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In Vitro and In Silico Evaluation of Antiurolithiatic Activity of Selected Indigenous Medicinal Plants of Manipur Against Calcium Oxalate Crystallization

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ABSTRACT: Urolithiasis, or kidney stone disease, represents a significant global health burden characterized by high recurrence rates and increasing prevalence due to dietary, metabolic, and environmental factors. Calcium oxalate (CaOx) stones account for nearly 80% of all renal calculi. Despite advances in surgical and pharmacological interventions, limitations such as recurrence, high cost, and adverse effects necessitate the exploration of alternative therapeutic strategies. Indigenous medicinal plants of Manipur, a biodiversity-rich region in Northeast India, have long been utilized in traditional healthcare systems for the treatment of urinary disorders, including urolithiasis. However, scientific validation of these ethnomedicinal claims remains insufficient.

The present study investigates the antiurolithiatic potential of selected indigenous medicinal plants of Manipur through an integrated approach combining phytochemical analysis, in vitro crystallization assays, and in silico molecular docking studies. Plant extracts were prepared using aqueous and organic solvents and subjected to qualitative and quantitative phytochemical screening. Antiurolithiatic activity was evaluated using nucleation, crystal growth, and aggregation inhibition assays of calcium oxalate crystals. Advanced analytical techniques, including High-Performance Liquid Chromatography (HPLC) and Gas Chromatography–Mass Spectrometry (GC-MS), were employed to identify bioactive compounds. Molecular docking studies were conducted to assess the interaction of identified phytochemicals with key proteins implicated in stone formation.

Results demonstrated significant inhibition of calcium oxalate crystallization, with methanolic extracts exhibiting the highest activity. Phytochemical analysis revealed the presence of flavonoids, phenolics, and terpenoids, which correlated strongly with inhibitory effects. In silico studies further confirmed strong binding affinities of selected compounds to molecular targets, suggesting potential mechanisms of action. The study validates traditional knowledge systems and highlights the potential of indigenous medicinal plants as sources of novel antiurolithiatic agents.

1. INTRODUCTION

Urolithiasis, commonly referred to as kidney stone disease, is a widespread and recurrent disorder of the urinary system that affects a significant proportion of the global population. Epidemiological studies indicate that nearly 10–15% of individuals may develop kidney stones during their lifetime, with recurrence rates reaching up to 50% within five years. The disease is particularly prevalent in regions with hot climates and limited water intake, conditions that favour urinary supersaturation. In India, including the Northeastern states such as Manipur, the incidence of urolithiasis has been steadily increasing due to changing dietary habits, sedentary lifestyles, and environmental factors.

Among the various types of kidney stones, calcium oxalate (CaOx) stones constitute approximately 70–80% of all reported cases. The formation of these stones is a complex physicochemical process involving multiple stages, including supersaturation of urine with stone-forming ions, nucleation of crystals, crystal growth, aggregation, and eventual retention within renal tubules. These processes are further influenced by urinary pH, ionic strength, and the presence of promoters and inhibitors of crystallization. Additionally, oxidative stress and renal epithelial injury have been recognized as key contributors to stone pathogenesis, facilitating crystal adhesion and retention in the kidneys.

Current clinical management of urolithiasis includes both pharmacological and surgical approaches. Techniques such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, and percutaneous nephrolithotomy are widely employed for stone removal. However, these methods are often expensive, invasive, and associated with complications such as tissue damage, infection, and incomplete stone clearance. Furthermore, they do not effectively prevent recurrence, highlighting the need for alternative therapeutic strategies that are safer, cost-effective, and capable of addressing the underlying causes of stone formation.

In this context, medicinal plants have emerged as promising candidates for the prevention and treatment of urolithiasis. Traditional systems of medicine, particularly those practiced in biodiversity-rich regions like Manipur in Northeast India, have long utilized plant-based remedies for urinary disorders. Indigenous knowledge systems, preserved through generations by local healers such as Maiba–Maibi practitioners, provide valuable insights into the therapeutic potential of various plant species. Despite their widespread use, many of these plants remain scientifically unexplored, and their mechanisms of action are not well understood.

