IJETIR

ISSN (Online) 2583-0554

International Journal of Emerging Technologies and Innovative Research (IJETIR)

Volume 5, Issue 3, March 2025

Impact Factor: 5.731

Evaluation of In Vitro Anti-Urolithiatic Activity of the Hydroalcoholic Extract of Artocarpus lakoocha Leaves

Ahmad Kaleem, Achla Vyas, Mohini Rathod, Dr. M. N. Noolvi, Shafiya Ansari, Zohra Shaikh

Department of Pharmacology

Shree Dhanvantary Pharmacy College, Kim , Surat, Gujarat, India.

kaleemshaikh536@gmail.com

Abstract: *Aim and Objective: Evaluation of In vitro Anti-urolithiatic activity of the hydroalcoholic extract of Artocarpus lakoocha leaves. Method and Material: These included nucleation, aggregation, and microscopic analysis. The extract's and cyctone effects on nucleation and aggregation slopes were measured spectrophotometrically, while crystal density was examined microscopically.*

Result:The hydroalcoholic extract significantly inhibited the nucleation and aggregation of CaOx crystals, reduced crystal density and size, these effects were comparable to Cystone. Discussion:The extract demonstrated strong anti-urolithiatic activity by inhibiting calcium oxalate crystallization,decreasing crystal density. These effects, similar to those of Cystone. Conclusion:The hydroalcoholic leaf extract of Artocarpus lakoocha shows promising in vitro anti- urolithiaticactivity.

Keywords: Anti- urolithiatic,Nucleation,Aggregation, Microscopic ,Artocarpus lakoocha.

I. INTRODUCTION

Urolithiatic conditions involving the formation of stones in the urinary tract, including the kidneys, bladder, or ureters. It is derived from "urolithiasis," which denotes the presence of urinary stones. Urolithiatic conditions can cause symptoms like pain, urinary obstruction, and infection. Treatment often focuses on removing or managing the stones, as well as preventing their recurrence through changes in diet, fluid intake, and addressing underlying health issues that contribute to stone formation. Urolithiatic disorders may result from factors such as dehydration, genetic predisposition, or dietary imbalances. Managing these conditions often involves medication, lifestyle changes, and in some cases, surgical interventions to remove larger stones.[1]

II. MATERIALS AND METHODS

Plant material

In September 2024, a specimen of Artocarpus lakoocha Buch.- Ham was collected from the region of Utraula (Uttar Pradesh) for The purpose of botanical study. The collection was under taken the supervision of and authenticated by Dr. Farzin M. Parabia, voucher number- numbered VNGSU/BVBRC/2024/09/TC-55 was dated 25/09/2024.Who authenticated speci men as Artocarpus lakoocha Buch.-Ham belonging to family Moraceae .

Extraction:

Fine bark powder of Artocarpus lakoocha Buch.-Ham collected from Utraula , Balrampur ,Uttar Pradesh ,India. Solvent such as 70% Ethanol and 30% distilled water of high purity was used.

Fig.1

IJETIR
ISSN (Online) 2583-0554
International Journal of Emerging Technologies and Innovative Research (IJETIR)
Volume 5, Issue 3, March 2025
Impact Factor: 5.731

The fine leaves powder was subjected to Hydro-alcoholic extraction using an ultrasonication-assisted method.Ultrasonic waves were applied to the mixture of leaves powder and ethanol using an ultrasonic bath sonicate The Filtration was performe using filter paper to obtain a clear extract for Experimental Reference.[2]

Evaluation of the in vitro anti-urolithiatic activity:

Preparation of experimental kidney stones (calcium oxalate (CaOx) stones) by homogenous precipitation: Solutions of CaCl2 and Na2C2O4 (50 mM) were mixed. The mixture was then heated at 60 °C in a water bath for 1 h and incubated overnight at 37 °C in an oven to form the CaOx crystals .[3]

Nucleation Assay:

The impact of *Artocarpus lakoocha* hydro-alcoholic leaf extract on calcium oxalate (CaOx) crystal formation was assessed through a nucleation assay, following the procedure outlined by D.M.G. Mosquera, et al.[3] A 5 mM solution of calcium chloride (CaCl2) and a 7.5 mM sodium oxalate (Na2C2O4) solution were prepared in a Tris-HCl (0.5 M) and NaCl (0.15 M) buffer at pH 6.5.

Different concentrations of the extract and Cystone® (from Himalaya Herbal Healthcare, India, batch no. 11700204) ranging from 100 to 1000 µg/mL were prepared in distilled water. For each concentration (100 µg/mL, 200 µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL, and 1000 µg/mL), 1 mL of the extract or Cystone® was mixed with 3 mL of CaCl2, followed by the addition of 3 mL of Na2C2O4 solution. The mixtures were incubated at 37°C for 30 minutes, then allowed to cool to room temperature. The optical density (OD) of the resulting mixtures was then measured at 620 nm using a spectrophotometer.

