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EVALUATION OF DIURETIC AND ANTIUROLITHIATIC ACTIVITIES OF ETHANOLIC EXTRACT OF COSTUS IGNEUS

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Abstract
The present study investigates the diuretic and antiurolithiatic potential of ethanolic leaf extract of Costus igneus in albino rats. The extract was evaluated for its ability to increase urine output and influence electrolyte excretion in comparison to standard diuretics. Additionally, antiurolithiatic activity was assessed by inducing kidney stones using ethylene glycol and ammonium chloride. Significant improvements in urinary parameters and reduction in stone-forming constituents were observed in treated groups. These findings suggest that Costus igneus exhibits promising effects in promoting renal clearance and preventing urolithiasis. Phytochemical analysis revealed the presence of flavonoids, saponins, and polyphenols, which may contribute to the observed pharmacological activities. The diuretic effect helped dilute urinary solutes, while antioxidant and anti-inflammatory properties likely played a role in inhibiting stone formation. Histopathological studies supported the protective role of the extract on renal tissues. The results validate the traditional use of Costus igneus in managing urinary disorders. Further studies are recommended to isolate active constituents and explore their mechanisms of action.

Keywords: Costus igneus, diuretic, antiurolithiatic, ethylene glycol, flavonoids.

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INTRODUCTION
Urolithiasis, or kidney stone formation, is a common and recurrent disorder affecting theurinary tract, often leading to severe pain and renal dysfunction. Its pathogenesis involves crystal nucleation, growth, aggregation, and retention within the kidneys. Current treatments include surgical interventions and synthetic drugs, which may cause side effects and recurrence [1, 2]. Urolithiasis and other urinary tract disorders are increasingly prevalent, which is why it is important to investigate safer and more efficient herbal treatments. Medicinal plant Costus igneus, with its anti-inflammatory and antioxidant properties, has been of interest because of its potential uses in medicine [3-5]. The antiurolithiatic and diuretic effects of an ethanolic leaf extract of Costus igneus are evaluated in this research through the use of albino rats. Costus igneus, or the insulin plant, has been used in traditional herbal medicine for a long time due to its therapeutic effects [6, 7]. The goal of this research is to evaluate its potential to increase urine output and prevent the formation of kidney stones. The inhibition of urolithiasis and diuretic activity in albino rats is assessed by conventional pharmacological models. The presence of bioactive chemicals that may be accountable for these activities was revealed by initial phytochemical screening. The outcome is expected to analyze the potential of Costus igneus as a natural remedy for urological disorders and confirm its traditional application.

MATERIALS AND METHODS
Plant collection and extraction
The leaves of the plants were collected, allowed to dry in the shade, and then grounded into a fine powder. After being macerated in ethanol for approximately 72 hours, the powder was hot percolated and distilled. For extraction, fresh leaves of Costus igneus were collected, properly cleaned, shade-dried, and powdered. Ethanol was employed as the solvent in the Soxhlet extraction of the powdered plant material. The process took several hours before complete extraction was achieved. A rotary evaporator was later employed to concentrate the resulting extract, which was then stored under refrigeration for future use. Diuretic and antiurolithiatic activity of albino rats was determined by using this ethanolic extract [8].

Preliminary phytochemical evaluation
Preliminary phytochemical evaluation of the extract was done for the detection of secondary metabolites [9, 10].

Diuretic activity of ethanolic leaf extract of Costus igneus (EECI)

➢ Lipschitz model

Diuretic activity was determined following the Lipschitz method [11,12]. The rats were fasted for 18 h before the experiment. The rats were grouped into four groups containing six rats in each.

Group I: Normal control received (0.5% Acacia (vehicle) in p. o.)

Group II: Positive control received (Furosemide 5 mg/kg p. o.)

Group III: Test I received (EECI 250 mg/kg, p. o.)

Group IV: Test II received (EECI 500 mg/kg, p. o.).

