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EXPLORING THE ETHNOBOTANICAL AND PHARMACOLOGICAL BASIS OF ZORNIA GIBBOSA IN UROLITHIASIS MANAGEMENT

Pravina Wagadre*, Dr. Vishal Shrivastava

LNCT Bhopal, Near Jk Hospital & Medical Research Centre, J. K. TOWN, C-SECTOR SARVADHARAM, Kolar Road, Bhopal-462042, Madhya Pradesh.

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***Corresponding Author**
Pravina Wagadre
LNCT Bhopal, Near Jk Hospital & Medical Research Centre, J. K. TOWN, C-SECTOR SARVADHARAM, Kolar Road, Bhopal-462042, Madhya Pradesh.

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ABSTRACT

Background: Urolithiasis is associated with renal biochemical disturbances and oxidative stress. The present dataset evaluates the ethnobotanical and pharmacological relevance of Zornia gibbosa for urolithiasis management using phytochemical, antioxidant, toxicity, and in vivo antiurolithiatic endpoints. **Methods:** Powdered plant material was extracted by Soxhlation using ethanol. Qualitative phytochemical tests were conducted, total phenolic content (TPC) was estimated using the Folin-Ciocalteu method, total flavonoid content (TFC) using the AlCl3 colorimetric method, and antioxidant activity using the DPPH radical scavenging assay. Antiurolithiatic activity was assessed in an ethylene glycol/ammonium chloride-induced rat model using serum creatinine, calcium, urea, and phosphorus levels. **Results:** The ethanolic extraction yielded 19.06 g from 400 g plant material, corresponding to 4.76% yield. Qualitative screening indicated the presence of flavonoids, tannins/phenolics, carbohydrates, alkaloids, glycosides, and saponins, whereas proteins/amino acids, steroids, and triterpenoids were not detected. TPC and TFC were reported as 49.6 mg gallic acid equivalents/g and 28.2 mg rutin equivalents/g, respectively. The extract showed concentration-dependent DPPH radical scavenging activity with an IC50 of 37.12 µg/mL, compared with 20.80 µg/mL for ascorbic acid. In the in vivo model, the disease control group showed higher serum creatinine, calcium, urea, and phosphorus than normal control. Extract treatment produced dose-dependent reductions, with the 400 mg/kg dose approaching the values observed for Cystone.

Conclusion: The supplied data support the antioxidant and antiurolithiatic potential of Zornia gibbosa. Confirmation requires complete raw replicate data, formal statistical testing, histopathology, urinary stone-forming parameters, and mechanistic validation.

KEYWORDS: Zornia gibbosa; urolithiasis; antiurolithiatic activity; DPPH; phenolics; flavonoids; nephroprotection; ethylene glycol model.

1. INTRODUCTION

Urolithiasis involves the formation or retention of calculi in the urinary tract and is frequently modeled experimentally using ethylene glycol-induced hyperoxaluria. The supplied dataset investigates whether Zornia gibbosa extract can counter biochemical changes associated with experimentally induced stone formation. The study integrates ethnobotanical rationale with phytochemical screening, antioxidant analysis, acute toxicity observations, and serum biochemical markers in a rat model.

The working hypothesis was that ethanol-soluble phytoconstituents, particularly phenolic and flavonoid compounds, may contribute to antioxidant activity and reduce renal biochemical disturbances induced by ethylene glycol/ammonium chloride. Because full literature references and raw statistical outputs were not provided, this manuscript presents the dataset as an evidence-based draft and identifies the required validation steps.

2. MATERIALS AND METHODS

2.1 Plant material and extraction

Four hundred grams of Zornia gibbosa plant material were collected from a localized region of Bhopal in October 2025 and authenticated by a botanist. After cleaning, material was shade-dried at room temperature for three days, oven-dried at 45°C until complete dryness, powdered, and stored in airtight containers. The powdered plant material was extracted by Soxhlation using ethanol. Extract yield was calculated as extract weight divided by plant material weight, multiplied by 100.

Table 1: Extraction summary.