Get smarter responses, upload files and images, and more.

The percentage inhibition for the extract and Cy stone® was calculated using the formula:

$$\%\text{Inhibition} = [1 - (\text{ODtest}/\text{ODcontrol}) \times 100]$$

where ODtest is the optical density of the Artocarpus lakoocha extract and ODcontrol the optical density of the negative control

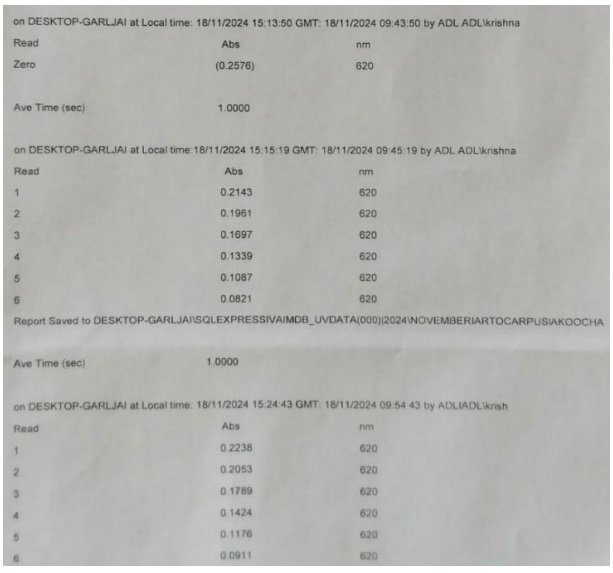


Fig.2

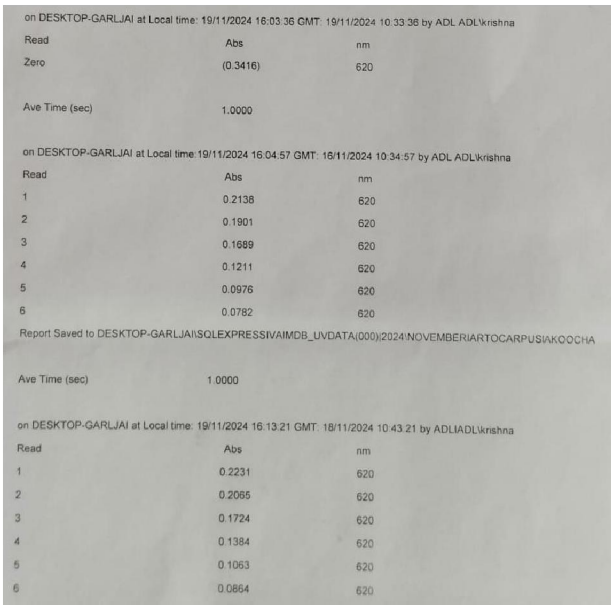
Microscopic Assay:

Microscopic analysis was conducted to evaluate the number, size, and morphology of the CaOx crystals formed in the absence or presence of the extract and Cystone®. This was done using an LED microscope at a 1000× magnification.[4]

IJETIR
ISSN (Online) 2583-0554
International Journal of Emerging Technologies and Innovative Research (IJETIR)
Volume 5, Issue 3, March 2025
Impact Factor: 5.731

Aggregation Assay:

The impact of the extracts and Cystone® on CaOx crystal aggregation was assessed following the method described by Bawari et al. (2018) [5]. Solutions of CaCl2 and Na2C2O4 (50 mM each) were mixed, heated to 60°C in a water bath for 1 hour, and then incubated overnight at 37°C. After drying, a CaOx crystal solution (0.8 mg/mL) was prepared in a 0.05 M Tris-HCl and 0.5 M NaCl buffer at pH 6.5. For each dilution (100–1000 µg/mL) of the *Artocarpus lakoocha* extract and Cystone®, 1 mL was added to 3 mL of the CaOx solution, vortexed, and incubated at 37°C for 30 minutes. The optical density (OD) of the final mixtures was measured at 620 nm, and the percentage inhibition of aggregation was calculated in the same manner as the nucleation assay.



on DESKTOP-GARLJAI at Local time: 19/11/2024 16:03:36 GMT: 19/11/2024 10:33:36 by ADL ADL\krishna

Read	Abs	nm
Zero	(0.3416)	620

Ave Time (sec) 1.0000

on DESKTOP-GARLJAI at Local time:19/11/2024 16:04:57 GMT: 19/11/2024 10:34:57 by ADL ADL\krishna

Read	Abs	nm
1	0.2138	620
2	0.1901	620
3	0.1689	620
4	0.1211	620
5	0.0976	620
6	0.0782	620

