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In Vitro Antiurolithiatic Activity by Inhibition of Calcium Oxalate Crystallization of Methanol Extract of *Imperata Cylindrica* L

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Abstract

Aim: The study investigates the *in vitro* antiurolithiatic potential of the methanolic extract of the whole *Imperata cylindrica* L. plant through its inhibitory effect on calcium oxalate crystallization. **Methods:** Antiurolithiatic activity was evaluated using a nucleation assay to test the extract's inhibitory effects on urolithiasis at concentrations ranging from 100 to 800 µg/mL. A standard antiurolithiatic drug, Cistone, was used as a reference. Phytochemical analysis revealed the presence of amino acids, proteins, carbohydrates, saponins, phenols, and flavonoids, while alkaloids and glycosides were not detected. **Results and Conclusion:** The extract showed dose-dependent inhibition, achieving 83.41% inhibition at 800 µg/mL, close to Cistone's inhibition of 88.98% at the same concentration *Imperata cylindrica* exhibits significant inhibitory activity against urolithiasis *in vitro*. These findings suggest potential therapeutic applications, although variability at higher concentrations indicates a need for further studies to enhance accuracy and explore optimization strategies, such as isolation of active components or combination therapies.

Keywords
Antiurolithiatic activity, Calcium oxalate crystallization, Imperata cylindrica, Nucleation assay, Urolithiasis.

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INTRODUCTION:

Renal stones develop inside the kidneys, a condition known as nephrolithiasis.[1] When these stones travel from the renal pelvis into the rest of the urinary system, encompassing the ureters, bladder, and urethra, it's termed as urolithiasis.[2] Urolithiasis' stands for ouron (urine) and lithos means stone. About 80% of cases of this condition are calcium-based stones, either calcium oxalate (CaOx) or a mixture of CaOx and calcium phosphate. It affects 2 to 20% in the population and has a recurrence rate of roughly 30% to 50% within 5 years.[3] Persistent kidney disease, end-stage renal disease, and a markedly elevated risk of cardiovascular diseases are among the important morbidities associated with kidney stones, based on recent studies.[4] Stones can arise from a broad range of metabolic or environmental abnormalities, such as cystinuria, hyperuricosuria, hyperoxaluria, hypocitraturia, hypercalciuria, and excessive urine acidity.[5] There are now effective medical treatments available that can address underlying disorders. These comprise potassium citrate for hypocitraturic calcium nephrolithiasis, sodium cellulose phosphate, thiazide, and orthophosphate for hypercalciuric nephrolithiasis, acetohydroxamic acid for infection stones, and d-penicillamine and α-mercaptopropionylglycine for cystinuria.[6,7] As individuals and healthcare systems increasingly recognize the limitations and risks associated with

conventional drugs, there is a growing interest in exploring plant-origin products as safer, more sustainable alternatives. However, it's important to approach the use of plant-origin products with caution, seeking guidance from healthcare professionals and relying on scientific evidence to inform treatment decisions.[8] The rising incidence and recurrence rates of urolithiasis underscore the necessity for diverse treatment options.[7] Herbal medicines often contain compounds with anti-inflammatory, antioxidant, diuretic, and lithotriptic properties, which can help dissolve stones, alleviate symptoms, and inhibit stone formation. Additionally, many herbal remedies possess nephroprotective effects, safeguarding renal function amidst the challenges posed by stone formation and treatment.[8]

The integration of herbal medicine into urolithiasis management can also offer a personalized approach, considering the heterogeneous nature of stone composition and individual patient characteristics. Furthermore, as the global healthcare system increasingly emphasizes preventive and holistic care, herbal medicine aligns well with this paradigm by promoting overall urinary tract health and addressing underlying risk factors for stone formation.[9]

Monohydrate (COM) or Whewellite and the dihydrate (COD) or Weddellite are the two forms of CaOx stones that are found. With a higher affinity for renal tubular cells than COD, COM is the thermodynamically most stable form and is more frequently found in clinical stones. As a result, COM is the root cause of kidney stones.[10]

Increased urine supersaturation triggers the onset of CaOx crystallization, leading to the formation of solid crystalline particles in the urinary system, where nucleation occurs as salts form clusters that grow larger with added components, ultimately resulting in the trapped and aggregated crystals that form kidney stones.[11]

The literature review of the plant i.e., *Imperata cylindrica* confirms that there are no reports related to the antiurolithiatic activity of methanolic extract of Imperata cylindrica whole plant. As from the literature review, it is found out that *Imperata cylindrica* is traditionally used in kidney stone, so it may have antiurolithiatic activity. The aim of the research was to perform Phytochemical screening of *Imperata cylindrica* and to explore the Antiurolithiatic activity of methanolic extract of *Imperata cylindrica*.