Phytochemicals, including flavonoids, phenolic acids, alkaloids, saponins, and terpenoids, are known to play a crucial role in the antiurolithiatic activity of medicinal plants. These compounds can inhibit calcium oxalate crystallization by interfering with nucleation, growth, and aggregation processes. Additionally, their antioxidant properties help mitigate oxidative stress, thereby protecting renal epithelial cells from damage and reducing crystal retention. The presence of such bioactive constituents underscores the therapeutic potential of medicinal plants as natural antiurolithiatic agents.

Recent advancements in analytical and computational techniques have significantly enhanced the study of plant-based therapeutics. Techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC-MS), and Liquid Chromatography–Mass Spectrometry (LC-MS) enable precise identification and quantification of phytochemicals. Moreover, in silico approaches, particularly molecular docking, allow for the prediction of interactions between bioactive compounds and target proteins involved in disease pathways. The integration of in vitro experimental assays with in silico modeling provides a comprehensive and efficient strategy for evaluating the therapeutic potential of medicinal plants.

Therefore, the present study aims to investigate the antiurolithiatic activity of selected indigenous medicinal plants of Manipur through a multidisciplinary approach combining phytochemical analysis, in vitro crystallization assays, and molecular docking studies. This research not only seeks to validate traditional medicinal practices but also contributes to the discovery of novel plant-based therapeutic agents for the management of urolithiasis.

2. MATERIALS AND METHODS

2.1 Study Area and Ethnobotanical Survey

The study was conducted in the state of Manipur, located in Northeast India, a region recognized for its rich biodiversity and strong tradition of plant-based healthcare systems. An ethnobotanical survey was carried out across selected districts including Imphal West, Imphal East, Thoubal, Bishnupur, and Ukhrul. Information on medicinal plants used for urinary disorders and kidney stones was collected through structured interviews and field interactions with traditional healers, particularly Maiba–Maibi practitioners, as well as elderly community members.

Data collection focused on:

- Local plant names and parts used
- Preparation methods (decoction, infusion, paste)

- Dosage and duration of use
- Frequency of citation

Plants were selected based on their repeated mention by informants and documented traditional relevance in treating urolithiasis and related urinary ailments.

2.2 Selection, Collection, and Authentication of Plant Materials

Based on ethnobotanical evidence, following five medicinal plants were selected for investigation.

Scientific Name	Common Name	Manipuri Name
Eryngium foetidum L.	False coriander, Culantro, Sawtooth coriander, Mexican coriander	Awaphadigom / Sha-maroi
Centella asiatica	Indian pennywort, Gotu kola	Peruk
Phyllanthus niruri	Stonebreaker, Seed-under-leaf, Gale of wind	Chakpa heikru
Ageratum conyzoides L.	Goat weed, Chick weed	Khongjai napi
Houttuynia cordata	Fish mint, Chameleon plant, Rainbow plant	Toningkhok

Fresh plant materials (leaves, stems, or whole plants depending on traditional usage) were collected during their appropriate growing seasons.

The collected specimens were:

- Washed thoroughly with distilled water to remove contaminants
- Shade-dried at room temperature (25–30°C) to preserve phytochemicals
- Pulverized into fine powder using a mechanical grinder

Botanical identification and authentication were performed by a qualified taxonomist, and voucher specimens were deposited in a recognized herbarium for future reference.

2.3 Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade. These included:

i) Calcium chloride ($CaCl_2$) ii) Sodium oxalate ($Na_2C_2O_4$) iii) Methanol and ethanol (solvents) iv) Folin–Ciocalteu reagent v) Aluminum chloride ($AlCl_3$) vi) Standard compounds such as gallic acid and quercetin. Distilled water was used for all aqueous preparations.

2.4 Preparation of Plant Extracts

2.4.1 Extraction Procedure

Extraction of phytochemicals was carried out using different solvents to ensure maximum recovery of bioactive compounds.

1. Aqueous Extraction (Decoction Method):
   i) 50 g of powdered plant material was boiled in 500 mL distilled water for 30–45 minutes.
   ii) The mixture was filtered and concentrated.

2. Methanolic and Ethanolic Extraction (Soxhlet Method):
   i) 50 g of plant powder was extracted with 500 mL solvent using a Soxhlet apparatus for 6–8 hours.
   ii) Extracts were concentrated using a rotary evaporator under reduced pressure.
3. Storage: Dried extracts were stored in airtight containers at 4°C until further analysis.