Plant	Solvent	Plant material (g)	Extract yield (g)	Yield (%)
Zornia gibbosa	Ethanol	400	19.06	4.76

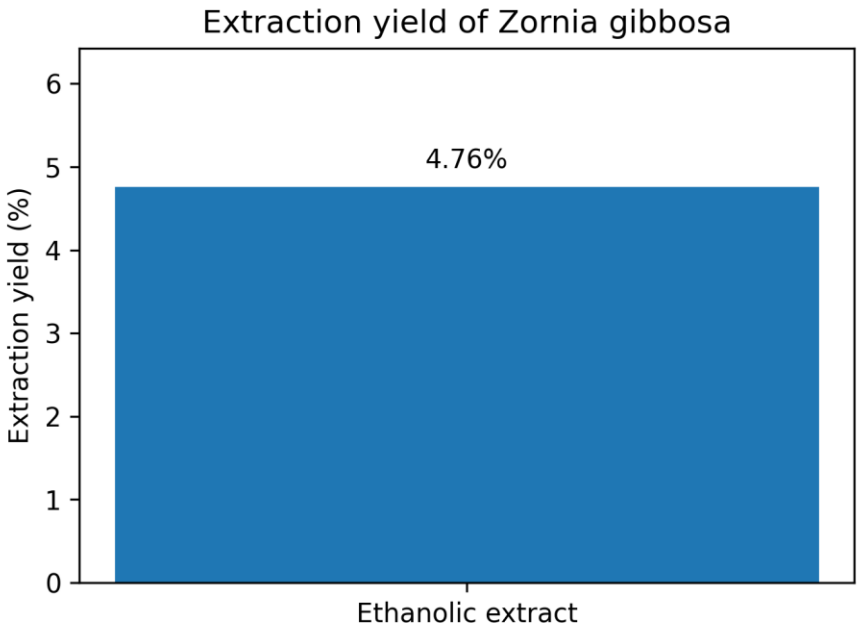

Figure 1: Percentage yield obtained from ethanolic Soxhlet extraction of Zornia gibbosa.

2.2 Qualitative phytochemical screening

The ethanolic extract was screened using standard qualitative tests for flavonoids, tannins/phenolic compounds, carbohydrates, alkaloids, proteins/amino acids, glycosides, triterpenoids/steroids, and saponins. The extracted results were coded as present (+) or absent (-).

Table 2. Qualitative phytochemical profile of the ethanolic extract.

Phytochemical class	Tests reported	Ethanolic extract
Flavonoids	Alkaline reagent; lead acetate	Present (+)
Tannins and phenolic compounds	Ferric chloride	Present (+)
Carbohydrates	Molisch's; Fehling's; Benedict's; Barfoed's	Present (+)
Alkaloids	Dragendorff's; Mayer's; Wagner's; Hager's	Present (+)
Proteins and amino acids	Biuret; ninhydrin	Absent (-)
Glycosides	Borntrager's; Legal's; Keller-Killiani	Present (+)
Triterpenoids and steroids	Salkowski's; Liebermann-Burchard	Absent (-)
Saponins	Foam test	Present (+)

2.3 Total phenolic and flavonoid content

Total phenolic content was estimated using the Folin-Ciocalteu method with gallic acid as standard. Absorbance was measured at 760 nm and values were expressed as mg gallic acid equivalents per gram of extract. Total flavonoid content was estimated using the aluminum

chloride colorimetric method with rutin as standard. Absorbance was measured at 510 nm and values were expressed as mg rutin equivalents per gram of extract.

Table 3: Calibration data for phenolic and flavonoid estimation.

Standard	Concentration (µg/mL)	Absorbance
Gallic acid	20	0.162
Gallic acid	40	0.195
Gallic acid	60	0.209
Gallic acid	80	0.245
Gallic acid	100	0.280
Rutin	20	0.182
Rutin	40	0.207
Rutin	60	0.260
Rutin	80	0.289
Rutin	100	0.339