MATERIAL AND METHODS

Collection and Authentication of plant material

According to the plant's literature review, *Imperata cylindrica*, there is no data on the antiurolithiatic activity of the plant's entire methanolic extract. The Plant of *Imperata Cylindrica* L based on their medicinal importance was collected from Haldia, West Bengal, India. The voucher specimen for the species was identified, and confirmation of Plant specimen is kept in the Central National Herbarium, Botanical Survey of India, at Botanic Garden, Howrah District, West Bengal under Ministry of Environment, Forest and Climate Change. Shade drying was carried out under natural air and surrounding's temperature (mean temperature = 25 °C) for 1-2 weeks. In the case of sun drying, leaves were dried into trays under direct sunlight at temperatures between 30 and 35 °C.

Preparation of extract

The selected plant material was extracted using the cold maceration method.[12] First, 100 g of dried powder was mixed with 500 ml of 70% methanol and thoroughly blended, then carefully transferred into a 500 ml volumetric flask. The solution was continuously shaken for 4 hours to promote complete dissolution, after which it was left undisturbed for 3 days to allow maximum extraction. The mixture was then filtered through filter paper to separate the liquid extract from any residual solids. Finally, the extract was dried and stored for future use.

Phytochemical Screening

To validate the presence of phytochemicals, tests for alkaloids, carbohydrates, glycosides, saponins, phenols, tannin, flavanoids, protein and amino acids, steroids, and terpinoids were conducted.

Nucleation Assay

Calcium chloride (CaCl2) (5m mol/lit) and sodium oxalate (Na2C2O4) solution (7.5m mol/lit) were prepared in Tris HCl (0.05 mol/lit) and sodium chloride (NaCl) (0.15mol/lit) buffer (pH- 6.5). 1ml extract was mixed with 3 ml calcium chloride solution followed by the addition of 3 ml sodium oxalate solution. Final mixture was incubated for 30 min at 37 ℃. The optical density (OD) of the mixture will be measured at 620 nm wavelength. [13,14]

% inhibition of nucleation = [1- (OD test/OD control) ×100]

RESULT

Phytochemical screening

The phytochemical tests of methanolic extract of *Imperata cylindrica* indicate the presence of carbohydrates, saponins, phenol, flavonoids, protein and amino acid and absence of alkaloids, glycosides (*Table no: 1*).

***Table No. 1*: Phytochemical analysis of Methanolic extracts of whole plant of Imperata cylindrica.**

Type of phytochemical constituent	Name of the tests	Methanolic Extract
Test for alkaloid	**Mayer's test**	**-**
	Wagner test	**-**
Test for carbohydrate	**Fehling's test**	**+**
Test for glycosides	**Borntrager's test**	**-**
Test for saponins	**Foam test**	**+**
Test for phenol	**Ferric chloride test**	**+**
Test for flavonoids	**Alkaline reagent test**	**+**
Test for proteins and amino Acid	**Ninhydrin test**	**+**

In-vitro antiurolithiatic activity by Nucleation assay method

The results of Anti urolithiatic activity by nucleation assay of methanolic extract of whole plant of *Imperata cylindrica* is summarized in *Table no 2* and *Fig no 1*. The inhibitory activity of *Imperata cylindrica* extract and Cistone (standard drug) was measured across a concentration range of 100 µg/mL to 800 µg/mL. The percentage inhibition for *Imperata cylindrica* ranged from 49.94% at 100 µg/mL to 83.41% at 800 µg/mL. The percentage inhibition for Cistone ranged from 52.26% at 100 µg/mL to 88.98% at 800 µg/mL.

