency of inhibitor was obtained by using the following formula given by Farook *et al.,* (2004) –

$$\% \text{ Inhibition} = \frac{\text{Wt. of ppt in blank set} - \text{wt. of ppt in experimental set}}{\text{Weight of ppt in blank set}} \times 100$$

Where, wt. = weight, ppt = precipitate

Data were expressed as mean values of three independent experiments as Mean ± Standard deviation.

In method proposed by Atmani *et al.* (2000), the study of crystallization without inhibitor and with it was undertaken in order to assess the inhibiting capacity of the plant extract. Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5 mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 mL of calcium chloride solution mixed with 100 mL of extracts at different concentrations. Crystallization was started by adding 950 mL of sodium oxalate solution. The temperature was maintained at 37ºC. The OD of the solution was monitored at 620 nm using spectrophotometer (Systronics digital spectrophotometer 166) after 30 minutes. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. Data was represented in percentage inhibition. The growth of crystals was expected due to the following reaction:

$$CaCl2 + Na2C2O4 \rightarrow CaC2O4 + 2NaCl$$

RESULT AND DISCUSSION:

In method by N.A.M. Farook *et al.* (2004) the aqueous extract was found to show maximum 41.93±0.07 % inhibition of calcium oxalate crystallization. Cystone a prescribed medicine for renal calculi showed highest inhibition (90.55±1.27%).

In method by Atmani *et al.* (2000) incubation of the metastable solutions of calcium chloride and sodium oxalate resulted in the formation of calcium oxalate crystals. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. The O.D. was monitored at 620nm after 30 minutes. The turbidity of solution in the presence of herb extract was lower in comparison to the control, showing that oxalate crystallization was less in the presence of extract. Data represents that % inhibition for calcium oxalate crystal formation was directly proportional to the increase in concentration of the plant extract, with minimum inhibition of 34.07% at 100 mg/ml to a maximum inhibition of 55.36% at 1000 mg/ml extract concentration. The reduction of stone forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing

salts such as calcium oxalate and calcium phosphate, thus aqueous extract of leaves of *Ageratum conyzoides* could be analyzed further *in vivo* and further characterization of its active compound could lead to a new candidate drug for the patients with urolithiasis.

CONCLUSION:

Most of plant species which have now been considered to be endangered or threatened were once available in abundance on this earth, but due to ignorance or over exploitation their existence is in danger. As *Ageratum conyzoides* L. is considered to be a weed growing in abundance during rainy season, common man mostly removes it from fields or gardens. But nature has endowed it with ample medicinal qualities which could be harnessed for alleviating many human diseases. Thus, steps could be undertaken to make people aware about its medicinal properties so as to conserve this plant species for future generations. Thus, this study provides a basis for utility of *Ageratum conyzoides* in the treatment of urinary stones specially kidney stone which is in accordance with earlier studies. Literature search has shown that no such work on antilithic potential of leaves of *Ageratum conyzoides* L. by above discussed two nucleation assays has been undertaken in Bhopal district. Thus to the best of our knowledge this is the first report on potent antilithic potential of leaves of *Ageratum conyzoides* L. found growing in Bhopal district.

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