



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

Konda Ravi Kumar¹, A.Prathyusha*².

¹ Professor, Department of Pharmaceutical Chemistry, Hindu College of Pharmacy, Amaravathi Road, Guntur-522002. A.P, India.

² Assistant Professor, Department of Pharmacology, Hindu College of Pharmacy, Amaravathi Road, Guntur-522002. A.P, India.

Article History	Abstract
Received on: 05-01-2020 Revised on : 06-03-2020 Accepted on : 07-03-2020	The claims of <i>Acalypha indica</i> .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 <i>Acalypha indica</i> .L. Urolithiasis is a common and a major cause of morbidity worldwide. <i>Acalypha</i> 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 <i>Acalypha indica</i> 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 <i>Acalypha indica</i> Linn.
Keywords <i>Acalypha indica</i> Linn., Urolithiasis, Invitro Evaluation, Methanolic and Ethanolic Extracts.	
*Corresponding Author Mrs. A.Prathyusha Email: apratyusha1990@gmail.com https://doi.org/10.37022/jpmhs.v3i1.15	

This article is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License. Copyright © 2020 Author(s) retain the copyright of this article.



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 abdomen^{1, 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 arial parts contain a cyanogenic glycoside called acalyphin (a 3-cyanopyridone derivative) as well as flavanoids, 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]. The chemicals that attract cats are the iridoid compounds isodihydronepetalactone and isoridomyrmecin [5].

MEDICINAL USES⁶⁻¹⁵

- 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

Plant Constituents Test/ Reagents	Methan oc extract	Ethanol ic extract	Wat er extr act
I. Alkaloids	-	-	-
1. Mayer's Reagent	+	+	-
2. Dragendorff Reagent	+	+	-
II. Carbohydrates			
1. Molish's test	+	+	-
2. Fehling soln.test	-	+	-
3. Benedict test	+	+	-
Glucose	+	-	-
Fructose	-	-	-
Galactose	+	+	-
Lactose	+	+	-
Starch	+	-	-
III. Glycosides			
1. Keller killani test	+	+	-
2. Legals test	+	+	-
3. Bontragers test	+	+	-
IV. Phytosterols			
1. Liebermann's test	+	-	-
2. Liebermann's Burchard's test	+	-	-
V. Phenolic Compounds			
1. Ferric Chloride test	-	+	-
2. Lieberman test	-	+	-
VI. Tannins	+	+	-
VII. Proteins			

1. Xanthoproteic test	+	+	-
2. Biuret test	+	+	-
VIII. Amino Acids	+	+	-
IX. Flavonoids	+	-	-
X. Gums & Mucilages			
Molish test	-	-	-

'+' Present, '-' Absent

Chemicals

Calcium oxalate, 0.1 M TRIS buffer, 1N Sulphuric acid, 0.9494 N KMnO₄.

Evaluation For Anti-Urolithiatic Activity¹⁶

Step-1: Preparation Of Experimental Kidney Stones (Calcium Oxalate Stones) By Homogenous Precipitation

Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H₂SO₄ were allowed to react in sufficient quantity of distilled water to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of (2N H₂SO₄), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipitates freed from traces of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60°C for 4 hours.

Step-2: Preparation Of Semi-Permeable Membrane From Farm Eggs

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 and the contents squeezed out completely from the decalcified 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.

Step-3: Estimation Of Calcium Oxalate By Titrimetry

Weighed accurately 1mg of the calcium oxalate and 10mg of the extract/compound/ and packed it together in semi permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate). Place the conical flask of all groups in an incubator, preheated to 37°C for 2 hours, for about 7-8 hours. Removed the contents of semi-permeable membrane from each group into a test tube Added 2 ml of

1N sulphuric acid and titrated with 0.9494 N KMnO₄. 0.9494 N KMnO₄ equivalents to 0.1898mg of 4 calcium.

The amount of undisclosed calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve.

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:

Tab.2: % Dissolution Of Calcium Oxalate Of Methanolic And Ethanolic Extracts

S.No	Group	% Dissolution of Calcium oxalate
1	Blank	0.00
2	Ethanolic extract	42±0.023559651
3	Methanolic extract	44±0.021549879

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 calcifying 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*.L in treating urolithiasis.