Report Saved to DESKTOP-GARLJAI\SQLEXPRESSIVAIMDB_UVDATA(000)\2024\NOVEMBER\ARTOCARPUS\AKOOCHA

Ave Time (sec) 1.0000

on DESKTOP-GARLJAI at Local time: 19/11/2024 16:13:21 GMT: 19/11/2024 10:43:21 by ADL\ADL\krishna

Read	Abs	nm
1	0.2231	620
2	0.2065	620
3	0.1724	620
4	0.1384	620
5	0.1063	620
6	0.0864	620

Fig.3

III. RESULT

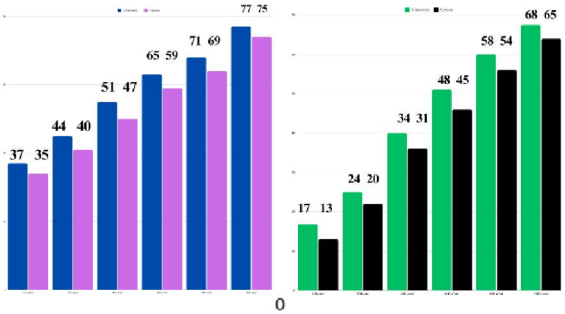
Evaluation of the in vitro anti-urolithiatic activity

In the nucleation assay, the addition of Na2C2O4 solution to the reaction mixture containing CaCl2 led to the formation of numerous CaOx crystals (fig.1&3) . Microscopic examination revealed that the control group predominantly exhibited large CaOx monohydrate (COM) crystals, which had either an ovoid shape . At higher concentrations, the extract (Fig. 2) and at lower concentrations, Cystone® (Fig. 3) encouraged the formation of Ovoid-shaped calcium oxalate crystals with a smoother morphology. Both the extract and Cystone® reduced the size and number of the CaOx crystals. The percentage reduction in the size of the CaOx crystals for the extract (77.08%) was similar to the reduction observed with Cystone® (74.70%).

In the aggregation assay, the extract significantly reduced the aggregation of preformed CaOx crystals. The percentage reduction in aggregation caused by the extract at 1000 µg/mL was 68.12%, compared to 64.63% for Cystone® (Fig. 2&4).

In Microscopic assay, the addition of Na2C2O4 solution to the reaction mixture containing CaCl2 led to the formation of numerous CaOx crystals (fig. 5). Microscopic examination revealed that the control group predominantly exhibited large CaOx crystals, which had either an ovoid shape. Both the extract and Cystone reduced the size and number of the CaOx crystals. The reduction in the Density and Size of the CaOx crystals for the was similar to the reduction observed with Cystone.

IJETIR
ISSN (Online) 2583-0554
International Journal of Emerging Technologies and Innovative Research (IJETIR)
Volume 5, Issue 3, March 2025
Impact Factor: 5.731



Nucleation Assay (Fig.3) Aggregation Assay (Fig. 4)

Extract (Blue)	Extract (Green)
Cystone (Purple)	Cystone (Black)
1- 100 µg/mL	1- 100 µg/mL
2- 200 µg/mL	2- 200 µg/mL
3- 400 µg/mL	3- 400 µg/mL
4- 600 µg/mL	4- 600 µg/mL
5- 800 µg/mL	5-800 µg/mL
6- 1000 µg/mL	6- 1000µg/mL