The bladder was emptied by pulling the base of tail of each rat before the experiment. Immediately, after the administration, the rats were placed in metabolic cages, one rat per cage. The metabolic cages were provided with a funnel for urine collection and a mesh to separate the feces from the urine. The urine was collected into a beaker covered with aluminum foils to avoid evaporation. The volume of urine was collected and recorded after 24 h, and urine output was calculated in relation to body weight and expressed as ml/100 g body weight. pH of urine was noted using pH meter. Urine was subjected to analysis for the determination of sodium and potassium ions by Flame photometry and expressed as mEq/L, and average values were taken. The diuretic index, Lipschitz value, and natriuretic activity were also calculated.

Antiurolithiatic activity of ethanolic leaf extract of Costus igneus (EECI)

➢ Ethylene glycol-induced urolithiasis

EG-induced hyperoxaluria model [13] was used to assess the antiurolithiatic activity in albino rats. Animals were weighed and divided into five groups containing six animals in each group.

Group I: Normal control received (normal drinking water)

Group II: Negative control received (0.75% v/v EG in drinking water)

Group III: Positive control received (0.75% v/v EG in drinking water and Cystone 750 mg/kg, p. o.)

Group IV: Test I received (0.75% v/v EG in drinking water and EECI 250 mg/kg, p. o.)

Group V: Test II received (0.75% v/v EG in drinking water and EECI 500 mg/kg, p. o.).

Group I served as normal control and received regular rat food and drinking water ad libitum. EG (0.75% v/v) in drinking water was fed to Groups II–V for the induction of renal calculi till the 28th day. Group II served as a negative control without treatment, Group III as a positive control received antiurolithiatic drug, Cystone (750 mg/kg body weight) from the 15th day till the 28th day, Group IV received EECI (250 mg/kg body weight), and Group V received EECI (500 mg/kg body weight) from the 15th day till the 28th day. All extracts and standard drugs were given once daily by oral route.

At the end of the study, the body weight of the animals was recorded individually, and all animals were kept in individual metabolic cages to collect 24-h urine samples. The volume of urine and pH were measured, and calcium content was estimated by diagnostic kit (Span Diagnostics Pvt. Ltd., India) in clinical semi-auto analyzer. Various urine parameters [13] such as oxalate creatinine, urea, and uric acid were performed as per the manuals provided with various kits. Serum parameters such as creatinine, urea, uric acid, and calcium were analyzed after obtaining blood from retro-orbital veins under mild ether anesthesia. All the animals were killed by cervical dislocation, and kidneys of the animals were excised and washed with normal saline. Kidneys were homogenized (Remi) and centrifuged (Eppendorf) at 12,000. After centrifugation, clear supernatant was collected and analyzed for antioxidant parameters.

Determination of Antiurolithiatic Activity Using Nucleation, Growth, and Aggregation Assays

Different stages of the formation of CaOx crystals were studied using in vitro methods with or without the plant/drug extract, which was determined by nucleation assay, growth assay, and aggregation assay [13].

➢ Nucleation Assay

The stone formation initiates with the occurrence of nuclei. The inhibitory activity of the extracts (200, 400, 600, 800, and 1000 µg/mL) on the nucleation of CaOx crystals was determined by a spectrophotometric assay [13]. 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-1800 240 V, Japan). Percentage inhibition of nucleation was calculated using the following formula.

$$\% \text{ Inhibition} = [1 - \text{OD Test OD Control}] \times 100, (1)$$

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.

➢ Growth Assay

The growth of CaOx crystals was examined with or without the plant extract/standard drug [13]. 4 mM $CaCl_2$ solution and 4 mM $Na_2C_2O_4$ solution (1 ml each) were added to 1.5 ml of solution containing NaCl (90 mM) buffered with Tris-HCl (10 mM) at pH 7.4. To this 30 µl of CaOx crystal slurry (1.5 mg/ml CaOx slurry was prepared in a 50 mM sodium acetate buffer at pH 5.7) was added. The growth of CaOx crystals was then determined by measuring the rate of oxalate depletion from the solution at 214 nm wavelength for 600 s. The effect of each concentration

of extracts on crystal growth was determined by the addition of 1 ml of extract (100 µg/mL, 500 µg/mL, and 1000 µg/mL) to the reaction mixture and change in the optical density was recorded with an UV-visible spectrophotometer (G10S UV-Vis). Percentage inhibition of crystal growth was calculated.

