Journal of Young Pharmacists, 2024; 16(2):223-228.
https://www.jyoungpharm.org

Original Article

Phytochemical Screening, Acute Toxicity and Evaluation of *in vitro* Antiurolithaitic Activity of Ethanolic and Methanolic Root and Leaf Extracts of *Acalypha indica* Linn.

Anil Kumar*, Ashish Kumar Sharma

Department of Pharmacology, NIMS Institute of Pharmacy, NIMS University Rajasthan, Jaipur, Rajasthan, INDIA.

ABSTRACT

Background: A plethora of novel therapeutic strategies, notably urolithiasis therapy, are currently being spoke of into traditional medicine. A particularly significant herbal remedy that is employed in indigenous medicine for its antiurolithiatic property is *Acalypha indica* Linn. Although *A. indica* is widely used in traditional medicine as an antiurolithiatic, there is currently no scientific data to substantiate this claim. Moreover, deriving inferences from the suggested theories and limited *in vitro* investigation casts doubt on its significance. Hence, this work focuses on the preliminary evaluation, acute oral toxicity and *in vitro* evaluation for antiurolithiatic activity of *A. indica*. **Materials and Methods:** Phytochemical evaluation was carried out using various standardized tests. Acute toxicity studies for Methanolic and Ethanolic extract of Roots and Leaves was conducted according to OECD guideline 420 using Wistar albino rats. The inhibitory activity of the extracts with concentrations of 100, 200, 400, 600, 800 and 1000 µg/mL on the nucleation of Calcium oxalate (CaOx) crystals was determined by a spectrophotometric assay. **Results:** A phytochemical screening resulted in identification of secondary metabolites such as carbohydrates, alkaloids, flavonoids, saponins and terpenoids with varying amounts in each extract. There were no signs of toxicity in the initial investigation on a single rat given a dosage of 2000 mg/kg of methanolic and ethanolic root and leaf extracts. The maximum inhibition was observed in Methanolic Root Extract (71.26%) at a concentration of 1000 mg/mL, which is followed by Ethanolic Leaf Extract (60.91%) at the same concentration. **Conclusion:** *Acalypha indica* Linn. was discovered to possess therapeutic value in the management of urolithiasis.

Keywords: *Acalypha indica*, Urolithiasis, *in vitro*, Nucleation Assay.

Correspondence:
Mr. Anil Kumar
Ph.D Research Scholar, Department of Pharmacology, NIMS Institute of Pharmacy, NIMS University Rajasthan, Jaipur, Rajasthan, INDIA.
Email: anil.kims@gmail.com

Received: 13-01-2024;
Revised: 28-02-2024;
Accepted: 04-03-2024.

INTRODUCTION

Urolithiasis, often referred to as renal calculi or kidney stones, is a condition in which accumulation of solid deposits of salts and minerals occurs in kidneys. The frequency of stones is influenced by dietary changes, hereditary, ethnic, environmental and topographical factors. The condition or ailment underlying the stone development essentially determines the likelihood of its recurrence. Accordingly, the proportion of cases of urinary calculi fluctuates between 1% to 20%.[1] There are growing evidence that nephrolithiasis increases the chance of developing chronic kidney disease.[2,3] Recommendations on future diagnosis and treatment are based on the chemical composition of the stone. Stones are frequently developed by integrating various compounds. There are four types of stones: pharmaceutical stones, infectious stones (magnesium ammonium phosphate, highly carbonated apatite), non-infectious stones (calcium oxalate, calcium phosphate and uric acid) and inherited stones (cystine, xanthine).[1] A subsequent recurrence rate of 26% was determined in five years by a recent comprehensive review of first-time stone predecessors.[4] Every individual's urolithiasis therapy strategy is unique and depends on a variety of factors. The size, quantity, position and composition of the stones are among the key factors that determine the course of therapy. The significant variability in lithiasis among patients makes it challenging to construct therapy algorithms that incorporate all of the aforementioned criteria. While there are several ways to relieve symptoms promptly, but none guarantee the prevention of a recurrence.[5] Furthermore, substantial, serious adverse events and post-treatment distress are associated with modern surgical and therapeutic interventions for kidney stones.[6]