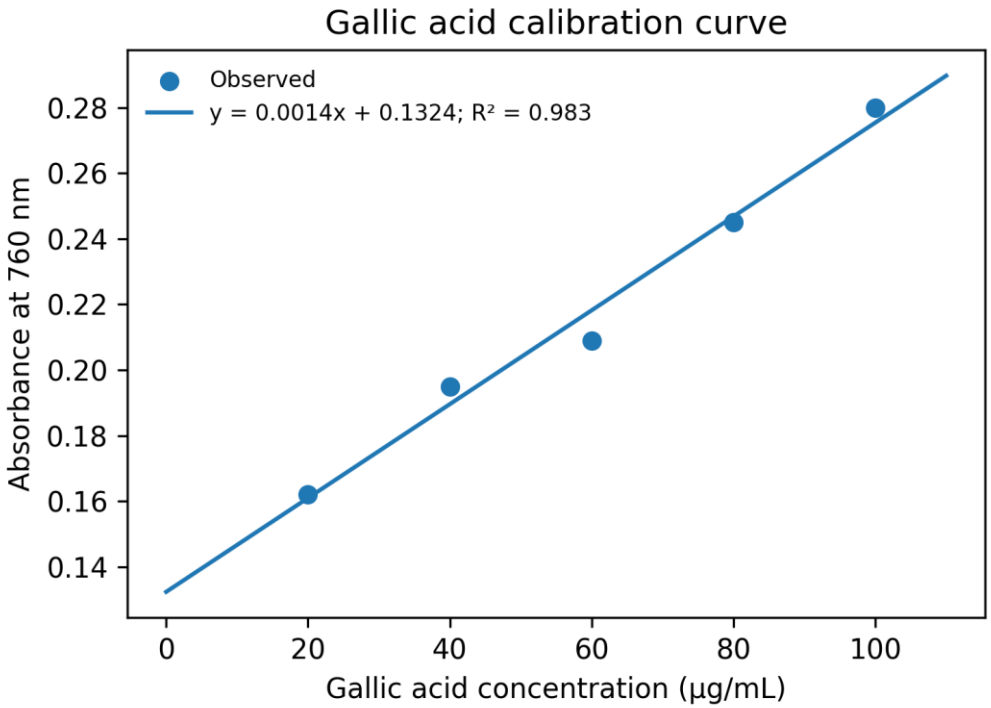

Figure 2: Gallic acid calibration curve for total phenolic content estimation. Linear fit: y = 0.0014x + 0.1324; R^2 = 0.983.

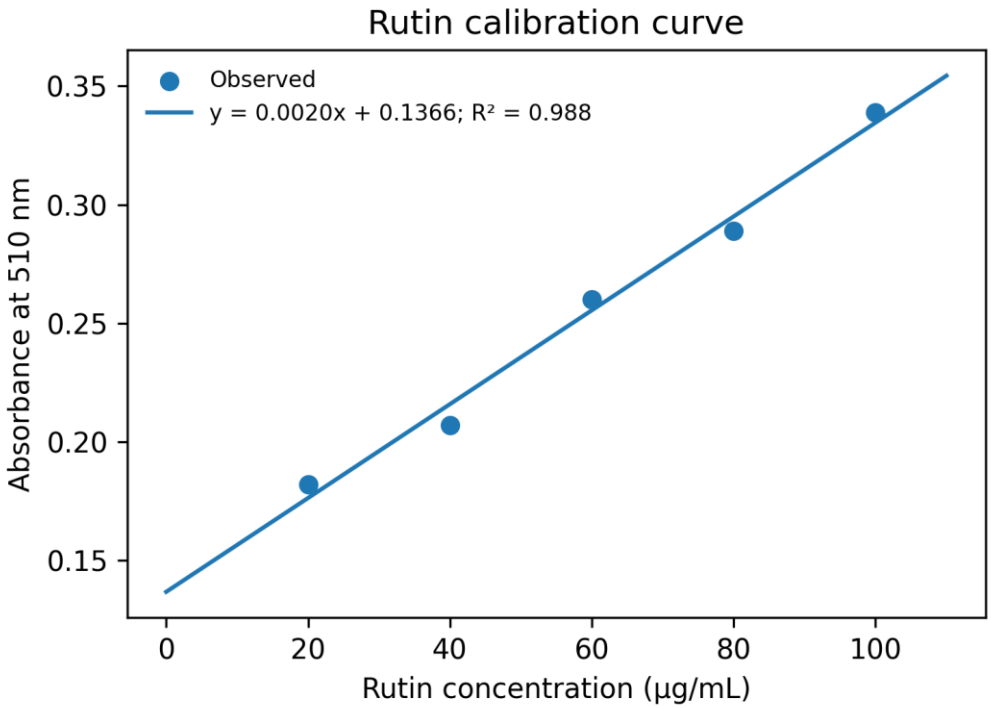

Figure 3: Rutin calibration curve for total flavonoid content estimation. Linear fit: y = 0.0020x + 0.1366; R^2 = 0.988.

