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Therapuetic prospects of *Lagenaria siceraria* seed *in vitro* antioxidant diuretic and urolithiatic activities

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Abstract
Lagenaria siceraria is a medicinal plant with history of therapeutic use in traditional medicine. The main aim of the present study was to evaluate the invitro antioxidant and possible diuretic and anti urolithiatic activities of ethanolic seed extract of *Lagenaria siceraria* on albino rats. The invitro antioxidant activity was performed using standard methods such as DPPH and Hydroxyl scavenging assay. The diuretic activity in albino rats was assessed by measuring the urine volume and electrolytes excretion with oral administration of seed extract at doses of 150 mg/kg and 300 mg/kg body weight. And the results so obtained were compared with standard drug Furosemide (5mg/kg) body weight. Further the anti urolithiatic activity was assessed using ethylene glycol induced urolithiasis model. The various different parameters such as urine volume, calcium oxalate, phosphate, creatinine and uric acid levels were also analyzed using biochemistry analyzer. The histopathological examination of kidney was also performed to evaluate the crystal deposition and tissue architecture.

Keywords: *Lagenaria siceraria*, antioxidant activity, diuretic activity, anti urolithiatic activity, biochemical parameters, kidney stones, histopathological studies

Introduction
Urolithiasis refers to the formation of stones (calculi) in the urinary system, which includes the kidneys, ureters, bladder, and urethra [16, 17]. These stones can vary in size and may cause significant discomfort as they move through the urinary tract. The condition is commonly known as kidney stones or renal calculi [1]. *Lagenaria siceraria* commonly known as bottle gourd belonging to the family Cucurbitaceae [11]. The natives of remote villages in east Godavari district traditionally use the seeds of *Lagenaria siceraria* for indigestion, bloating, biliary cholic and constipation relief. It is also known for promoting urine flow [1, 2] and aiding in conditions like edema [2]. This medicinal plant is being used as folkloric medicine and the seeds of *Lagenaria siceraria* is effective in Genito urinary tract disorders based on the folkloric usage by natives of East Godavari district region [15]. The authors made a sincere attempt to evaluate the Antioxidant, diuretic and Anti urolithiatic activities.

Materials and Methods
Extraction
The dried seeds of the medicinal plant *Lagenaria siceraria* is collected and debris is removed, washed and dried under shade and then subjected to pulverization and coarse powder is collected and subjected to extraction process using solvent ethanol. The powder was macerated in ethanol for period of 5 days and subjected to hot percolation followed by distillation. The obtained solution was filtered and the filtrate was concentrated, dried and kept in a desiccator.

Chemicals and glassware
All chemicals and glassware used for the experiment are of standard quality and of analytical grade.

FTIR Studies
FTIR spectroscopy was carried out to identify the functional groups present in the ethanolic fruit extract of *Lagenaria siceraria*.

The spectra were obtained within the range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} using an FTIR spectrometer. The generated spectra were examined to detect characteristic peaks corresponding to specific functional groups, providing information about the chemical constituents of the extract.

Animals used

Albino rats of either sex weighing between 160-225 grams were selected for the study, Animals were selected for the study, Animals were procured from NIN (National institute of Hyderabad) and all the animals were fed with standard diet with adequate water. The selected animals are very healthy and are acclimatized to laboratory conditions. Animals were grouped and each group comprising 3 animals and all the rats were allocated in metabolic cages.

Experimental Design

Determination of *in vitro* antioxidant activity using Dpph scavenging method

DPPH (1,1 diphenyl, 2-picryl hydrazyl) was used to study the free radical scavenging effect of *Lagenaria siceraria* seed extract. Different concentrations of extract (50,100,300,500 µg/ml) were prepared in methanol. 1 ml of extract at various concentrations (50,100,300,500µg/ml) is added to 0.1 ml of prepared Dpph solution and kept in dark for 30 minutes[6]. Incubated samples were checked for absorbance at 517nm using the following equation and expressed as percent inhibition and the obtained results were compared with that of the standard Gallic acid of concentration (2.5µg/ml) [7].

$$\% RSA = [(A_0-A_1/A_0)] *100$$

Where,

A_0 indicates control absorbance
A_1 indicates sample absorbance

Hydroxyl radical scavenging assay method:

For each individual concentration of extract, 1ml of the reagent solution was added, followed by the sequential addition of 1 ml of 1.5Mm $Feso_4$, 0.7 ml of 6mM H_2O_2, and 0.3ml of 20mM sodium salicylate [8]. The resulting mixture was incubated for approximately 1 hour, after which the absorbance was measured at 562nm and% inhibition is calculated by using the formula and compared with that of standard Gallic acid (2.5µg/ml) [9].

$$\% RSA = [(A_0-A_1/A_0)] *100$$

Where,

A_0 indicates control absorbance
A_1 indicates sample absorbance

Diuretic Activity

The diuretic activity of a substance was assessed using the Lipschitz method, following approval from the Institutional Animal Ethical Committee (IAEC). The study involved rats divided into four groups of three animals each. Prior to the experiment, the rats were fasted for 18 hours and water was withheld. To ensure adequate hydration, a priming dose of normal saline (25 ml/kg) was administered to all rats [12, 13]. The groups were then subjected to different treatments to evaluate the diuretic effects of the substance.