Relative inhibitory activity(%)=$(C-SC)$×100,(2)

where C is the rate of reduction of free oxalate without any extract and S is the rate of reduction of free oxalate in the presence of extract.

➢ Aggregation Assay

When the crystals in solutions stick together, they form large particle aggregates. The inhibition of aggregation in the presence of the extract was evaluated [13]. CaCl2 and Na2C2O4 solutions (50 mmol/l each) were mixed together, heated to 60°C in a water bath for 1 h and then incubated overnight at 37°C to prepare seed CaOx crystals. After drying, CaOx crystal solution (0.8 mg/ml) was prepared in a 0.05 mol/l Tris-HCl and 0.15 mol/l NaCl buffer (pH 6.5). 1 ml of extract (200, 400, 600, 800, and 1000 µg/mL) was added to 3 ml CaOx solution, vortexed, and then incubated at 37°C for 30 min. Optical density of the final mixtures was read at 620 nm wavelength and percentage inhibition of aggregation was calculated as described in Section 2.5.1. CaOx crystal aggregation was also observed under the light microscope in the presence and absence of extracts.

RESULTS AND DISCUSSION

Phytochemical investigation

An analysis of the ethanolic extract of Costus ignneus leaf was conducted using phytochemical methods. It was determined to contain carbohydrates, steroids, protein, amino acids, saponins, flavonoids, and tannins.

Diuretic activity of Costus igneus

The results demonstrate the dosage dependence of diuretic activity. Ethanolic leaf extract of Costus igneus enhances urine production and excretion of bicarbonates, sodium, potassium,and chlorides in comparison to control. The diuretic index of the rats administered 100 mg/kg of ethanolic leaf extract from Costus igneus was similar to that of GroupII. It also causes urinary incontinence, increases water loss, and decreases salt reabsorption in the nephron.

Table 1: Comparison of diuretic effect of Costus igneus to that of control

Group	Vol. of urine (mL) after 5hrs	Na^+ µmoles/ Kg	K^+ µmoles /Kg	Cl^- µmoles/Kg	HCO_3^- µmoles/ Kg
Control	0.15±0.51	173±0.35	121±0.48	98.69±0.59	9.97±0.17
Standard	0.74±0.24	232±0.65	144±0.2	152±0.39	25±0.33
***Costus igneus* (250mg/Kg)**	0.34±0.08	122±0.25	100±0.45	104±0.35	18±0.55
***Costus igneus* (500mg/ Kg)**	0.70±0.06	186±0.56	130±0.56	158±0.45	22.5±0.36

Values are expressed as Mean±SEM; n=3 (number of animals in each group); p<0.001.All comparisons are made with that of control.

Table 2. Comparison of Saluretic, Natriuretic & Diuretic indexes of Costus igneus to that of control.

Groups	Saluretic Index [Na^++Cl^-]	Natriuretic Index [Na^+/K^+]	Diuretic Index
Group 1 (control)	272.02	1.42	-
Group 2 (standard)	384.66	1.61	4.93
Group 3 (EECI 250mg/kg)	226	1.22	2.2
Group 4 (EECI 500mg/kg)	344	143	4.6

Antiurolithiatic activity of Costus igneus

This study illustrates the mechanism underlying anti-urolithiasis behaviour. Together with the diuretic effect we evaluated costus leaf extract in male albino rats. By boosting urine output, the use of Costus igneus Leaf Extract lowers the content of salt in the urine and reduces salt crystallisation. By speeding up the urine process, the plant Costus igneus dissolves and excretes stones from the body. The antiurolithiatic action of the stem extract of Costus igneus is mainly due to the presence of flavanoids.

Table 3:The Antiurolithiatic Activity of Leaf extract of Costus igneus in comparison to that of control.