Table 4: Quantitative phytochemical and antioxidant summary.

Endpoint	Reported value	Unit/Expression
Total phenolic content	49.6	mg gallic acid equivalents/g extract
Total flavonoid content	28.2	mg rutin equivalents/g extract
DPPH IC50 - ascorbic acid	20.80	µg/mL
DPPH IC50 - Z. gibbosa extract	37.12	µg/mL

2.4 DPPH radical scavenging assay

Antioxidant activity was determined by the DPPH free radical scavenging assay. A 0.1 mM DPPH solution was prepared in ethanol and mixed with test solutions at graded concentrations. After incubation in the dark at room temperature for 30 min, absorbance was measured at 517 nm. Ascorbic acid served as the reference antioxidant.

Table 5: DPPH radical scavenging activity.

Sample	Concentration (µg/mL)	Absorbance	Inhibition (%)
Ascorbic acid	20	0.479	51.810
Ascorbic acid	40	0.431	56.639
Ascorbic acid	60	0.346	65.191
Ascorbic acid	80	0.285	71.327
Ascorbic acid	100	0.144	85.513
Z. gibbosa extract	20	0.510	46.031
Z. gibbosa extract	40	0.452	52.169

Sample	Concentration (µg/mL)	Absorbance	Inhibition (%)
Z. gibbosa extract	60	0.437	53.756
Z. gibbosa extract	80	0.402	57.460
Z. gibbosa extract	100	0.356	62.328

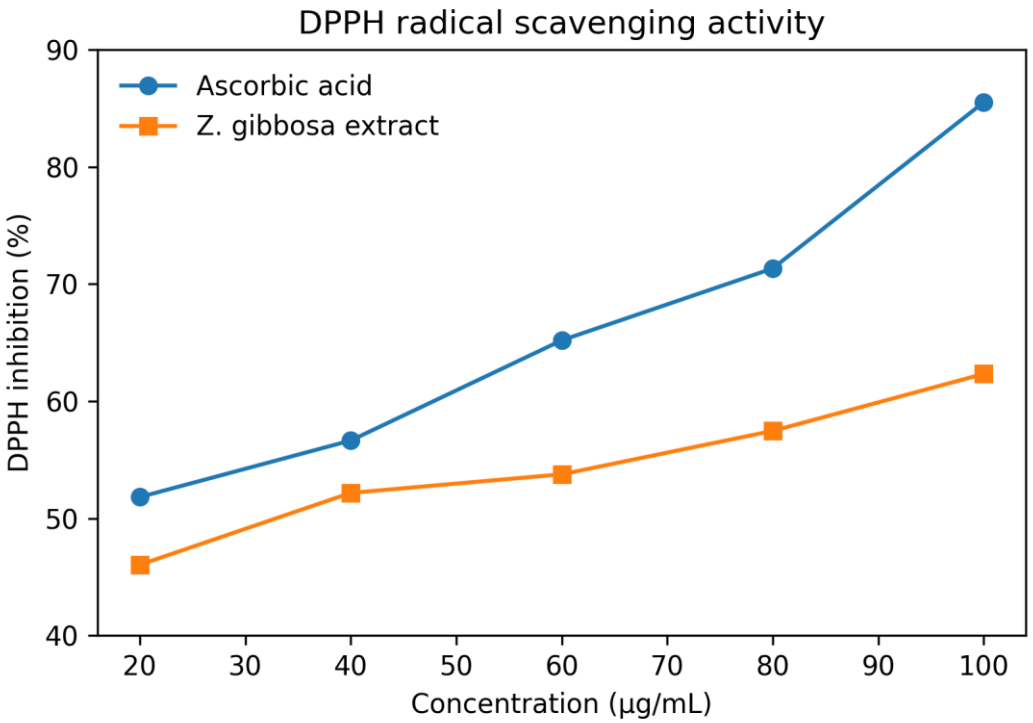

Figure 4: Concentration-dependent DPPH radical scavenging activity of ascorbic acid and Zornia gibbosa ethanolic extract.