- **Group 1:** Control (treated with vehicle 0.5% acacia)
- **Group 2:** Treated with 150 mg/kg of *Lagenaria siceraria* seed extract
- **Group 3:** Treated with 300 mg/kg of *Lagenaria siceraria* seed extract
- **Group 4:** Furosemide (5 mg/kg) body weight

Following administration, each rat was placed in a metabolic cage to collect urine and feces separately. The cage setup included a funnel and collection dish for urine, while a mesh allowed for the separation of faces. To prevent evaporation, the urine collection dish was covered with aluminium foil. The total urine volume was measured after 5 hours. Subsequently, the urine samples were analysed to determine the levels of various ions [19, 20], including:

Sodium (Na+) and Potassium (K+) ions: Measured using flame photometry[21].

Chloride (Cl-) and Bicarbonate (HCO3-) ions: Quantified through titrimetric analysis.

This analysis was conducted over a 24-hour period to assess the diuretic effects and electrolyte excretion patterns in the rats.

Anti Urolithiatic Activity

The anti-urolithiatic activity of *Lagenaria siceraria* seed extract was evaluated in Albino rats with ethylene glycol-induced hyperoxaluria. The study involved four groups of rats:

- **Group 1 (Normal Control):** Received standard rat food and drinking water.
- **Group 2 (caliculi induced):** Received 0.75% v/v ethylene glycol in drinking water for 28 days to induce renal calculi.
- **Group 3 (Test Group):** Received ethylene glycol for 28 days and extract *Lagenaria siceraria* (300 mg/kg) from the 15th to 28th day.
- **Group 4 (Standard Treatment):** Received ethylene glycol for 28 days and Cystone (750 mg/kg) from the 15th to 28th day [12, 13].

Estimation of biochemical parameters

On the 28th day of the experiment, urine samples were collected and analysed to determine the levels of calcium, phosphate, and oxalate [4, 5]. Blood was drawn from the tail vein to assess serum calcium, creatinine, and uric acid using a biochemical analyser [34, 36, 37].

Histopathological Studies

On the 29th day of the experiment, the rats were anesthetized with diethyl ether and then sacrificed. Their kidneys were collected for histopathological examination.

Results

FTIR Findings

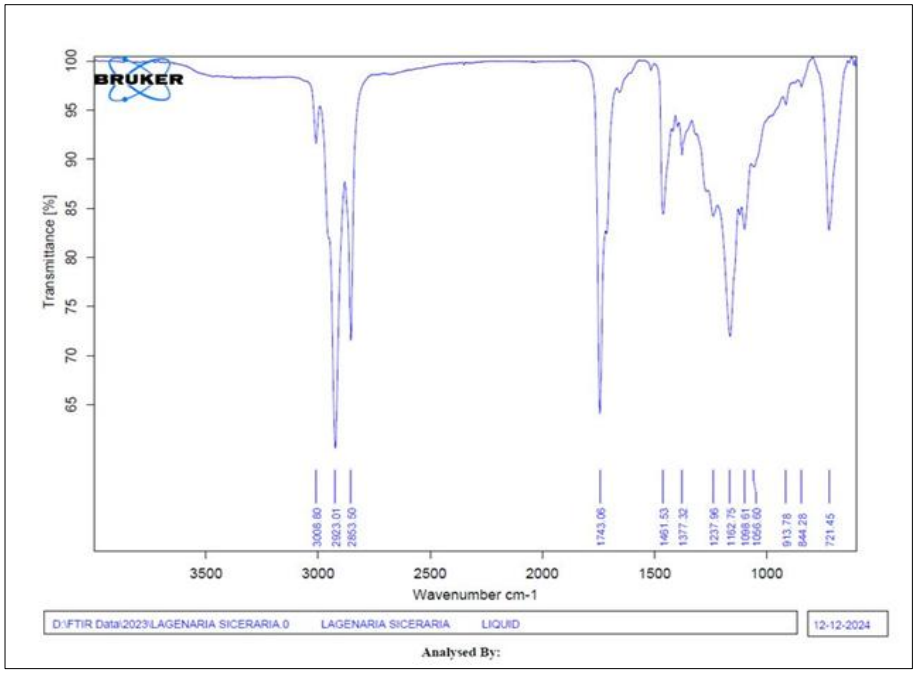

Fig 1: FTIR Spectrum of *Lagenaria siceraria*

FTIR Spectral Findings

The FTIR spectrum of the ethanolic seed extract of *Lagenaria siceraria* exhibited several diagnostic bands that corroborate the presence of key phytochemical classes. A pair of strong aliphatic C-H stretching vibrations at 2923 and 2853 cm^{-1} confirms abundant -CH_2-and-CH_3- groups, consistent with long-chain aliphatic backbones in polysaccharides, steroidal saponins, and lipids. A pronounced carbonyl band at 1743 cm^{-1} is characteristic of ester C=O linkages, supporting the existence of glycosidic esters such as those found in cardiac glycosides or acylated flavonoids. Multiple C-O and C-O-C stretching bands between 1238 and 1056 cm^{-1} further validate extensive ether and alcohol functionalities, indicative of sugar moieties in glycosides and phenolic hydroxyl groups in flavonoids and tannins. A shoulder at 3008 cm^{-1}, attributable to =C-H stretching, points to unsaturated or aromatic components-most likely flavonoid aglycones while out-of-plane C-H bending at 913 and 844 cm^{-1} confirms substitution patterns on aromatic rings characteristic of flavonoids and tannins. Finally, the methylene rocking vibration at 721 cm^{-1} underscores the presence of long aliphatic chains, in agreement with steroidal saponins and other lipidic constituents. Together, these FTIR spectral features provide a cohesive fingerprint of carbohydrates, glycosides, flavonoids, tannins, and saponins in the seed extract.