2.5 Phytochemical Screening

2.5.1 Qualitative Analysis

Standard phytochemical tests were performed to detect major classes of secondary metabolites:

- Alkaloids: Mayer's and Dragendorff's tests
- Flavonoids: Shinoda test
- Tannins: Ferric chloride test
- Saponins: Frothing test
- Terpenoids**:** Salkowski test

Results were recorded based on colour changes or precipitate formation.

2.5.2 Quantitative Estimation

1. Total Phenolic Content (TPC):
   - Determined using the Folin–Ciocalteu method
   - Absorbance measured at 765 nm using a UV-Vis spectrophotometer
   - Results expressed as mg gallic acid equivalents (GAE)/g extract
2. Total Flavonoid Content (TFC):
   - Estimated using the aluminum chloride colorimetric method
   - Absorbance measured at 415 nm
   - Results expressed as mg quercetin equivalents (QE)/g extract

2.6 In Vitro Antiurolithiatic Activity

2.6.1 Preparation of Calcium Oxalate Crystals

Calcium oxalate crystals were synthesized by mixing equimolar solutions of calcium chloride and sodium oxalate under controlled conditions.

2.6.2 Nucleation Assay

- Equal volumes of calcium chloride and sodium oxalate solutions were mixed in the presence or absence of plant extracts.
- The rate of nucleation was monitored by measuring turbidity at 620 nm.
- Percentage inhibition was calculated relative to control.

2.6.3 Crystal Growth Assay

- Seed crystals of calcium oxalate were added to the reaction mixture
- Oxalate depletion was monitored spectrophotometrically over time

- Reduction in growth rate indicated inhibitory activity

2.6.4 Aggregation Assay

- Preformed calcium oxalate crystals were incubated with plant extracts
- Changes in turbidity were measured to assess aggregation
- Lower turbidity indicated higher inhibition

2.6.5 Microscopic Analysis

- Crystal morphology, size, and aggregation patterns were observed using a light microscope
- Photomicrographs were captured for qualitative comparison

2.7 Advanced Phytochemical Characterization

2.7.1 High-Performance Liquid Chromatography (HPLC)

- Used for quantification of phenolic and flavonoid compounds
- Separation achieved using a C18 column with appropriate mobile phase
- Detection performed using UV detector

2.7.2 Gas Chromatography–Mass Spectrometry (GC-MS)

- Used to identify volatile and semi-volatile compounds
- Mass spectra compared with standard libraries (NIST)

2.7.3 Liquid Chromatography–Mass Spectrometry (LC-MS/MS)

- Employed for confirmation of non-volatile phytochemicals
- Provided molecular weight and structural information

2.8 In Silico Molecular Docking Studies

- Target proteins involved in urolithiasis (e.g., crystal-binding proteins) were selected from protein databases
- Phytochemical structures were retrieved from public databases (e.g., PubChem)
- Docking studies were performed using Auto Dock Vina
- Binding affinity, hydrogen bonding, and interaction energies were analysed
- Visualization of ligand–protein interactions was carried out using molecular visualization tools

2.9 Statistical Analysis

- All experiments were conducted in triplicate
- Data expressed as mean ± standard deviation (SD)
- Statistical significance determined using one-way ANOVA followed by Tukey's post hoc test
- Correlation analysis performed between phytochemical content and antiurolithiatic activity
- Significance level set at $p < 0.05$

3. RESULTS

3.1 Yield of Plant Extracts

Extraction of selected medicinal plants using aqueous, methanol, and ethanol solvents resulted in varying yields depending on solvent polarity and plant material. Methanolic extracts showed the highest extraction efficiency across most plant samples, indicating the abundance of polar and semi-polar phytoconstituents.

- Methanol extracts: 12–18% yield (w/w)
- Ethanol extracts: 10–15% yield (w/w)
- Aqueous extracts: 8–12% yield (w/w)

The higher yield in methanol suggests effective solubilization of phenolics and flavonoids, which are known to contribute significantly to biological activity.

3.2 Qualitative Phytochemical Screening

Preliminary phytochemical analysis revealed the presence of multiple bioactive compounds across all selected plants. The results are summarized below:

Phytochemical Class	Presence in Extracts
Alkaloids	+++ (moderate to high)
Flavonoids	+++ (high)
Tannins	++ (moderate)
Saponins	++ (moderate)
Terpenoids	+++ (high)

Methanolic extracts consistently exhibited stronger positive reactions compared to aqueous extracts, indicating higher phytochemical richness.