2.5 Acute oral toxicity observations

The source text states that acute oral toxicity was assessed according to OECD Guideline 423. Animals were observed for skin, eye, mucous membrane, salivation, stool, urine, sleep, behavior, sensory-motor activity, mortality, body weight, and food intake across 14 days. The toxicity table supplied in the source text appears to contain inconsistent dose labeling; therefore, only the observed outcome pattern is summarized here.

Table 6: Summary of acute toxicity observations reported in the dataset.

Parameter category	Observation summary
Mortality	No mortality reported across the 14-day observation period; all animals recorded as alive.
Behavior, sleep, salivation, urine, sensory-motor activity	Generally recorded as normal throughout the observation period.
Skin/eye/mucous membrane/stool	Minor transient findings were recorded, including hair fall, flaky eye appearance, runny nose, discolored or hard stool.
Body weight	Reported values ranged from 175 g to 193 g across

Parameter category	Observation summary
	observation days.
Interpretive caution	Dose labels in the source toxicity section are inconsistent and should be verified against the laboratory record before submission.

2.6 Experimental antiurolithiatic model

Male rats were allocated into five groups (n = 6 per group). Urolithiasis was induced using 0.75% ethylene glycol and 1% ammonium chloride in drinking water for 28 days. Cystone at 750 mg/kg served as standard treatment, and Zornia gibbosa extract was administered orally at 200 and 400 mg/kg.

Table 7: Experimental grouping for antiurolithiatic evaluation.

Group	Designation	Treatment	Duration
I	Normal control	Saline 5 mL/kg; standard diet and water	28 days
II	Disease/lithiatic control	0.75% ethylene glycol + 1% ammonium chloride	28 days
III	Standard drug	0.75% ethylene glycol + 1% ammonium chloride + Cystone 750 mg/kg orally	28 days
IV	Extract low dose	0.75% ethylene glycol + 1% ammonium chloride + extract 200 mg/kg orally	28 days
V	Extract high dose	0.75% ethylene glycol + 1% ammonium chloride + extract 400 mg/kg orally	28 days

2.7 Biochemical endpoints

Serum creatinine, serum calcium, serum urea, and serum phosphorus were used as biochemical endpoints of renal dysfunction and antiurolithiatic response. Results are reported as mean ± SEM. The source data indicate that assays were performed in triplicate; however, raw replicate data and p-values were not provided.

3. RESULTS

3.1 Extraction yield and phytochemical profile

Soxhlet extraction with ethanol produced 19.06 g extract from 400 g plant powder, corresponding to a yield of 4.76% (Table 1; Figure 1). Qualitative screening showed a broad profile of secondary metabolites, including flavonoids, tannins/phenolics, carbohydrates, alkaloids, glycosides, and saponins (Table 2). Proteins/amino acids, steroids, and triterpenoids were recorded as absent.

3.2 Phenolic/flavonoid content and antioxidant activity

The gallic acid and rutin calibration datasets showed positive linear relationships between concentration and absorbance (Figures 2 and 3). The extract contained 49.6 mg gallic acid equivalents/g and 28.2 mg rutin equivalents/g. In the DPPH assay, both ascorbic acid and the extract exhibited concentration-dependent radical scavenging. The extract had a higher IC50 than ascorbic acid (37.12 versus 20.80 µg/mL), indicating lower potency than the reference antioxidant but measurable antioxidant activity within the tested concentration range.

3.3 Acute oral toxicity

No mortality was reported during the 14-day observation period. Most behavioral and physiological observations were recorded as normal. Minor transient observations included hair fall, flaky eye appearance, runny nose, and stool changes. Because the dose labels in the source table are internally inconsistent, the toxicity findings should be verified before regulatory or journal submission.