Anti-Oxidant Activity

The antioxidant activity of ethanolic seed extract of *Lagenaria siceraria* was evaluated using standard protocols DPPH scavenging assay and hydroxyl radical scavenging assay.

DPPH scavenging assay

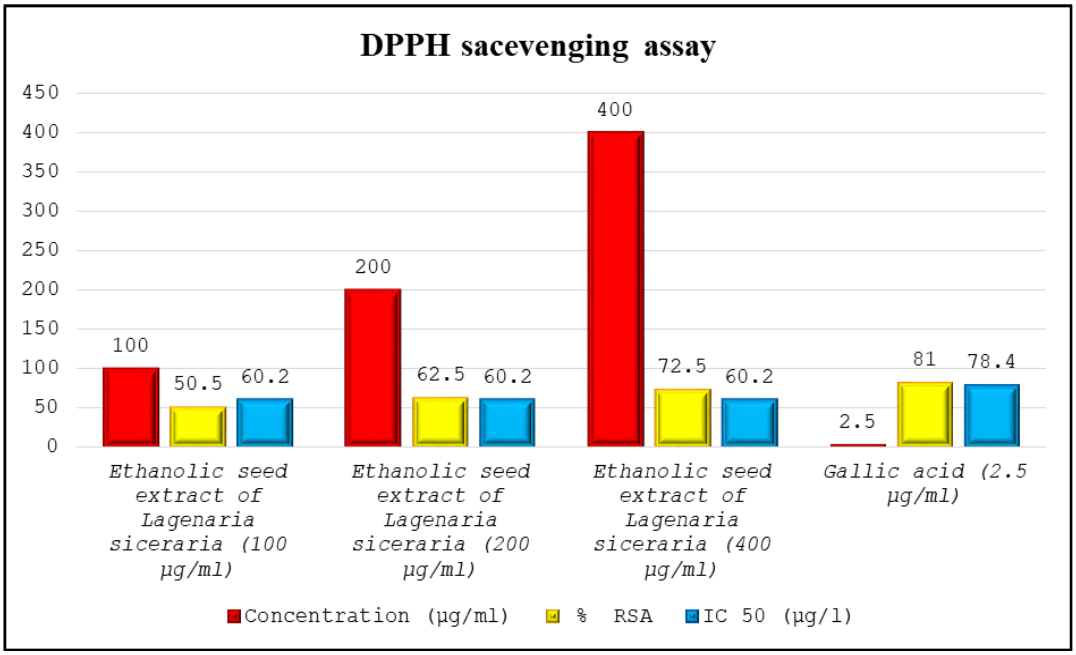

Fig 2: DPPH radical scavenging assay

The antioxidant potential of the ethanolic seed extract of *Lagenaria siceraria* was evaluated by the DPPH radical scavenging assay (Figure 1). The extract exhibited a concentration-dependent increase in radical scavenging activity. At concentrations of 100, 200, and 400 µg/ml, the extract showed% RSA values of 50.5, 62.5, and 72.5, respectively, with an IC_{50} of 60.2 µg/ml. In comparison, the standard gallic acid (2.5 µg/ml) demonstrated 81% RSA with an IC_{50} of 7.84 µg/ml.

Hydroxyl radical scavenging assay

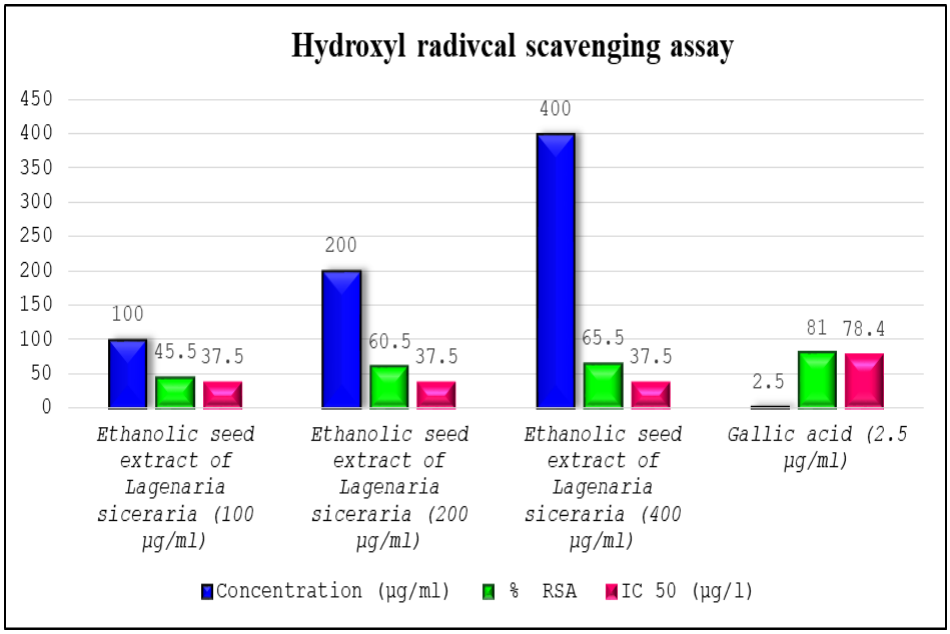

Fig 3: Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of the ethanolic seed extract of *Lagenaria siceraria* was quantitatively evaluated at three concentrations: 100, 200, and 400 µg/ml, using Gallic acid (2.5 µg/ml) as the reference standard as shown in the figure 3. The percentage of hydroxyl radical scavenging activity (%RSA) demonstrated a clear dose-dependent trend. At 100 µg/ml, the extract exhibited 45.5% scavenging activity, which increased to 60.5% at 200 µg/ml and further to 65.5% at 400 µg/ml. The IC_{50} value of the extract was determined to be 37.5 µg/ml, indicating moderate scavenging potential. In contrast, the standard Gallic acid displayed a substantially higher% RSA of 81% with an IC_{50} value of 78.4 µg/ml. These graphs suggest that while the *Lagenaria siceraria* seed extract possesses significant hydroxyl radical quenching ability.