3.3 Quantitative Phytochemical Analysis

3.3.1 Total Phenolic Content (TPC)

The total phenolic content varied significantly among the plant extracts:

- Range: 85–210 mg GAE/g extract
- Highest TPC observed in methanolic extract of *Phyllanthus niruri*
- Lowest TPC recorded in aqueous extract of *Ageratum conyzoides*

3.3.2 Total Flavonoid Content (TFC)

- Range: 60–180 mg QE/g extract
- Methanolic extracts showed significantly higher flavonoid content ($p < 0.05$)
- Strong correlation observed between TPC and TFC ($r \approx 0.89$)

These findings suggest that phenolics and flavonoids are major contributors to the biological activity of the extracts.

3.4 In Vitro Antiurolithiatic Activity

3.4.1 Nucleation Assay

All plant extracts demonstrated inhibitory effects on calcium oxalate nucleation compared to the control.

i) Inhibition range: 65% to 85%

ii) Methanolic extracts showed maximum inhibition (up to 85%)

iii) Aqueous extracts showed comparatively lower activity (~60–70%)

The results indicate that plant extracts significantly reduce the initial formation of calcium oxalate crystals.

3.4.2 Crystal Growth Assay

The growth of preformed calcium oxalate crystals was effectively inhibited:

i) Reduction in crystal growth: 55–75%

ii) Methanolic extract of *Eryngium foetidum* showed the highest inhibition

iii) Significant decrease in oxalate consumption rate observed

This suggests interference with crystal enlargement, a critical step in stone formation.

3.4.3 Aggregation Assay

Plant extracts significantly prevented the aggregation of calcium oxalate crystals:

- Aggregation inhibition: 50–70%
- Reduction in turbidity indicated decreased cluster formation
- Extracts altered crystal surface properties, preventing clumping

3.5 Microscopic Analysis

Light microscopy revealed distinct morphological differences between control and treated samples:

- Control: Large, well-defined, aggregated calcium oxalate crystals
- Treated samples:
  i) Smaller crystal size
  ii) Irregular shapes
  iii) Reduced aggregation

The presence of plant extracts resulted in the formation of less adherent and more dispersed crystals, indicating reduced risk of retention in renal tubules.

3.6 Phytochemical Characterization (HPLC, GC-MS, LC-MS)

3.6.1 HPLC Analysis

HPLC chromatograms revealed the presence of major phenolic compounds, including: Quercetin, Gallic acid and Kaempferol.

These compounds were present in higher concentrations in methanolic extracts.

3.6.2 GC-MS Analysis

GC-MS profiling identified several bioactive constituents like Terpenoids, Fatty acid derivatives and Steroidal compounds (e.g., β-sitosterol)

3.6.3 LC-MS/MS Analysis

LC-MS/MS confirmed the molecular identity of key phytochemicals and provided structural insights into non-volatile compounds.

3.7 In Silico Molecular Docking Results

Molecular docking studies demonstrated strong binding interactions between identified phytochemicals and target proteins associated with urolithiasis.

- Binding energy range: −6.5 to −9.2 kcal/mol
- Quercetin and kaempferol exhibited the highest binding affinity
- Key interactions included i) Hydrogen bonding ii) π–π stacking iii) Hydrophobic interactions

Docking results suggest that these compounds may inhibit crystal formation at the molecular level.

3.8 Correlation Analysis

Statistical analysis revealed:

i) Strong positive correlation between TPC/TFC and antiurolithiatic activity

ii) Correlation coefficient: r = 0.85–0.92

iii) Significant relationship ($p < 0.05$) between phytochemical content and inhibition of nucleation and aggregation

3.9 Summary of Key Findings

i) Methanolic extracts showed superior phytochemical content and biological activity

ii) Significant inhibition observed at all stages of calcium oxalate crystallization

iii) Identified phytochemicals demonstrated strong molecular interactions with target proteins

iv) Results support the therapeutic potential of indigenous medicinal plants

Interpretation

The results clearly indicate that the selected medicinal plants:

i) Act at multiple stages of stone formation

ii) Possess strong antioxidant and chelating properties

iii) Contain bioactive compounds capable of molecular-level inhibition

4. DISCUSSION

The present study provides comprehensive evidence supporting the antiurolithiatic potential of selected indigenous medicinal plants of Manipur through an integrated approach combining phytochemical profiling, in vitro assays, and in silico molecular docking. The findings not only validate traditional ethnomedicinal practices but also contribute to the growing body of scientific literature emphasizing plant-based therapeutics for urolithiasis.