A plethora of novel therapeutic strategies, notably urolithiasis therapy, are currently being spoke of into traditional medicine. As per the World Health Organization, the utilization of traditional medicine in conjunction with modern medical remedies can yield multiple benefits for a variety of medical illnesses.[7] These

OPEN ACCESS

EPUBL editing. publishing. technology

DOI: 10.5530/jyp.2024.16.29

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

advantages include expanded treatment options, hospice care for the management of modern medical treatment adverse effects, enhanced psychological and emotional wellness and heightened patient satisfaction. A particularly significant herbal remedy that is employed in indigenous medicine for its various benefits such as antiurolithiatic, anti-venomous, wound-healing and anti-malarial properties is *Acalypha indica* Linn., which is a member of the Euphorbiaceae family. Although *A. indica* is widely used in traditional medicine as an antiurolithiatic, there is currently no scientific data to substantiate this claim. Moreover, deriving inferences from the suggested theories and limited *in vitro* investigation casts doubt on its significance. It's crucial to assess the active ingredient and the part of the plant that is most effective in treating urolithiasis. This glaring gap has highlighted the requirement for scientific validation. Hence, this work focuses on the preliminary evaluation, acute oral toxicity and *in vitro* evaluation for antiurolithiatic activity of *A. indica*.

MATERIALS AND METHODS

A. indica plant was collected from various places in and around the areas of Mandya district of Karnataka state. Whole plants of it were collected and identified by comparing with herbarium specimens. The procured plants of *A. indica* was sent to "Central Ayurveda Research Centre", Bengaluru for the authentication process. Upon thorough examination and verification, the board certified that the plant specimens were authentic as per the requirements (Ref: RRCBI-18572). After authentication, leaves and roots were sorted from the whole-plant. The leaves and roots were air dried and mashed until it turned into powder. The powder was vacuum dried at 25°C and 50% humidity, thus preventing any oxidation. The powdered roots and leaves of the plants were stored in air-tight container and to prevent accidental hygroscopy and a desiccator was added. The powder was used for the extraction process and the extraction was done using methanol and ethanol using Soxhlet apparatus. A basic phytochemical analysis of plants extracts (Roots and leaves) for phytoconstituents was performed using the standard phytochemical screening procedures mentioned by Sofowara 1993.[8]

Acute toxicity studies for Methanolic and Ethanolic extract of Roots and Leaves was conducted according to OECD guideline 420[9] using Wistar albino rats. The extracts were dissolved in Tween 80 before administration into the animals. Female Adult Wistar albino rats weighing around 180-200 g were used for the testing acute oral toxicity. They remained in polypropylene cages at 25±2°C with relative humidity 45-55% under 12 hr light and dark cycles. All the animals were adjusted to the laboratory surroundings for a week earlier use. They were nourished with standard animal feed and water *ad libitum*. The animals were divided into 5 groups; each group containing 6 animals. The initial dose was chosen at 2000 mg/kg with the consideration that there were *in vivo* and *in vitro* toxicity data. One animal in each group is from the sighting study dosed at 2000 mg/Kg. Animals (e.g. with the rat, food but not water should be withheld) were fasted overnight prior to administration. Group I was considered as control, whereas group II to V acted as test groups. After administration of the extracts, food was withheld for a further 3 to 4 hr. The animals were then individually observed (with special attention during the first 4 hr) for possible behavioural changes, allergic reactions (skin rash, itching), eyes and mucous membrane, or any alterations unceasingly and mortality for next 24 hr and the observation was continued for 14 days. The Institutional Animal Ethics Committee clearance was obtained prior conducting the study (NIMSUR/IAEC-01/2022/02).