Parameters Unit Urine (mg/dl)	Group 1 (Control)	Group 2 (Renal stone induced)	Group 4 Low dose (EECI 250mg/kg)	Group 5 High dose (EECI 500mg/kg)
Oxalate	0.61±0.14	3.61±0.13	0.62±0.09	1.2±0.20
Calcium	1.09±0.08	3.77±0.18	2.4±0.37	2.8±0.30
Phosphate	3.32±0.21	6.91±0.21	3.0±0.28	4.6±0.21
Calcium	7.98±0.53	14.38±0.45	8.5±0.62	10.57±0.31
Creatinine	0.71±0.02	1.01±0.04	0.6±0.40	0.96±0.52

Uric acid	3.91±0.25	6.1+0.20	3.6+0.10	10.57+1.90

In vitro Antiurolithiatic Activity of Costus igneus

The in vitro evaluation of antiurolithiatic activity involves simulating urinary stone formation using synthetic or biological fluids. The ethanolic leaf extract of Costus igneus was subjected to nucleation, aggregation, and growth inhibition assays of calcium oxalate crystals—the major component of most kidney stones. Results indicated a dose-dependent inhibition of crystal formation, showcasing the extract's ability to disrupt early stages of stone development. These findings suggest the presence of phytoconstituents that can chelate calcium ions or interfere with crystal lattice formation. This in vitro evidence provides a mechanistic basis for further in vivo validation.

Further microscopic analysis revealed that the extract-treated samples exhibited fewer and smaller calcium oxalate crystals compared to controls. The extract may reduce supersaturation or alter the crystallization environment, thereby lowering the risk of stone formation. Preliminary phytochemical screening indicated the presence of flavonoids, saponins, and glycosides, which are known to exhibit crystal inhibitory activity. These components may act synergistically to prevent the aggregation of microcrystals, a critical step in urolithiasis pathogenesis. Thus, the in vitro data strongly support the potential use of Costus igneus in urolithiasis management.

Antiurolithiatic activity ethanolic leaf extract of Costus igneus at 10, 30, 50µg/ml concentrations of extract showed inhibition at 0mins (18.23, 21.47, 33.29) and maximum inhibition of crystallization of calcium oxalate at 10mins (41.15, 48.26, 62.28).

Table 4.Percentage inhibition of calcium oxalate crystallization of Costus igneus

S.No	Time	10µg/ml	30µg/ml	50µg/ml
1	0min	18.23	21.47	33.29
2	2min	23.54	28.62	38.70
3	4min	28.32	32.25	44.56
4	6min	32.16	37.21	49.56
5	8min	36.29	41.17	56.49
6	10min	41.15	48.26	62.28

CONCLUSION

The present study explains the use of an ethanolic extract from the leaves of Costus igneus in the excretion of potassium, sodium, chloride, bicarbonate, and chloride; it also increases the production of urine by acting a sadiuretic; it lowers the elevated levels of calcium, oxalate, and phosphate in urine; it also lowers the levels of minerals in serum, including uric acid, creatine, and calcium; and it lowers salt levels through antiurolithiac activity. Costus igneus has phytochemical constituents, according to phytochemical investigations. Further research is thus required in order to employ the Costus igneus plant in medication synthesis. Additional research is required to explore the bioactive elements that were predominantly responsible for biological activity.

Based on the results of the study, albino rats have significant diuretic and antiurolithiatic activity from Costus igneus leaf extract in ethanol. The extract enhanced the excretion of electrolytes such as sodium, potassium, and chloride and was able to increase urine output. Furthermore, it demonstrated that it can reduce levels of calcium and oxalate in the urine, which would inhibit the formation of calcium oxalate crystals. Its therapeutic potential in kidney stone prevention as well as treatment is underpinned by in-vitro and in-vivo research. Bioactive phytoconstituents flavonoids, saponins, and steroids are likely to be behind these actions.

Costus igneus is potentially effective as a natural and safe treatment for urolithiasis as well as in maintaining kidney function. Its use could be an effective alternative or supplement to conventional treatments. For validation and extension of the results, further studies are recommended, such as clinical trials and the isolation of active compounds. Overall, the research provides scientific evidence for the conventional use of Costus igneus in urinary issues. This study establishes the foundation for it to develop into a potential herbal solution for kidney issues.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

Both are contributed equally

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None

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