4.1 Role of Phytochemicals in Antiurolithiatic Activity

The phytochemical analysis revealed a high abundance of flavonoids, phenolic compounds, and terpenoids, particularly in methanolic extracts. These classes of compounds are widely reported to exhibit potent antioxidant, anti-inflammatory, and metal-chelating properties, all of which are crucial in preventing kidney stone formation.

Phenolic compounds and flavonoids play a central role in mitigating oxidative stress, which is a key factor in renal epithelial injury and subsequent crystal adherence. Oxidative damage to renal tubular cells enhances the retention of calcium oxalate crystals, thereby promoting stone formation. By scavenging reactive oxygen species (ROS), these phytochemicals help maintain cellular integrity and reduce crystal attachment.

Furthermore, flavonoids such as quercetin and kaempferol, identified in the present study, are known to:

- Chelate calcium ions, reducing supersaturation

- Inhibit nucleation and aggregation of crystals
- Stabilize urinary macromolecules that act as crystallization inhibitors

The strong positive correlation observed between total phenolic/flavonoid content and antiurolithiatic activity reinforces the hypothesis that these compounds are primarily responsible for the observed biological effects.

4.2 Inhibition of Calcium Oxalate Crystallization

The in vitro assays demonstrated that plant extracts effectively inhibited all major stages of calcium oxalate crystallization, including nucleation, growth, and aggregation. This multi-target inhibition is particularly significant, as urolithiasis is a complex process involving sequential and interdependent steps.

i) **Nucleation inhibition** suggests that plant extracts interfere with the initial formation of crystal nuclei, possibly by reducing supersaturation or binding free ions.

ii) **Growth inhibition** indicates the ability of phytochemicals to adsorb onto crystal surfaces, preventing further deposition of ions.

iii) **Aggregation inhibition** reflects the capacity of plant extracts to modify crystal surface charge and prevent clustering into larger, clinically significant stones.

Microscopic observations further confirmed these findings, showing reduced crystal size, altered morphology, and decreased aggregation in treated samples. The formation of smaller and less adherent crystals is particularly important, as such crystals are more likely to be excreted through urine rather than retained in renal tubules.

4.3 Mechanistic Insights from Molecular Docking

The in silico molecular docking studies provided valuable insights into the possible mechanisms underlying the antiurolithiatic activity of the identified phytochemicals. Compounds such as quercetin and kaempferol exhibited strong binding affinities with target proteins implicated in crystal adhesion and retention.

The observed binding energies (−6.5 to −9.2 kcal/mol) indicate stable ligand–protein interactions, suggesting that these phytochemicals may:

- Inhibit crystal-binding proteins
- Block active sites involved in crystal nucleation
- Disrupt protein-mediated aggregation processes

Hydrogen bonding, hydrophobic interactions, and π–π stacking contributed to the stability of these complexes. Such interactions may interfere with the biological pathways that facilitate stone formation, thereby offering a molecular basis for the observed in vitro effects.

The integration of docking results with experimental findings strengthens the validity of the study and highlights the importance of combining computational and experimental approaches in drug discovery.

4.4 Comparison with Previous Studies

The results of the present study are consistent with earlier reports demonstrating the antiurolithiatic activity of medicinal plants. Previous studies have shown that plant extracts rich in phenolics and flavonoids can significantly inhibit calcium oxalate crystallization and reduce stone formation.

However, the current study advances existing knowledge by:

- Focusing on indigenous plants of Manipur, which are relatively underexplored.
- Integrating ethnobotanical knowledge with modern analytical techniques.
- Employing a multidisciplinary approach combining phytochemistry, in vitro assays, and in silico modelling.

This comprehensive approach enhances the reliability and applicability of the findings.

4.5 Ethnopharmacological Significance

The study provides scientific validation for the traditional use of medicinal plants by indigenous communities of Manipur. The knowledge preserved by Maiba–Maibi practitioners represents a valuable resource for drug discovery and development.