The inhibitory activity of the extracts with concentrations of 100, 200, 400, 600, 800 and 1000 µg/mL on the nucleation of Calcium oxalate (CaOx) crystals was determined by a spectrophotometric assay as described by Bawari *et al.*[10] Solution of Calcium Chloride ($CaCl_2$) and Sodium Oxalate ($Na_2C_2O_4$) 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. 1 mL of each concentration was mixed with 1 mL $CaCl_2$ solution followed by the addition of 1 mL $Na_2C_2O_4$ solution. Final mixtures were incubated for 30 min at 37°C. The Optical Density (OD) of the mixtures was measured at 620 nm with an UV-visible spectrophotometer (UV-1900I, SHIMADZU, Japan). Percentage inhibition of nucleation was calculated using the following formula:

$$\% \, inhibition = \left[1 - \frac{OD\ Test}{OD\ Control}\right] X\ 100$$

Where % Inhibition is percentage of inhibition, OD Test is optical density with plant extract/standard drug and OD Control is optical density without plant extract/standard drug. CaOx crystallization was observed under a light microscope in the presence and absence of extracts. Cystone, a standard herbal therapy in kidney stones was used as a standard/control. The basic calculations were performed using Microsoft Excel and the add-on facility of graph design was used to create the graphs.

RESULTS

The study involved the extraction of compounds from the roots and leaves of *Acalypha indica*, commonly known as Indian Acalypha. Two different solvents, methanol and ethanol, were used for extraction, resulting in Methanolic Root Extract (MRE), Methanolic Leaf Extract (MLE), Ethanolic Root Extract (ERE) and Ethanolic Leaf Extract (ELE). The yields of these extracts were determined, with MRE and MLE yielding 5.76% and 14.83%, respectively, while ERE and ELE yielded 3.0% and 13.0%, respectively.

After obtaining the extracts, a phytochemical screening was conducted to identify secondary metabolites present in each extract. The results revealed the presence of various secondary metabolites, including carbohydrates, alkaloids, flavonoids,

Table 1: Phytochemical Constituents of roots and leaves of *A. indica* using its methanolic and ethanolic extracts.

Phytochemicals	MRE	MLE	ERE	ELE
Carbohydrates	+	-	+	+++
Proteins	-	+	-	+
Alkaloids	+	+ +	+	+
Phenol	-	+	+	+
Saponin	-	+	-	+++
Tannin	-	-	+	+++
Flavonoids	-	+ +	+	+++
Glycosides	-	+	-	+
Steroids	-	+	-	+
Terpenoids	-	-	+	++

– indicates absence of the constituents, + indicates the presence, ++ indicates moderate presence and +++ indicates high presence of constituents.

saponins and terpenoids. These compounds play important roles in the biological activities and therapeutic potential of plant extracts. The findings indicated that both methanol and ethanol leaf extracts contained higher amounts of phytoconstituents compared to their respective root extracts. This suggests that the leaves of *Acalypha indica* may be a richer source of bioactive compounds compared to the roots. The differences in phytoconstituent content between the methanol and ethanol extracts could be attributed to the varying solubility of different compounds in these solvents.

The results, as summarized in Table 1, provide valuable information about the chemical composition of the extracts, which can contribute to understanding the potential medicinal properties of *Acalypha indica*. MRE indicated the presence of carbohydrates and alkaloids, MLE indicated proteins, alkaloids, phenols, saponins, flavonoids, glycosides and steroids. ERE showed the presence of carbohydrates, alkaloids, phenols, tannins, flavonoids and terpenoids and ELE indicated high presence of carbohydrates, saponins, tannins and flavonoids.