Diuretic Activity

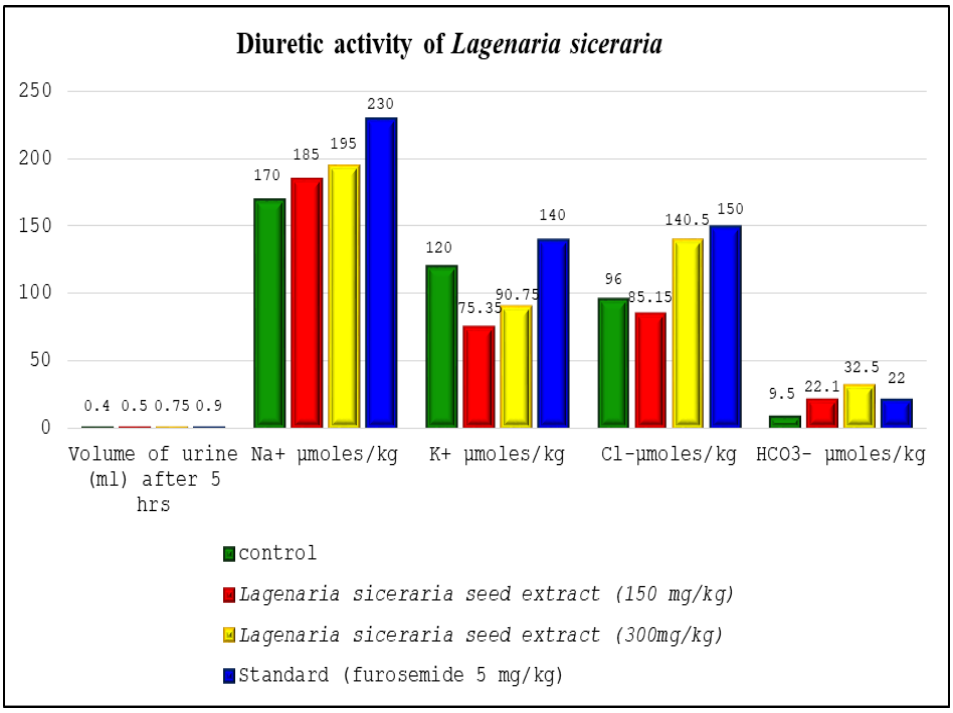

Fig 4: Diuretic activity of *Lagenaria siceraria*

Figure 5 represents the diuretic activity of *Lagenaria siceraria* seed extract was evaluated by measuring urine volume and electrolyte excretion (Na^+, K^+, Cl^-, and HCO_3^-) after 5 hours as shown in the figure 4. The extract at doses of 150 mg/kg and 300 mg/kg showed a dose-dependent increase in urine output compared to the control group (0.4 mL for control, 0.5 mL for 150 mg/kg, 0.75 mL for 300 mg/kg, and 0.9 mL for standard furosemide). Sodium excretion significantly increased from 170 µ mol/kg in control to 185 µ mol/kg and 195 µ mol/kg for the 150 mg/kg and 300 mg/kg groups, respectively, while the standard drug Furosemide produced the highest sodium excretion of 230 µ mol/kg. Similarly, potassium and chloride levels were also elevated in treated groups, with K^+ excretion reaching 75.35, 90.75, and 140 µ mol/kg and Cl^- excretion at 85.15, 96, and 150 µ mol/kg for 150 mg/kg, 300 mg/kg, and Furosemide groups, respectively. The bicarbonate levels also showed a moderate increase, with the 300 mg/kg extract and Furosemide yielding comparable values (32.5 µ mol/kg and 22 µ mol/kg, respectively).