3.4 Serum biochemical markers in the antiurolithiatic model

Table 8: Serum biochemical markers in ethylene glycol/ammonium chloride-induced urolithiasis model. Values are mean ± SEM.

Group	Creatinine (mg/dL)	Calcium (mg/dL)	Urea (mg/dL)	Phosphorus (mg/dL)
Normal saline	0.62 ± 0.02	5.74 ± 0.07	10.23 ± 0.33	3.35 ± 0.06
Disease control	1.02 ± 0.06	8.66 ± 0.13	30.16 ± 2.15	5.87 ± 0.13
Cystone 750 mg/kg	0.75 ± 0.03	6.13 ± 0.05	11.37 ± 0.07	4.14 ± 0.04
Extract 200 mg/kg	0.95 ± 0.05	7.19 ± 0.04	15.25 ± 1.45	4.78 ± 0.09
Extract 400 mg/kg	0.79 ± 0.04	6.53 ± 0.06	13.15 ± 0.12	4.35 ± 0.05

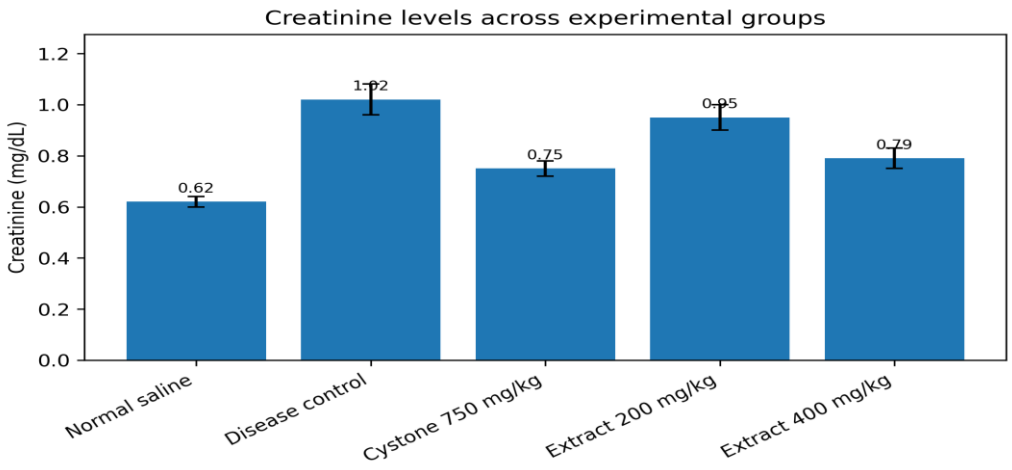

Figure 5: Serum creatinine level across experimental groups. Bars represent mean values and error bars represent SEM as reported in the supplied dataset.