The initial investigation involved assessing the potential toxicity of methanolic and ethanolic root and leaf extracts of *Acalypha indica*. A single rat was administered a relatively high dosage of 2000 mg/kg of each extract and the results revealed no signs of toxicity. This indicates that the tested extracts did not cause adverse effects at the given concentration within the observed timeframe. Specifically, there were no reported deaths and no notable behavioural abnormalities were observed in any of the groups studied. The absence of mortality and abnormal behaviour is a crucial indicator of the safety of the extracts at the tested dosage. This suggests that the extracts, whether derived from the roots or leaves and regardless of the solvent used (methanol or ethanol), did not induce acute toxic effects under the conditions of the experiment.

Furthermore, the evaluation extended to both methanolic and ethanolic leaf extracts of *Acalypha indica* and similar results were obtained. At doses up to 2000 mg/kg body weight, neither the methanolic nor the ethanolic leaf extracts showed signs of toxicity. This consistent lack of adverse effects across different extracts and solvents strengthens the conclusion that the tested extracts are safe within the specified dosage range.

In the context of *in vitro* antiurolithiatic activity, various extracts of *A. indica* were evaluated using a nucleation assay, with Cystone serving as the standard reference. The extracts included Methanolic Root Extract (MRE), Methanolic Leaf Extract (MLE), Ethanolic Root Extract (ERE) and Ethanolic Leaf Extract (ELE). The assessment involved computing the percentage of inhibition in comparison to the standard, Cystone. The results of this antiurolithiatic activity are summarized in Table 2. The findings indicate that the extracts of *A. indica* exhibited a range of inhibitory effects on nucleation, suggesting potential antiurolithiatic properties. The percentage of inhibition varied among the extracts and concentrations tested. Notably, the Methanolic Root Extract (MRE) demonstrated the highest inhibition at a concentration of 1000 mg/mL, reaching 71.26%. This suggests that, at this concentration, MRE effectively interfered with nucleation, showcasing a strong antiurolithiatic potential (Figure 1).

Following closely, the Ethanolic Leaf Extract (ELE) also displayed significant inhibitory activity, recording a percentage inhibition of 60.91% at the same concentration (1000 mg/mL). This indicates that ELE has promising antiurolithiatic properties as well, though slightly less potent than MRE at this specific concentration. Conversely, the Ethanolic Root Extract (ERE) exhibited the minimum percentage of inhibition, especially at a concentration of 100 mg/mL, where it recorded 19.05%. This suggests that ERE, particularly at lower concentrations, may have a comparatively weaker antiurolithiatic effect compared to the other extracts. These results provide insights into the potential antiurolithiatic activity of *A. indica* extracts and highlight the importance of the specific extract and concentration in determining efficacy (Figure 2).

Table 2: *In vitro* Percentage Inhibition of Methanolic and Ethanolic Roots and Leaf Extracts of *A. indica*.

Concentration mg/mL	% Inhibition of MRE	% Inhibition of MLE	% Inhibition of ERE	% Inhibition of ELE
100	38.10	38.10	19.05	28.57
200	57.14	45.71	45.71	45.71
400	63.83	55.32	57.45	55.32
600	64.71	56.86	58.82	56.86
800	69.57	63.77	63.77	65.22
1000	70.11	66.67	67.82	68.97