Anti Urolithiatic Activity

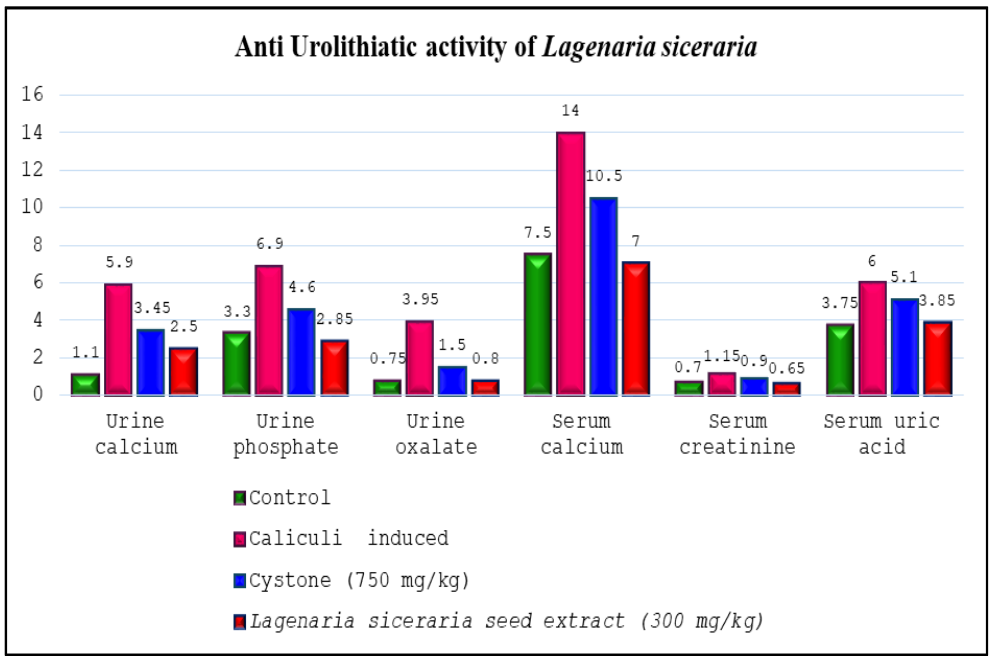

Fig 5: Anti urolithiatic activity of *Lagenaria siceraria*

Figure 5 present the parameters measured include Urine calcium, Urine oxalate, Urine phosphate, Serum calcium, Serum creatinine, and Serum uric acid.

Effect on Urine Parameters

- **Urine Calcium:** The Calculi Induced group shows a marked increase in urine calcium (5.9) compared to the Control (1.1). Both the Standard Drug (Cystone) (2.5) and the *Lagenaria siceraria* seed extract (3.45) significantly reduced the elevated urine calcium, with Cystone being slightly more effective.
- **Urine Oxalate:** Similar to calcium, urine oxalate is highly elevated in the Calculi Induced group (6.9). The *Lagenaria siceraria* seed extract (4.6) and the Standard Drug (Cystone) (3.3) both caused a reduction, with Cystone showing a greater effect.
- **Urine Phosphate:** The Calculi Induced group exhibits a high urine phosphate level (3.95). Treatment with Cystone (2.85) and the *Lagenaria siceraria* seed extract (0.75) resulted in a decrease. Notably, the *Lagenaria siceraria* seed extract showed a reduction in urine phosphate, even below the Control level (0.7).

Effect on Serum Parameters

- **Serum Calcium:** The Calculi Induced group shows the highest serum calcium level (14), indicating hypercalcemia associated with the disease model. Both the Standard Drug (Cystone) (10.5) and the *Lagenaria siceraria* seed extract (7.5) caused a substantial reduction in serum calcium, with the seed extract performing better than the standard drug and bringing the level closer to the Control (7).
- **Serum Creatinine:** Serum creatinine, a marker of renal function, is elevated in the Calculi Induced group (1.15). Treatment with Cystone (0.9) and the *Lagenaria siceraria* seed extract (0.7) resulted in a reduction, suggesting an improvement in renal function. The seed extract lowered creatinine closer to the Control value (0.7).
- **Serum Uric Acid:** The Calculi Induced group shows elevated serum uric acid (6.1). Both the Standard Drug (Cystone) (3.85) and the *Lagenaria siceraria* seed extract (3.75) caused a significant decrease, bringing the levels closer to the Control (3.1).

Histopathology Studies

Figure 6 explains about the histopathological examination of kidney sections from the Control group (Group I) observes that normal renal architecture, with well-defined glomeruli, intact tubular epithelial lining, and no any inflammatory infiltration was observed. Kidneys from the ethylene glycol-treated group (Group II) showed marked pathological alterations, including extensive tubular epithelial cell degeneration, tubular dilation and interstitial inflammatory infiltration. Numerous birefringent crystal deposits were observed within the tubular lumen, indicating calcium

oxalate crystal formation and confirming successful induction of urolithiasis. In the *Lagenaria siceraria* treated group (400 mg/kg; Group III), a notable reduction in crystal deposition was observed compared to the ethylene glycol group. The tubular epithelium exhibited partial restoration with mild degenerative changes and minimal inflammatory infiltration, indicating considerable nephroprotective activity of the extract. The standard Cystone-treated group (75 mg/kg; Group IV) demonstrated near-normal renal histoarchitecture, with minimal presence of intraluminal crystals. Tubules and glomeruli appeared intact, and signs of degeneration or inflammation were negligible.