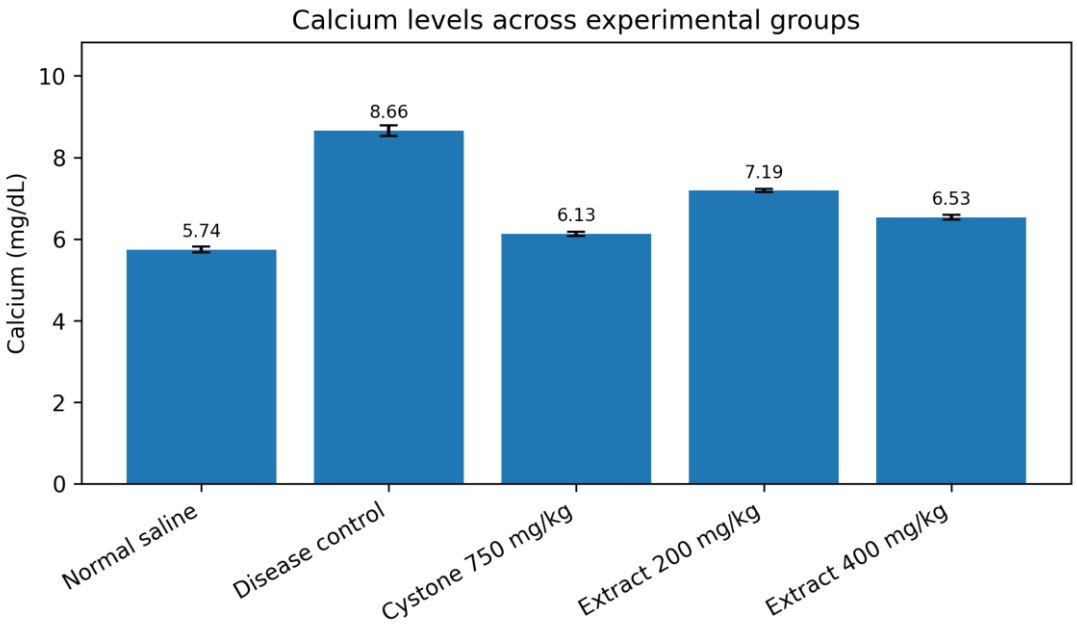

Figure 6: Serum calcium level across experimental groups. Bars represent mean values and error bars represent SEM as reported in the supplied dataset.

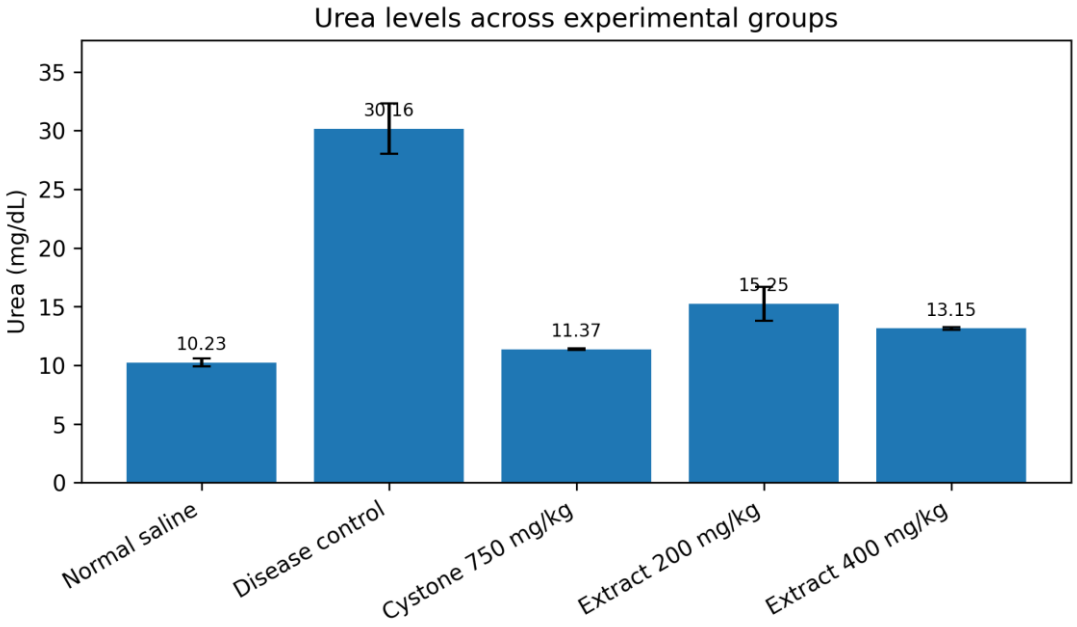

Figure 7: Serum urea level across experimental groups. Bars represent mean values and error bars represent SEM as reported in the supplied dataset.