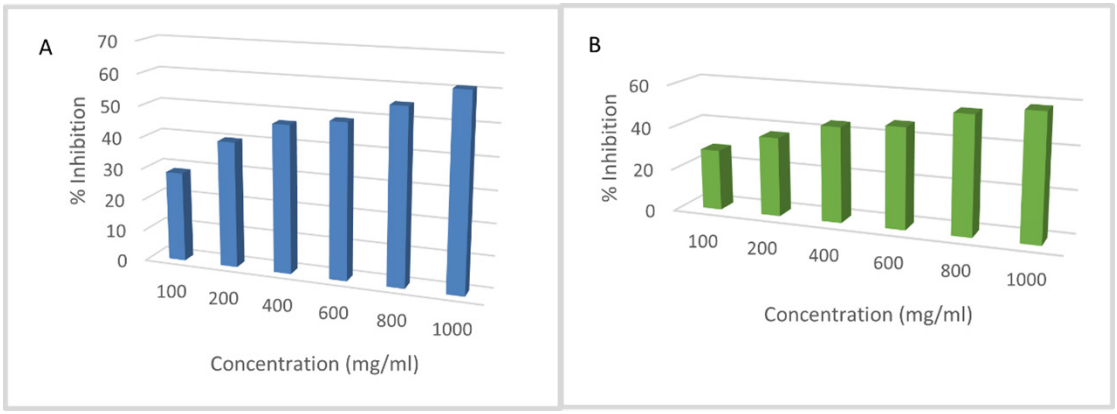

Figure 1: The *in vitro* Percentage Inhibition of A: ELE and B: MLE.

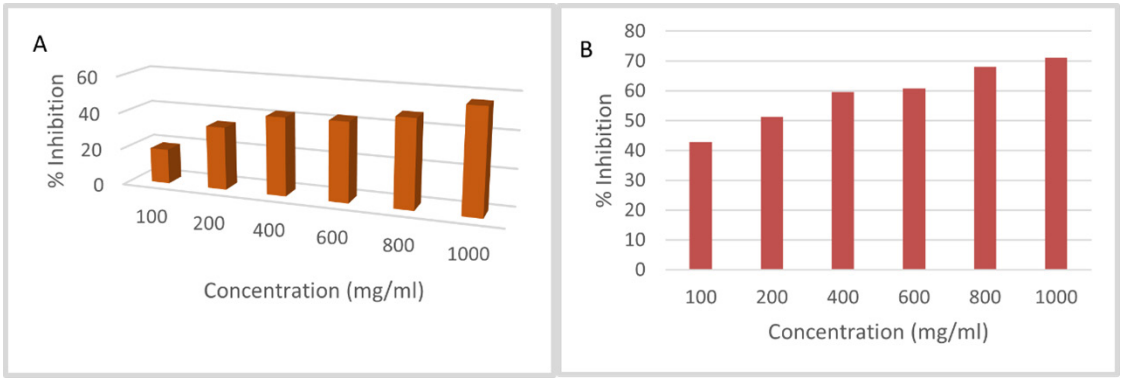

Figure 2: The *in vitro* Percentage Inhibition of A: ERE and B: MRE.

DISCUSSION

Prior to the development of modern-day medicines, humanity relied mostly on Earth's resources for health management. *A. indica* is closely associated with Ayurvedic medicine, which is practised by elder Indian generations.[11] The extensive research and investigation of the literature revealed the Indian Acalypha's potent antiurolithiatic properties.[12-14] The purpose of this study was to identify the phytochemicals found in the plant and assess the antiurolithiatic efficacy of ethanolic and methanolic extracts of *A. indica* leaves and roots *in vitro*.

The results of preliminary phytochemical screening of plant resulted in presence of carbohydrates and alkaloids in MRE; proteins, alkaloids, phenols, saponins, flavonoids, steroids in MLE; carbohydrates, alkaloids, phenols, tannins, flavonoids, terpenoids in ERE; and carbohydrates, saponins, tannins, flavonoids, terpenoids in ELE. This was seen in studies conducted by Mohan C *et al.*[15] and Nazri NM *et al.*[16] The acute oral toxicity of the MRE, MLE, ERE and ELE did not result in any significant toxicities and hence was found to be safe up to 2000mg/kg body weight of rats.

The extracts of *Acalypha indica* exhibits promising antiurolithiatic properties attributed to the diverse array of phytochemicals present. Proteins in MLE may influence crystal growth and structure maintenance, while alkaloids, known for their diuretic properties, contribute to the elimination of urinary stones. Phenols and flavonoids, acting as antioxidants, can mitigate oxidative stress associated with urolithiasis and influence crystallization processes. Saponins, with their detergent-like

action, may disperse crystals, preventing agglomeration. Steroids, potentially possessing anti-inflammatory effects, could alleviate inflammation linked to stone formation. Carbohydrates, alkaloids, phenols, tannins, flavonoids and terpenoids in *Acalypha indica* collectively play roles in energy metabolism, diuretic effects, antioxidation, anti-aggregation, astringency and anti-inflammation, contributing synergistically to the overall antiurolithiatic efficacy of the plant extract. This holistic phytochemical composition underscores the potential of *Acalypha indica* as a natural remedy for preventing and managing urolithiasis.