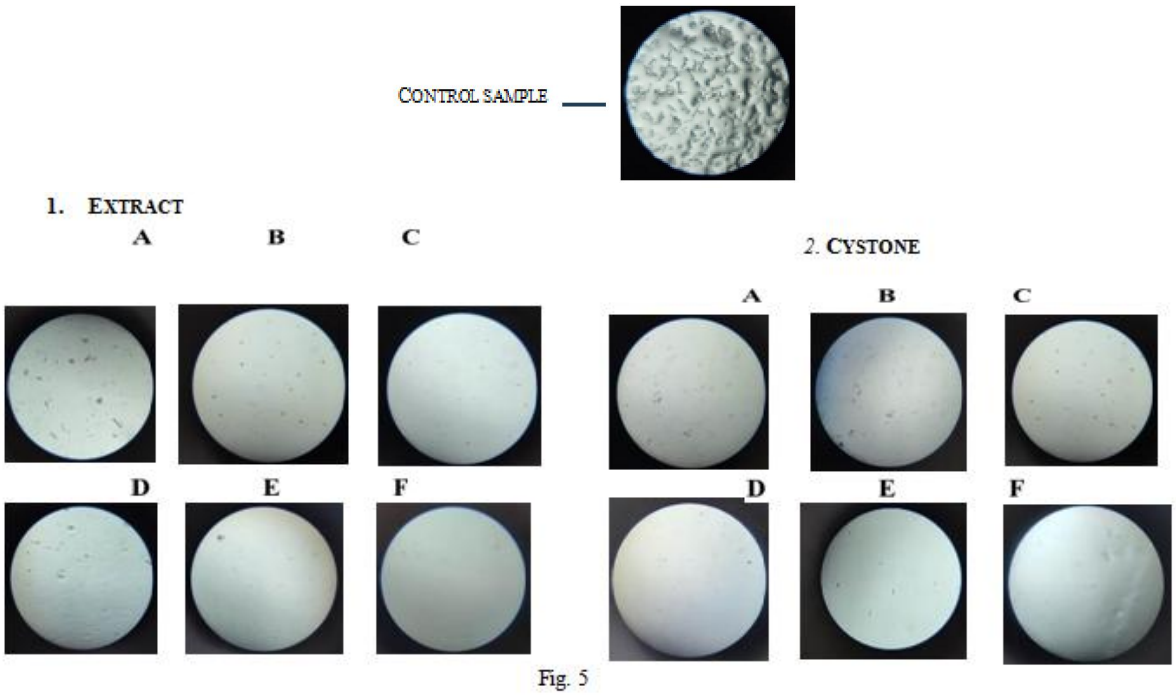


Fig. 5

Microscopic images:

The Artocarpus lakoocha extract exhibited a dose-dependent effect on calcium oxalate (CaOx) crystals, where an increase in concentration led to a reduction in both the size and density of the crystals. Representative photographs of CaOx crystals, observed under the light microscope, clearly demonstrated this trend at different concentrations of the extract, compared to the control sample.

1. Artocarpus lakoocha Extract
2. Cystone (Standard Drug)
3. X Control Sample

The concentrations were (Both) :(A) 100 µg/mL, (B) 200 µg/mL, (C) 400 µg/mL, (D) 600 µg/mL, (E) 800 µg/mL, (F) 1000 µg/mL

IV. DISCUSSION

Artocarpus lakoocha has been reported to possess notable antiurolithiatic properties. The hydroalcoholic leaf extract of this plant has demonstrated the ability to inhibit the formation of kidney stone crystals, particularly calcium oxalate crystals. These findings suggest that Artocarpus lakoocha could be a promising natural option for both preventing and managing kidney stones, offering a potential alternative to conventional treatments.

V. CONCLUSION

The findings of the present study clearly demonstrate the antiurolithiatic potential of the Artocarpus lakoocha leaf extract against calcium oxalate (CaOx) urolithiasis in vitro. The extract exhibited inhibition of all phases of CaOx stone formation, including nucleation, aggregation, and crystal growth, when compared to the standard treatment, Cystone. The hydroalcoholic leaf extract of Artocarpus lakoocha shows promising in vitro anti-urolithiatic activity, highlighting its potential for both preventing and treating urolithiasis. Additionally, the administration of the extract significantly reduced the development of urolithiasis in a rat model, further supporting its therapeutic potential.

VI. ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to Shree Dhanvantary Pharmacy College, Surat, for providing the financial support and necessary facilities required to conduct this research. Additionally, the authors are thankful to the Ribosome Research Centre for performing the UV spectrophotometry and microscopic analysis, which were essential to the success of this study.

REFERENCES

[1]. Suvarna Y, Rahaman SK. In Vitro–In Vivo evaluation of antiurolithiatic activity of piperine from Piper nigrum. Research Journal of Pharmacy and Technology. 2019;13(1):63-8.

[2]. Quoc T, Pham L. Ultrasound-assisted extraction of phenolic compounds from Alpinia galanga (L.) willd. rhizome. Agriculturae Conspectus Scientificus. 2021 Dec 16;86(4):323-8.

[3]. Sujatha D, Singh K, Vohra M, Kumar KV, Sunitha S. Antilithiatic Activity of phlorotannin rich extract of Sarghassum Wightii on Calcium Oxalate Urolithiais–In Vitro and In VivoEvaluation. International braz j urol. 2015 May;41:511-20.

[4]. Mosquera DM, Ortega YH, Quero PC, Martínez RS, Pieters L. Antiurolithiatic activity of Boldoa purpurascens aqueous extract: An in vitro and in vivo study. Journal of ethnopharmacology. 2020 May 10;253:112691.

[5]. Mosquera DM, Ortega YH, Quero PC, Martínez RS, Pieters L. Antiurolithiatic activity of Boldoa purpurascens aqueous extract: An in vitro and in vivo study. Journal of ethnopharmacology. 2020 May 10;253:112691.

[6]. Bawari S, Sah AN, Tewari D. Antiurolithiatic activity of Daucus carota: an in vitro study. Pharmacognosy journal. 2018;10(5).