References

1. Kala, Chandra Prakash; Sajwan (2007). "Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through

- institutional networking and capacity building". *Current Science*. 93 (6): 797-806.
2. Saad, Bashar & Said, Omar (2011). *Greco-Arab and Islamic Herbal Medicine: Traditional System, Ethics, Safety, Efficacy, and Regulatory Issues*. John Wiley & Sons. p. 80. ISBN 9780470474211.
3. *Acalypha indica* L. Indian *Acalypha*, on India Biodiversity Portal (<http://indiabiodiversity.Org/species/show/32852>). Accessed 31.07.2017.
4. Hong, Francis (2004). "History of Medicine in China" (PDF). *McGill Journal of Medicine*. 8 (1): 7984. Archived from the original (PDF) on 2013-12-01.
5. Scaffidi, Adrian; Algar, Dave; Bohman, Björn; Ghisalberti, Emilio L; Flematti, Gavin (2016). "Identification of the Cat Attractants Isodihydronepetalactone and Isoiridomyrmecin from *Acalypha indica*". *Australian Journal of Chemistry*. 69(2): 169. doi: 10.1071/CH15476 (<https://doi.org/10.1071%2FCH15476>).
6. M Aminuar Rahman, Sitiesh C Bachar and Mohammed Rahmatullah, Analgesic and Antiinflammoraty activity of methanolic extract of *Acalypha indica* L., *Pak. J. Pharm. Sci.*, Vol.23, No.3, July 2010, pp.256-258
7. B. Chengaiah, K. Mahesh Kumar, M. Alagusundaram, C. Sasikala, C. Madhusudhan Chetty, In- vitro Antihelmintic activity of root of *Acalypha Indica* Linn. *International Journal of PharmTech Research*, Vol.1, No.4, pp 1499-1502, Oct-Dec 2009.
8. Lakshmi, S, Antibacterial potential of *Acalypha Indica* against human pathogens, *International Journal of Current Research*, Vol. 1, pp. 001-004, January, 2010.
9. Omar, R., Ali Rahman, Z., Latif, M.T., Lihan, T. and Adam J.H, In Vitro Antibacterial and antifungal properties of *Acalypha indica* (KUCING GALAK) Proceedings of the Regional Symposium on Environment and Natural Resources 10-11th April 2002 Vol 1: 503-508.
10. Renu Gupta, Bandana Thakur, Pushpendra Singh, H.B. Singh, V.D. Sharma, V.M. Katoch & S.V.S. Chauhan, Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates, *Indian J Med Res* 131, June 2010, pp 809-813.
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
12. S. Sharma, T. Singh and R. Vijayvergia, Molluscicidal activity of some medicinal plants, *Journal of Herbal Medicine and Toxicology* 3 (2) 155-157 (2009).
13. Ernie H. Purwaningsih, Nurhadi Ibrahim, Hamdani Zain , Arjo Tedjo, Neuro-protection and neuro-therapy effects of *Acalypha indica* Linn. Water extract ex vivo on musculus gastrocnemius Frog, *MAKARA, KESEHATAN*, VOL. 12, NO. 2, December 2008: 70-75
14. K. Rajendran1, Annie Shirwaikar, Maneesh Mehta, R. Vijaya Bharathi, In vitro and in vivo anti-snake venom (*Daboia russelli*) studies on various leaf extracts of *Acalypha indica* Linn, *International Journal of Phytomedicine* 2 (2010) 217-220
15. S. Kalimuthu, P. Rajesh, V. Rajesh Kannan, B. Balamurugan, T.M. Chandrasekar, Antiulcer activity of Methanolic extract of *Acalypha indica* Linn. (Euphorbiaceae) by Pylorous Ligtore and Swim Stress Induced Ulceration, *Journal of Pharmacy Research* 2010, 3(11), 2779-2783.
16. Byahatti,Pai Vasanth Kumar. Effect of phenolic compounds from *Bergenia cliata* (Haw). *Sternb.leaves on experimental kidney stones*. *Ancient Science of Life* 2010;30(1):14-17.