By employing *in vitro* techniques-which were ascertained by nucleation assay—different phases of the development of calcium oxalate crystals were investigated in conjunction with the plant preparation. The development of the stone first occurs with the nuclei coalesce. The inhibitory action on different stages of CaOx crystallisation was identified by the duration course of the turbidity observed in counterfeit urine at varied doses. The most important stage in the production of stones is called nucleation, which starts when the stone salts in the solution combine to create loose clusters that can become larger by incorporating other elements.[17] CaOx crystals are generated by immersing artificial urine containing sodium oxalate and calcium chloride.[18] The turbidity of the reaction solutions was measured and compared to the control to assess the impact of plant extracts on CaOx crystallisation.

Alkaloids, identified in *A. indica*, have been linked to the prevention of crystal nucleation, possibly through interference with crystal growth pathways. Phenolic compounds, known for their antioxidant properties, may play a crucial role in mitigating oxidative stress associated with urolithiasis and could directly influence crystal nucleation. Flavonoids, recognized for their diverse biological activities, might contribute to the inhibitory action by influencing the solubility of minerals in urine and preventing the aggregation of crystals. The combination of these phytochemicals, along with other constituents like saponins and terpenoids, could act synergistically in hindering the nucleation process.[13] These findings not only provide valuable insights into the molecular mechanisms underlying the antiurolithiatic effects but also emphasize the potential of *Acalypha indica* extracts as a natural intervention for preventing the formation of urinary stones.

These data suggest that the plant acts more as a preventative measure than a cure, which can greatly lower the likelihood of recurrence of stone formation. Further *in vivo* research is being performed to acquire a better understanding of the plant's putative mode of action and the dose at which it demonstrates antiurolithiatic efficacy.

CONCLUSION

Acalypha indica Linn. was discovered to possess therapeutic value in the management of urolithiasis. There were no adverse events detected in the acute toxicity investigations even after 14 days of monitoring. The antiurolithaitic activity of *A. indica* was demonstrated by the considerable nucleation inhibitory properties observed in the methanolic and ethanolic roots and leaf extracts during the *in vitro* nucleation experiment.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Girish B S, Department of Pharmacy Practice, PES College of Pharmacy, Bangalore and Dr. Joel M. Johns, Department of Pharmacy Practice, PES College of Pharmacy, Bangalore for screening and reorganizing the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Mr. Anil Kumar: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing Original draft along with editing. Dr. Ashish Kumar Sharma: Supervision, Validation, Resources and Project Administration.

ABBREVIATIONS

OECD: The Organization for Economic Cooperation and Development; **CaOx:** Calcium Oxalate, **$CaCl_2$:** Calcium Chloride; **$Na_2C_2O_4$:** Sodium Oxalate, **OD:** Optical Density; **UV:** Ultraviolet; **MRE:** Methanolic Root Extract; **MLE:** Methanolic Leaf Extract; **ERE:** Ethanolic Root Extract; **ELE:** Ethanolic Leaf Extract.

