Evaluation of invitro antiurolithiatic activity of ethanolic and methanolic leaf extracts of acalypha indica linn.

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Abstract
The claims of Acalypha indica.L antiurolithiatic effect have not been established experimentally. The present study is designing to evaluate the invitro anti-urolithiatic activity of Ethanolic and methanolic leaf extracts of Acalypha indica.L. Urolithiasis is a common and a major cause of morbidity worldwide. Acalypha is widely utilized in traditional medicine systems. Traditional communities in India use this plant as an analgesic, anti-inflammatory, anti-helminthic, anti-bacterial, anti-fungal, anti-tubercular, antioxidant, molluscicidal, anti-venom, anti-ulcer, etc. So far, the claims of Acalypha indica antiurolithiatic activity have not been confirmed experimentally. The present study is designed to evaluate the invitro anti-urolithiatic activity of Ethanolic and methanolic leaf extracts of Acalypha indica Linn.

Introduction
Urolithiasis is a pathological condition of the Genitourinary System which is referred to as formation of calculi or stones within the urinary tract. This includes the formation of stones in the kidneys and the ureters obstructing the flow of urine and causing pain and other symptoms. In some cases, Urolithiasis may also be formed in the bladder or urethra. Urolithiasis also known as Kidney stone disease is when a Solid piece material (kidney stone) occurs in the urinary tract¹. Kidney stones typically form in the kidney and leave the body in the urine stream [1]. A small stone may pass without causing symptoms [1]. If a stone grows to more than 5 millimetres (0.2 in) it can cause blockage of the ureter resulting in severe pain in the lower back or abdomen1, 2. A stone may also result in blood in the urine, vomiting, or painful urination [1]. About half of people will have another stone within ten years.

Classification
Kingdom : Plantae
Sub kingdom: Tracheobionta
Super division: Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Rosidae
Order : Malpighiales
Family : Euphorbiaceae
Genus : Acalypha L.

vernacular names
Hindi : Kuppi, Khokli
Telugu: Kuppichettu
English: Copperleaf, Indian acalypha, Indian-nettle
Tamil: Kuppiameni
Sanskrit: Harita-manjari
Malayalam: Kuppiameni
Kannada: Kuppigida

Geographical Distribution
Acalypha indica occurs widely throughout the Old World tropics. In Africa, it occurs in Nigeria in West Africa and further widely throughout tropical Africa and the Indian Ocean islands. It also occurs in India, South East Asia, Yemen, and Oceania. It has been introduced to the New World Tropics [3].

Fig.1: Acalypha indica Linn.

CHEMICAL CONSTITUENTS
The aerial parts contain a cyanogenic glycoside called acalyphin (a 3-cyanopyridone derivative) as well as flavonoids, such as kaempferol glycosides mauritianin, clitorin, nicotiflorin, and biorobin. Tannins, β-sitosterol, acalyphamide, aurantiamide, succinimide and flindersin (a pyanoquinolinone alkaloid) have also been isolated [4].

MEDICINAL USES*15
- Analgesic activity
- Anti-inflammatory activity
- Anti-helminthic activity
- Anti-bacterial and Anti-fungal activity
- Anti-tubercular activity
- Anti-oxidant activity
- Molluscicidal activity
- Neuro-protective and Neuro-Therapy Activity
- Post-coital Antifertility Activity
- Anti-venom Activity
- Antiulcer Activity

Methods And Materials
Plant Material
Acalypha indica plants were collected from various places in and around the areas of Guntur. Whole plants of it were collected and identified by comparing with herbarium specimens. The leaves were collected from the plants. The leaves were air-dried and powdered.

Preparation of Ethanolic and methanolic leaf extracts of Acalypha indica by maceration About 50gms of dry powder was extracted with 150mL methanol and 150mL ethanol by macerating individually for 7days. The two extracts were filtered using Wattman filter paper, No. 1. The extracts were concentrated on a water bath and residues were dried in desiccators. All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phytoconstituents. TLC studies were done for identifying the presence of constituents which are detected in chemical tests and to know how many extracts are present in each extracts. This separated parameter is subjected for physical, chemical and spectral study (UV). And positive results for two fractions were taken for pharmacological evaluation.

PRILIMINARY PHYTOCHEMICAL ANALYSIS
Phytochemicals of the selected plants were carried out by using ethanolic and powdered form of the plant following Harborne (1973) Trease and Evans (1989).

Tab.1: Phytochemical tests for constituents

<table>
<thead>
<tr>
<th>Plant Constituents Test/ Reagents</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1. Mayer’s Reagent</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2. Dragendorff Reagent</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>II. Carbohydrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Molish’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2. Fehling soln.test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3. Benedict test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fructose</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III. Glycosides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Keller killani test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2. Legals test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3. Bontragers test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IV. Phytosterols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Liebermann’s test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. Liebermann’s Burchard’s test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V. Phenol Compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Ferric Chloride test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2. Lieberman test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>VI. Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>VII. Proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 of the 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.

**Results and Discussion**

The invitro Anti-Urolithiatic activity was performed by comparing different extracts of leaves of *Acalypha indica*. % Dissolution of Calcium oxalate table is given below:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>% Dissolution of Calcium oxalate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>Ethanolic extract</td>
<td>42±0.023559651</td>
</tr>
<tr>
<td>3</td>
<td>Methanolic extract</td>
<td>44±0.021549879</td>
</tr>
</tbody>
</table>

A result obtained showed that the methanolic extract of leaf has the higher capacity to inhibit the crystal formation and aggregation as compared to ethanolic extract of leaf.

In the present study, the anti-califying properties of *Acalypha indica* were explored by in vitro method. After nucleation, crystal growth is the next major step of stone formation. The driving force for crystallization is a reduction in the potential energy of the atoms or molecules when they form bonds to each other. The crystal growth process starts with the nucleation stage when several atoms or molecules in a supersaturated liquid start to form clusters. Nucleation is the formation of a solid crystal phase in a solution. It is an essential step in renal stone formation. The inhibitory potency of the *Acalypha indica* was tested on the growth of the most commonly occurring kidney stones, calcium oxalate monohydrate. A concentration dependent inhibition was observed using *Acalypha indica*.

**Conclusion**

We expected that this investigation would provide encouragement for further exploration into new drugs for the prevention and treatment of urolithiasis. The present investigations provide useful information on antiurolithiatic activity of leaves of *Acalypha indica*. The extract showed dissolution of stones (calcium oxalate and calcium phosphate). Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of the plant *Acalypha indica* in treating urolithiasis.

**References**

1. Kala, Chandra Prakash; Sajwan (2007). "Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through
11. Beena Joy, Molly Mathew, Anti-oxidant studies and chemical investigation of ethanol extract of Acalypha indica Linn. Recent Progress in Medicinal Plants, Volume 27