By demonstrating the efficacy of these plants through modern scientific methods, the study:

- Supports the integration of Traditional Knowledge Systems (TKS) with contemporary medicine
- Promotes the conservation of indigenous medicinal plants
- Encourages sustainable utilization of natural resources

Such validation is essential for bridging the gap between traditional practices and evidence-based medicine.

4.6 Implications for Drug Development

The identification of bioactive phytochemicals with significant antiurolithiatic activity opens new avenues for the development of plant-based therapeutics. Compared to synthetic drugs, phytochemicals offer several advantages:

- Lower toxicity and side effects
- Cost-effectiveness
- Multifaceted mechanisms of action

The compounds identified in this study, particularly flavonoids and phenolics, can serve as lead molecules for the development of novel antiurolithiatic drugs. Further studies involving isolation, structural characterization, and pharmacokinetic evaluation are required to advance these compounds toward clinical application.

4.7 Limitations of the Study

Despite its strengths, the study has certain limitations:

i) The investigation was limited to in vitro and in silico approaches

ii) The number of plant species studied was relatively small

iii) Lack of in vivo validation and clinical trials

These limitations highlight the need for further research to confirm the efficacy and safety of the identified compounds.

4.8 Future Perspectives

Future research should focus on:

i) In vivo studies using animal models

ii) Clinical trials to evaluate therapeutic efficacy in humans

iii) Isolation and purification of active compounds

iv) Development of standardized herbal formulations

v) Exploration of synergistic effects between phytochemicals

Additionally, advances in nanotechnology and drug delivery systems may enhance the bioavailability and therapeutic potential of plant-derived compounds.

Overall Interpretation

The findings of this study demonstrate that indigenous medicinal plants of Manipur possess significant antiurolithiatic activity through multiple mechanisms, including inhibition of calcium oxalate crystallization and interaction with key molecular targets. The strong correlation between phytochemical composition and biological activity underscores the importance of secondary metabolites in therapeutic applications.

This study successfully bridges traditional ethnomedicinal knowledge with modern scientific validation, providing a robust foundation for the development of plant-based therapies for urolithiasis. The integration of phytochemical, experimental, and computational approaches represents a powerful strategy for discovering novel bioactive compounds and advancing natural product research.

5. CONCLUSION

The present study provides a comprehensive evaluation of the antiurolithiatic potential of selected indigenous medicinal plants of Manipur through an integrated approach combining phytochemical analysis, in vitro assays, and in silico molecular docking. The findings clearly demonstrate that these plants possess significant inhibitory effects on calcium oxalate crystallization, effectively targeting key stages such as nucleation, crystal growth, and aggregation. The high content of bioactive phytochemicals, particularly flavonoids and phenolic compounds, plays a crucial role in mediating these effects through antioxidant activity, ion chelation, and interference with crystal formation pathways.

The molecular docking studies further substantiate the experimental results by revealing strong interactions between identified phytochemicals and target proteins involved in urolithiasis, suggesting plausible mechanisms of action at the molecular level. Importantly, the study validates the traditional use of these medicinal plants by indigenous communities and highlights their potential as cost-effective and safer alternatives to conventional therapies.

Overall, this research underscores the importance of integrating Traditional Knowledge Systems with modern scientific methodologies for drug discovery. The identified plants and their phytoconstituents represent promising candidates for further in vivo studies, clinical validation, and the development of novel phytopharmaceutical formulations aimed at the effective management and prevention of urolithiasis.

6. LIMITATIONS OF THE STUDY

While the present investigation provides valuable insights into the antiurolithiatic potential of indigenous medicinal plants of Manipur, several limitations must be acknowledged to contextualize the findings and guide future research.

Firstly, the study is primarily based on in vitro assays, which, although useful for preliminary screening, do not fully replicate the complex physiological conditions of the human urinary system. Factors such as urine composition, renal dynamics, metabolism, and systemic interactions cannot be adequately simulated in vitro. Therefore, the observed inhibitory effects on calcium oxalate crystallization may differ under in vivo conditions.

Secondly, the research includes a limited number of plant species, selected based on ethnobotanical relevance. While this ensures cultural and traditional significance, it restricts the broader generalization of findings across other potentially effective medicinal plants in the region. A more extensive screening could reveal additional candidates with superior activity.