REFERENCES

1. Tzelves L, Türk C, Skolarikos A. European association of urology urolithiasis guidelines: where are we going?. European Urology Focus. 2021;7(1):34-8.
2. Chien TM, Lu YM, Li CC, Wu WJ, Chang HW, Chou YH. A retrospective study on sex difference in patients with urolithiasis: who is more vulnerable to chronic kidney disease?. Biology of sex Differences. 2021;12(1):40.
3. Hsu HH, Ueno S, Miyakawa H, Ogawa M, Miyagawa Y, Takemura N. Upper urolithiasis in cats with chronic kidney disease: prevalence and investigation of serum and urinary calcium concentrations. Journal of Feline Medicine and Surgery. 2022;24(6):e70-5.
4. Ferraro PM, Curhan GC, D'Addessi A, Gambaro G. Risk of recurrence of idiopathic calcium kidney stones: analysis of data from the literature. Journal of nephrology. 2017;30:227-33.
5. Tzelves L, Berdempes M, Mourmouris P, Mitsogiannis I, Skolarikos A. Optimal delivery of follow-up care for the prevention of stone recurrence in urolithiasis patients: improving outcomes. Research and Reports in Urology. 2022:141-8.
6. Abou Chakra M, Dellis AE, Papatsoris AG, Moussa M. Established and recent developments in the pharmacological management of urolithiasis: an overview of the current treatment armamentarium. Expert opinion on pharmacotherapy. 2020;21(1):85-96.
7. Traditional Medicine. [Internet] https://www.who.int/news-room/questions-and-answers/item/traditional-medicine#:~:text=T%26CM%20can%20offer%20several%20benefits,being%2C%20and%20increased%20patient%20satisfaction, Accessed 06 December, 2023.
8. Sofowora A. Medicinal plants and traditional. medicine in Africa Spectrum books Ltd., Ibadan Nigeria. 1993.

9. Ridgway P. Revised fixed dose procedure: OECD Test Guideline 420. RAPPORTI ISTISAN. 2002;41:27-31.
10. Bawari S, Sah AN, Tewari D. Antiurolithiatic activity of *Daucus carota*: an *in vitro* study. Pharmacognosy journal. 2018;10(5).
11. Zahidin NS, Saidin S, Zulkifli RM, Muhamad II, Ya'akob H, Nur H. A review of *Acalypha indica* L. (Euphorbiaceae) as traditional medicinal plant and its therapeutic potential. Journal of ethnopharmacology. 2017;207:146-73.
12. Konda RK, Prathyusha A. Evaluation of invitro antiurolithiatic activity of ethanolic and methanolic leaf extracts of *Acalypha indica* Linn. UPI Journal of Pharmaceutical, Medical and Health Sciences. 2020:05-8.
13. Prathyusha A. Evaluation of Invitro Antiurolithiatic Activity of Ethanolic and Methanolic Leaf Extracts of *Acalypha indica* Linn. Asian Journal of Hospital Pharmacy 2020;5:8.
14. Arumugam N, Ramaswamy A, Prabhu SV. *In vitro* antilithiasis activity and cytoprotective properties of *Acalypha indica* extracts. Current Applied Science and Technology. 2021:240-54.
15. Mohan C, Dinakar S, Anand T, Elayaraja R, SathiyaPriya B. Phytochemical, GC-MS analysis and Antibacterial activity of a Medicinal Plant *Acalypha indica*. Int J Pharm Tech Res. 2012;4(3):1050-4.
16. Nazri NM, Hazali N, Ibrahim M, Masri M, Ayob MK. Preliminary studies on *Acalypha indica*: proximate analysis and phytochemical screening. Int. J. Pharm. Pharm. Sci. 2016;8:406-8.
17. Aggarwal KP, Narula S, Kakkar M, Tandon C. Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. BioMed research international. 2013; 2013.
18. Zaki S, Jahan N, Kalim M, Islam G. *In vitro* antilithiatic activity of the hydro-alcoholic extract of *Cinnamomum zeylanicum* Blume bark on calcium oxalate crystallization. Journal of integrative medicine. 2019;17(4):273-81.

Cite this article: Kumar A, Sharma AK. Phytochemical Screening, Acute Toxicity and Evaluation of *in vitro* Antiurolithaitic Activity of Ethanolic and Methanolic Root and Leaf Extracts of *Acalypha indica* Linn. J Young Pharm. 2024;16(2):223-8.