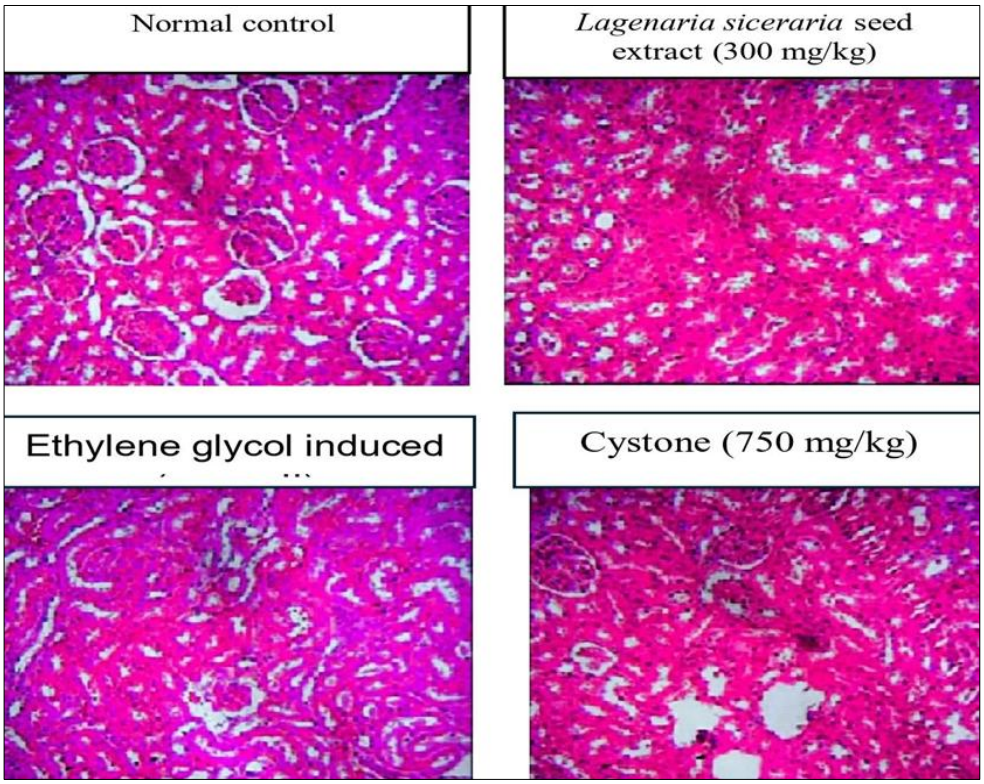

Fig 6: Histopathological observation of *Lagenaria siceraria*

Discussion

The FTIR spectral analysis of the ethanolic fruit extract of *Lagenaria siceraria* confirmed the presence of diverse phytoconstituents by showing distinct absorption bands for multiple functional groups. These compounds are well-known to exhibit diuretic activity. They are thought to work by, increasing renal blood flow, enhancing glomerular filtration rate, modulating electrolyte excretion, Specifically, flavonoids and saponins are key contributors, likely by promoting natriuresis (sodium excretion) and kaliuresis (potassium excretion), which helps reduce water retention and increase overall urinary volume. In addition to its diuretic action, the extract shows promise for preventing urolithiasis (kidney stone formation). These same compounds can inhibit various phases of stone development, including the nucleation, growth, and aggregation of crystals. The carbonyl and hydroxyl groups in flavonoids and phenolics possess calcium-chelating properties, which helps lower the degree of calcium salts that are supersaturated in the urine. Saponins have been shown to break down mucoprotein matrices and reduce crystal adherence to the renal epithelium. Collectively, these mechanisms help decrease calcium oxalate crystallization, a finding that is supported by *in vitro* (laboratory) inhibition data. The ethanolic *Lagenaria siceraria* seed extract clearly demonstrated a significant diuretic effect. This was evidenced by the test group's increased urinary excretion of key electrolytes and stone-forming minerals: calcium, phosphate, and oxalate, when compared to the control group. This enhanced clearance of electrolytes suggests a specific mechanism of action for the extract.

The ethanolic seed extract of *Lagenaria siceraria* displayed significant antioxidant activity as evidenced by its DPPH radical scavenging potential. The activity increased in a concentration-dependent manner, reaching 72.5% scavenging at 400 µg/ml, which is relatively close to the standard gallic acid (81% at 2.5 µg/ml). Although the extract required higher concentrations to achieve comparable activity, the IC_{50} value (60.2 µg/ml) indicates substantial free radical quenching ability. These findings suggest the presence of phytochemicals such as flavonoids, phenolics, or other secondary metabolites contributing to the observed antioxidant effect. Thus, the results support the therapeutic potential of *Lagenaria siceraria* seeds in mitigating oxidative stress-related disorders.

The results indicate that the ethanolic seed extract of *Lagenaria siceraria* possesses substantial hydroxyl radical scavenging activity, which increases proportionally with concentration. This dose-dependent enhancement suggests the presence of bioactive compounds capable of donating hydrogen atoms or electrons to neutralize free radicals. The extract's scavenging efficiency at 400 µg/mL, though lower than that of gallic acid, demonstrates its potential as a natural antioxidant source. The observed activity may be attributed to the presence of polyphenolic compounds, flavonoids, and other phytochemicals commonly found in *L. siceraria* seeds. These findings support previous reports on the antioxidant potential of *L. siceraria* and suggest its

usefulness in preventing oxidative stress-related cellular damage.