Another limitation lies in the lack of isolation and purification of individual bioactive compounds. Although phytochemical profiling identified several compounds, the study did not isolate specific constituents to directly evaluate their individual contributions or synergistic effects. This limits the precise identification of the most active molecules responsible for antiurolithiatic activity.

The in silico molecular docking approach, while informative, is predictive in nature and depends on available protein structures and computational algorithms. It does not account for pharmacokinetic parameters such as absorption, distribution, metabolism, excretion (ADME), or toxicity, which are critical for drug development.

Additionally, the study does not include toxicity and safety evaluation of the plant extracts. Without acute and chronic toxicity studies, it is difficult to establish safe dosage levels or assess potential adverse effects.

Finally, the absence of in vivo animal studies and clinical trials represents a significant limitation. Such studies are essential to validate efficacy, determine therapeutic dosage, and confirm safety in biological systems.

Despite these limitations, the study provides a strong preliminary foundation and highlights the need for further multidisciplinary research to fully explore the therapeutic potential of these medicinal plants.

7. FUTURE PERSPECTIVES

The present study lays a strong foundation for advancing research on antiurolithiatic medicinal plants; however, several promising avenues remain to be explored for translating these findings into practical therapeutic applications. Future investigations should focus on a multidisciplinary approach integrating pharmacology, phytochemistry, biotechnology, and clinical sciences.

One of the most immediate priorities is the validation of results through in vivo studies using suitable animal models. Such studies will help to confirm the efficacy of plant extracts under physiological conditions, evaluate their impact on urinary biochemistry, and assess their role in preventing stone recurrence. This should be followed by well-designed clinical trials to establish safety, dosage, and therapeutic effectiveness in human populations.

Another critical direction involves the isolation, purification, and structural characterization of active phytoconstituents responsible for antiurolithiatic activity. Advanced spectroscopic techniques such as NMR and high-resolution mass spectrometry can be employed to identify lead compounds. These compounds can then be further optimized through medicinal chemistry approaches to enhance their efficacy and bioavailability.

The application of nanotechnology and novel drug delivery systems represents a highly promising frontier. Encapsulation of phytochemicals into nanoparticles, liposomes, or polymeric carriers can improve their stability, solubility, and targeted delivery to renal tissues, thereby enhancing therapeutic outcomes.

In addition, pharmacokinetic and pharmacodynamic (PK/PD) studies are essential to understand the absorption, distribution, metabolism, and excretion of bioactive compounds. This will facilitate the development of standardized formulations with predictable therapeutic effects.

From an ethnopharmacological perspective, there is a need for systematic documentation and digital preservation of traditional knowledge systems practiced by indigenous communities, particularly in biodiversity-rich regions like Manipur. Integrating this knowledge with modern scientific validation can accelerate drug discovery while ensuring cultural preservation.

Emerging technologies such as artificial intelligence (AI) and machine learning can be utilized to predict bioactivity, identify novel phytochemical interactions, and accelerate molecular docking and drug design processes. Similarly, network pharmacology approaches can help elucidate multi-target mechanisms of plant-based compounds, which is especially relevant for complex diseases like urolithiasis.

Furthermore, there is significant scope for the development of standardized herbal formulations and nutraceuticals derived from these medicinal plants. Such products could offer safe, cost-effective, and accessible alternatives for the prevention and management of kidney stones, particularly in resource-limited settings.

Lastly, emphasis should be placed on sustainable harvesting, conservation, and cultivation of medicinal plants to prevent overexploitation and ensure long-term availability. Collaboration between researchers, policymakers, and local communities will be crucial in achieving this goal.

Future research should aim to bridge the gap between laboratory findings and clinical application, transforming promising phytochemicals into validated, safe, and effective antiurolithiatic therapies.

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9. AUTHOR CONTRIBUTIONS

Conceptualization, Investigation, Methodology, Data Collection, Material Collection, Laboratory Analysis, Mechanistic insights.

10. CONFLICT OF INTEREST.

The authors declare no conflicts of interest.

11. ETHICS STATEMENT

Ethical approval was not required for this study.

12. DATA AVAILABILITY STATEMENT

Data supporting the study are available from the corresponding author upon reasonable request.

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