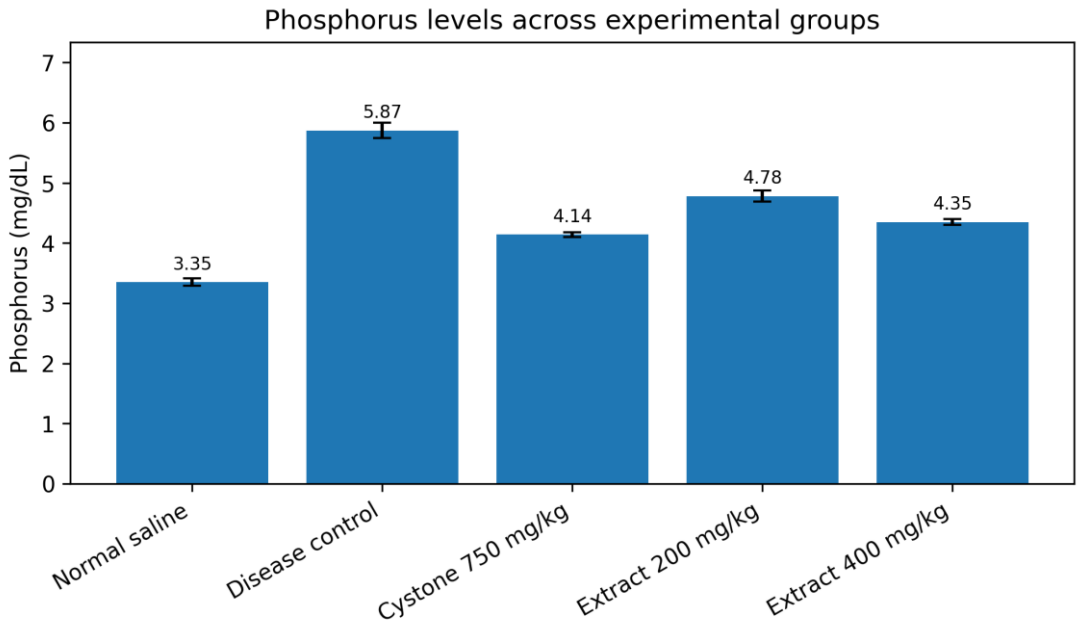

Figure 8: Serum phosphorus level across experimental groups. Bars represent mean values and error bars represent SEM as reported in the supplied dataset.

The disease control group had higher serum creatinine, calcium, urea, and phosphorus than normal control. Extract treatment produced dose-dependent numerical reductions in all four markers. The 400 mg/kg extract group approached the standard Cystone group more closely than the 200 mg/kg group, particularly for creatinine, urea, and phosphorus.

4. DISCUSSION

The dataset suggests that ethanol extracted a chemically diverse fraction from Zornia gibbosa, with qualitative evidence for phenolics/flavonoids, alkaloids, glycosides, and saponins. The quantitative phenolic and flavonoid values provide a plausible chemical basis for the DPPH radical scavenging response. Because oxidative stress contributes to renal injury in urolithiasis models, antioxidant activity may be relevant to the observed biochemical protection; however, this remains a mechanistic hypothesis unless oxidative stress biomarkers, histopathology, and stone/crystal burden data are provided.

In the ethylene glycol/ammonium chloride model, elevated creatinine and urea in the disease control group indicate renal functional disturbance. Increased calcium and phosphorus are consistent with altered mineral handling in the model. Treatment with Zornia gibbosa extract reduced these markers numerically, with the high dose showing stronger effects than the low dose. The pattern is consistent with a dose-dependent antiurolithiatic or nephroprotective

effect, but the supplied dataset does not include inferential statistics. Therefore, the statement should be presented as a numerical trend unless ANOVA and post-hoc test outputs confirm statistical significance.

Several limitations should be addressed before submission. First, individual animal-level data were not supplied, preventing independent statistical verification. Second, the toxicity section contains inconsistent extract type and dose labels, which require reconciliation. Third, histopathological kidney assessment, urinary oxalate/citrate/calcium, kidney crystal burden, and oxidative stress markers were not included in the supplied dataset. Fourth, no chromatographic fingerprinting or compound identification data were provided, so attribution to specific phytoconstituents remains provisional.

5. CONCLUSION

The supplied experimental data indicate that ethanolic extract of Zornia gibbosa contains multiple bioactive phytochemical classes, measurable phenolic and flavonoid content, and concentration-dependent DPPH radical scavenging activity. In an ethylene glycol/ammonium chloride-induced urolithiasis model, extract treatment produced dose-dependent numerical reductions in serum creatinine, calcium, urea, and phosphorus, with the 400 mg/kg dose approaching the standard Cystone group. These observations support further investigation of Zornia gibbosa as a candidate antiurolithiatic plant extract. Robust confirmation will require full raw data, statistical testing, kidney histopathology, urinary stone-forming parameters, and phytochemical characterization.

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