The results indicate that *Lagenaria siceraria* seed extract possesses significant diuretic activity, which increases proportionally with dosage. The elevated urinary output and enhanced excretion of sodium, potassium, and chloride ions suggest that the extract promotes natriuresis and kaliuresis, similar to the mechanism of loop diuretics like furosemide. The higher dose (300 mg/kg) demonstrated a more pronounced diuretic effect, approaching the activity of the standard drug, indicating strong dose dependency. The mild increase in bicarbonate excretion further supports the diuretic potential, though it suggests minimal interference with acid-base balance. These findings imply that the diuretic effect of *Lagenaria siceraria* may be attributed to the presence of bioactive phytoconstituents such as flavonoids, saponins, and alkaloids, which could act on renal tubular transport mechanisms. Thus, the study provides experimental evidence supporting the traditional use of *Lagenaria siceraria* in managing fluid retention and related disorders. The ethanolic seed extract of *Lagenaria siceraria* exhibited potent anti urolithiatic activity. The significant increase in urinary solutes (calcium, oxalate, and phosphate) and serum analytes (calcium, creatinine, and uric acid) in the Calculi Induced group confirms the successful establishment of the urolithiasis model, which mimics the condition of kidney stone formation (nephrolithiasis) and associated renal impairment. *Lagenaria siceraria* produced meaningful Diuretic/Litholytic effect by increasing urine flow and thus promoting the washout of crystals, or a litholytic action that dissolves pre-formed crystals. The reduction in serum calcium by the *Lagenaria siceraria* extract is particularly noteworthy, outperforming the standard drug. This suggests the extract may be involved in regulating systemic calcium metabolism (e.g., affecting bone resorption, intestinal absorption, or renal reabsorption), thereby reducing the load of calcium presented to the kidneys for excretion. The decrease in elevated serum creatinine and serum uric acid indicates that the *Lagenaria siceraria* seed extract possesses a Reno protective effect. Reduced creatinine suggests an improvement in the Glomerular Filtration Rate (GFR), which is typically compromised during stone formation and associated crystal-induced injury. The decrease in uric acid may also suggest a potential for preventing uric acid stones or an anti-inflammatory effect. The *Lagenaria siceraria* seed extract at 300mg/kg demonstrated comparable anti-urolithiatic activity to the standard drug Cystone (750mg/kg).

Tables

Anti-oxidant activity

DPPH scavenging assay

Table 1: Effect of ethanolic seed extract of *Lagenaria siceraria* on DPPH scavenging assay

Sample	Concentration (µg/ml)	% RSA (DPPH Scavenging Assay)	IC_{50} (µg/l)
Ethanolic seed extract of *Lagenaria siceraria*	100	50.50±0.45	60.20
	200	62.50±0.35	
	400	72.50±0.30	
Gallic acid	2.5	81±0.55	78.10

Values are expressed as Mean ± SEM, N=3

Table 1 represents the antioxidant potential of the ethanolic seed extract of *Lagenaria siceraria* as evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The results are expressed as the percentage of radical scavenging activity (% RSA) at different concentrations of the extract (100, 200, and 400 µg/ml) and compared with the standard antioxidant, Gallic acid. The ethanolic seed extract exhibited a concentration-dependent increase in DPPH radical scavenging activity. At 100 µg/ml, the extract showed 50.50±0.45% RSA, which increased to 62.50±0.35% and 72.50±0.30% at 200 µg/ml and 400 µg/ml, respectively. This indicates that higher concentrations of the extract possess greater antioxidant potential. The IC_{50} value (the concentration required to scavenge 50% of DPPH radicals) for the ethanolic extract was 60.20 µg/ml, demonstrating moderate antioxidant activity.

Table 2: Effect of ethanolic seed extract of *Lagenaria siceraria* by using Hydroxyl radical scavenging assay

Sample	Concentration (µg/ml)	% RSA (Hydroxyl Radical Scavenging Assay)	IC_{50} (µg/l)
Ethanolic seed extract of *Lagenaria siceraria*	100	45.50±0.35	37.50
	200	60.50±0.45	
	400	65.50±0.45	
Gallic acid	2.5	81±0.55	78.10

Values are expressed as Mean ± SEM, N=3

The hydroxyl radical scavenging activity of the ethanolic seed extract of *Lagenaria siceraria* was evaluated and the results are presented in Table 2. The extract exhibited a concentration-dependent increase in radical scavenging activity. At concentrations of 100, 200, and 400 µg/mL, the percentage scavenging activities were 45.50±0.35%, 60.50±0.45%, and 65.50±0.45%, respectively. The IC_{50} value of the extract was found to be 37.50 µg/mL, indicating a moderate antioxidant potential. In comparison, the standard antioxidant Gallic acid showed 81±0.55% scavenging activity with an IC_{50} value of 7.81 µg/mL, demonstrating higher antioxidant efficacy. Although the activity of the ethanolic seed extract was lower than that of the standard, the results clearly indicate that *Lagenaria siceraria* possesses significant hydroxyl radical scavenging ability. The values are expressed as Mean ± SEM for N= 3, confirming the reliability of the findings.

Diuretic activity

Table 3: Diuretic effect of ethanolic seed extract of *Lagenaria siceraria* seed extract

Group	Volume of Urine (ml) after 5 hrs.	Na^+ (µmoles/kg)	K^+ (µmoles/kg)	Cl^- (µmoles/kg)	HCO_3^- (µmoles/kg)
Control	0.40±0.40	170±0.30	120±0.45	96±0.55	9.50±0.15
Lagenaria siceraria seed extract (150 mg/kg)	0.50±0.20	185±0.50	75.30±1.50	85.15±2.30	22.10±0.40
Lagenaria siceraria seed extract (300 mg/kg)	0.75±0.30	195±1.15	90.75±2.10	140.50±3.50	32.50±0.30
Standard	0.90±0.15	230±0.50	140±0.40	150±0.30	22±0.20

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.