Table No. 2 Percentage inhibition of different concentration of Imperata cylindrica extract and Cystone

Concentration of Sample	% Inhibition Imperata cylindrica test	% Inhibition Cistone standard
100µg	**49.94±0.380**	**52.255±1.585**
200µg	**54±0.400**	**56.505±0.835**
400µg	**62.815±2.215**	**73.48±2.160**
600µg	**79.015±0.995**	**84.13±1.210**
800µg	**83.41±3.00**	**88.975±1.565**

Results are expressed as mean ± SEM. Level of significance $P < 0.005$; as compared with Cystone group between the same dose levels.

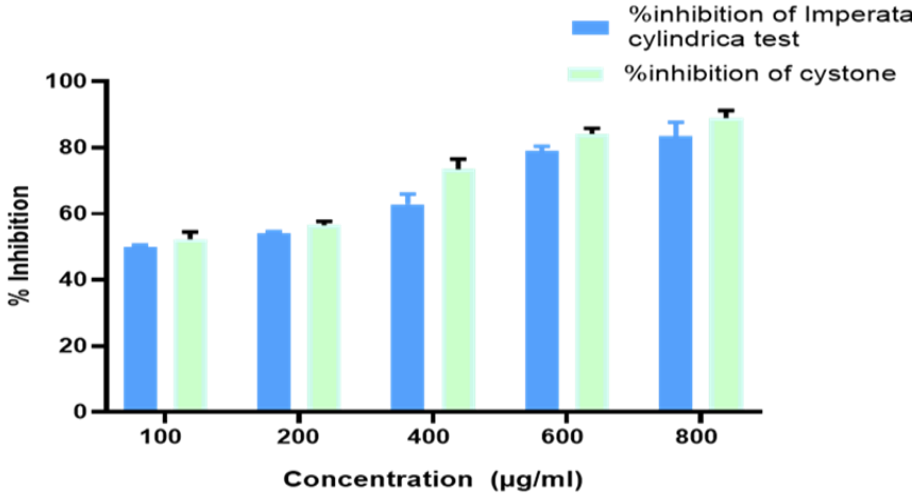

Fig No.1: Nucleation assay of methanolic extract of whole plant of Imperata cylindrica

DISCUSSION:

Phytochemical assays on a methanolic extract of the entire *Imperata cylindrica* plant reveal the presence of amino acids, proteins, carbohydrates, saponins, phenol, and flavonoids, but alkaloids and glycosides are absent. A concentration range of 100 µg/mL to 800 µg/mL was used to test the inhibitory activity of Cistone (standard medication) and Imperata

cylindrica extract. The percentage inhibition for *Imperata cylindrica* ranged from 49.94% at 100 µg/mL to 83.41% at 800 µg/mL. The percentage inhibition for Cistone ranged from 52.26% at 100 µg/mL to 88.98% at 800 µg/mL. *Imperata cylindrica* extract exhibits dose-dependent inhibition, indicating that higher concentrations result in more potent inhibitory effects. At a concentration of 800 µg/mL, the inhibition reaches 83.41%, which is close to the inhibition observed with the Cistone standard (88.98%). The effectiveness of Imperata cylindrica may be improved with more modification (such as the isolation of active components or combination therapy). This suggests that further study into the bioactive ingredients' mechanisms of action should be conducted on it. The data exhibits significant variability, particularly at higher concentrations (e.g., 83.41% ± 3.00% at 800 µg/mL for *Imperata cylindrica*). This indicates the necessity for additional works to enhance the data's reliability. Additional experiments focusing on identifying the active components of *Imperata cylindrica* could help in reducing the concentration required for significant inhibition.

CONCLUSION:

The methanolic extract of *Imperata cylindrica* demonstrates significant, dose-dependent inhibitory activity against calcium oxalate crystallization *in vitro*, with inhibition reaching 83.41% at 800 µg/mL, closely approaching the efficacy of the standard antiurolithiatic drug, Cistone (88.98% at 800 µg/mL). These results suggest that *Imperata cylindrica* possesses considerable antiurolithiatic potential. However, observed variability in inhibition at higher concentrations indicates the need for further studies to enhance data consistency and reliability. Investigating the mechanisms of action and isolating specific active components may not only clarify the plant's bioactivity but could also reduce the concentration needed for optimal inhibitory effects. This supports the potential for *Imperata cylindrica* as an alternative or complementary treatment for urolithiasis with further optimization.

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