The table 3 represents the diuretic effect of the ethanolic seed extract of *Lagenaria siceraria* in comparison with a control group and a standard drug. In the control group, urine volume after 5 hours was the lowest (0.44±0.40 ml) with minimal electrolyte excretion. Administration of the seed extract at 150 mg/kg produced a slight increase in urine volume (0.50±0.20 ml) and a moderate rise in sodium, potassium, chloride, and bicarbonate excretion, indicating a mild diuretic effect. At 300 mg/kg, the extract produced a more pronounced effect with a significant rise in urine output (0.75±0.30 ml) along with marked increases in electrolyte excretion (Na^+ 195±1.15, K^+ 90.75±2.10, Cl^- 140.5±3.50, and HCO_3^- 32.5±0.30 µmoles/kg), suggesting dose-dependent activity. The standard drug showed the highest diuretic response with a urine volume of 0.90±0.15 ml and the greatest electrolyte excretion, showing its strong diuretic potential.

Table 4: Saluretic, Natriuretic, and diuretic indexes of *Lagenaria siceraria* ethanolic seed extract

Group	Saluretic Index [Na^+ / Cl^-]	Natriuretic Index [Na^+ / K^+]	Volume of Urine (ml) after 5 hrs.	Diuretic Index
Control	250±1.5	1.50±0.70	0.65±0.02	–
Lagenaria siceraria seed extract (150 mg/kg)	228±0.50	1.30±0.30	0.75±0.10	1.80
Lagenaria siceraria seed extract (300 mg/kg)	290±0.65	1.65±0.25	0.90±0.05	2.25
Furosemide (5 mg/kg)	384±0.60	1.60±0.80	3.40±0.10	4.80

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

The Table 4 summarizes the saluretic, natriuretic, and diuretic indices of the ethanolic seed extract of *Lagenaria siceraria* in comparison with control and a standard drug (Furosemide). In the control group, the saluretic index ([Na^+ + Cl^-]) was 250±1.5, the natriuretic index ([Na^+/K^+]) was 1.05±0.70, urine volume after 5 hours was 0.65±0.02 ml, and the calculated diuretic index was considered baseline (1.0). Treatment with seed extract at 150 mg/kg produced a saluretic index of 228±0.50, a natriuretic index of 1.30±0.30, and a urine volume of 0.75±0.10 ml, corresponding to a diuretic index of 1.80, indicating a mild increase in diuretic activity compared to control. At 300 mg/kg, the extract produced higher values with a saluretic index of 290±0.65, a natriuretic index of 1.65±0.25, urine volume of 0.90±0.05 ml, and a diuretic index of 2.25, suggesting a dose-dependent enhancement of saluretic and diuretic effects. The standard drug Furosemide (5 mg/kg) showed the most pronounced effect with the highest saluretic index (384±0.60), natriuretic index (3.40±0.10), urine volume (1.60±0.80 ml), and diuretic index (4.80), showing its potent diuretic action.

Anti urolithiatic activity

Table 5: *In vivo* anti-urolithiatic activity of *Lagenaria siceraria* seed extract

Group	Urine Calcium	Urine Phosphate	Urine Oxalate	Serum Calcium	Serum Creatinine	Serum Uric Acid
Control	1.10±0.05	3.30±0.20	0.75±0.15	7.50±0.50	0.70±0.02	3.75±0.25
Caliculi induced	5.90±0.15	6.90±0.20	3.95±0.15	14.00±0.50	1.15±0.04	6.00±0.20
Lagenaria siceraria seed extract (350 mg/kg)	3.45±0.30	4.60±0.20	1.50±0.20	10.50±0.02	0.90±0.05	5.10±0.15
Cystone (750 mg/kg)	2.50±0.02	2.85±0.20	0.80±0.03	7.00±0.25	0.60±0.25	3.85±0.15

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.

The Table 5 presents the effects of different treatments on urinary and serum biochemical parameters in experimental groups, specifically focusing on urolithiasis (stone formation). Control group shows normal levels of urine calcium, phosphate, oxalate, and serum calcium, creatinine, and uric acid. Calculi-induced group shows a significant rise in urinary calcium (5.9±0.15), phosphate (6.9±0.20), and oxalate (3.95±0.15), as well as elevated serum calcium (14.4±0.50), creatinine (1.15±0.04), and uric acid (6.0±0.2). This indicates stone formation and impaired renal function. *Lagenaria siceraria* seed extract (350 mg/kg) treated group shows marked improvement: urinary calcium (3.45±0.30), phosphate (4.6±0.20), and oxalate (1.5±0.20) decreased compared to the calculi group. Serum parameters also improved, with calcium (10.5±0.02), creatinine (0.90±0.05), and uric acid (5.1±0.15) approaching near-normal levels. Cystone (750 mg/kg) treated group (standard drug) further reduced urinary calcium (2.5±0.02), phosphate (2.85±0.20), and oxalate (0.8±0.03), while normalizing serum calcium (7.4±0.25), creatinine (0.6±0.25), and uric acid (3.85±0.15). This group shows the best protective effect against urolithiasis.

Conclusion

The present study provides compelling evidence that *Lagenaria siceraria* seed extract possesses notable

antioxidant, diuretic, and anti-urolithiatic properties. The extract demonstrated significant free radical scavenging activity, suggesting its potential to mitigate oxidative stress a key contributor to renal pathophysiology. Its diuretic effect, characterized by increased urinary output and altered electrolyte excretion, supports its role in promoting renal clearance and reducing urinary stasis. Furthermore, the anti-urolithiatic activity observed in experimental models indicates the extract's ability to inhibit phosphate levels, calcium oxalate crystallization and aggregation, thereby preventing stone formation and facilitating dissolution of existing calculi. These findings validate the ethnomedicinal use of *Lagenaria siceraria* seeds in traditional systems of medicine for managing kidney-related ailments.

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