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In silico evaluation of the anti-urolithiasis potential of *Kalanchoe pinnata* using Network Pharmacology and Molecular Docking approaches

Daniel Raj N*, Chantra I, Dhanusu Raman N, Hema G, Prakash PN, Tamilarasi G*
Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India

Abstract
Urolithiasis, a multifactorial disorder characterized by the formation of renal calculi, remains a persistent global health challenge. The present study explored the anti-urolithiasis potential of *Kalanchoe pinnata* through network pharmacology and molecular docking approaches. Phytoconstituents were selected from literature and the IMPPAT database and screened for ADME and drug-likeness properties using the SwissADME tool. Target prediction and disease-associated gene identification were performed using SwissTargetPrediction and GeneCards databases, respectively. The intersecting targets were analyzed through STRING and Cytoscape to identify hub genes. Functional enrichment analyses via Metascape revealed key pathways, including PI3K-AKT, apoptosis, and oxidative stress regulation, linked to urolithiasis pathogenesis. Molecular docking studies demonstrated that quercetin and kaempferol exhibited strong binding affinities with hub proteins AKT1, BCL2L1, CASP3, and MMP9, confirming their multi-target therapeutic potential. These findings suggest that *Kalanchoe pinnata* exerts anti-urolithiasis effects by modulating oxidative stress, inflammation, and apoptosis pathways, providing a scientific basis for its traditional use in renal disorder management.

Keywords: *Kalanchoe pinnata*, urolithiasis, Network Pharmacology, Molecular Docking, phytochemicals

Introduction

Urolithiasis, commonly referred to as kidney stone disease, is a prevalent urinary disorder resulting from the crystallization of minerals and salts within the urinary tract. Factors such as oxidative stress, inflammation, apoptosis, and metabolic imbalance contribute to its complex pathophysiology. Current therapeutic interventions, including lithotripsy and pharmacological treatments, often present limitations like recurrence, side effects, and high cost. Hence, there is a growing need for safer and more effective plant-based alternatives.

Kalanchoe pinnata (Crassulaceae), a medicinal herb widely used in traditional systems of medicine, has been reported to exhibit antioxidant, anti-inflammatory, and nephroprotective activities. However, its molecular mechanisms against urolithiasis remain unclear. Network pharmacology, integrated with molecular docking, offers a comprehensive strategy to elucidate the multi-target interactions of phytochemicals and their associated pathways in complex diseases.

This study aimed to investigate the molecular mechanisms underlying the anti-urolithiasis potential of Kalanchoe pinnata using a network pharmacology framework coupled with *in silico* docking validation. By identifying key bioactive compounds, target proteins, and signaling pathways, the research provides insight into the herb's therapeutic role and establishes a foundation for future pharmacological studies.

Network Pharmacology

Network pharmacology is a molecular-level approach that uses computational methods to analyze drug-disease interactions, allowing for a more comprehensive understanding of complex diseases like urolithiasis, and provides insights into the anti-urolithiasis effects of phytochemicals through key signaling pathways [1].

Steps in Network Pharmacology Study

1. Identification of Ligand and Disease-Associated Proteins

The ligand is identified as the key drug molecule. Disease-related proteins associated with urolithiasis are obtained from public databases such as GeneCards (https://www.genecards.org/). The overlapping targets between Phytochemicals and urolithiasis-associated proteins are identified using the Venny tool (https://bioinfogp.cnb.csic.es/tools/venny/) [2]

2. Protein-Protein Interaction (PPI) Network Analysis

The identified disease-associated proteins are analyzed using STRING (https://string-db.org/) to construct a PPI network [3].

3. Network Construction Using Cytoscape

The PPI data obtained from STRING is imported into Cytoscape (https://cytoscape.org/) for visualization. Cytoscape software is used to analyze Hub genes (key regulators in the network), Degree centrality (proteins with the highest interactions), and Topological parameters that highlight critical drug-target interactions.

4. Target Validation Using BIOVIA Discovery Studio

The PPI network's top hub proteins are loaded into BIOVIA Discovery Studio for pharmacophore modeling and binding site prediction. Molecular docking studies are performed to assess binding affinity and drug-target interactions, confirming Phytochemicals' effectiveness in Urolithiasis by assessing predicted targets [4].

Results and Discussion

Network Pharmacology Analysis of *Kalanchoe pinnata* Against Urolithiasis

The present study employed a network pharmacology strategy to explore the multi-targeted therapeutic potential

of *Kalanchoe pinnata* in the management of urolithiasis. Phytochemicals were selected from the literature and the IMPPAT database, and their pharmacokinetic properties were evaluated using the SwissADME platform.

1. ADME Screening and Drug-Likeness Evaluation

All selected phytochemicals exhibited favorable ADME profiles, including high gastrointestinal (GI) absorption and molecular weight, suggesting good oral bioavailability and potential systemic distribution. Furthermore, these compounds adhered to major drug-likeness rules as Lipinski's Rule of Five, HB donor, and acceptor, indicating a high likelihood of pharmacological effectiveness with minimal absorption or permeability issues.

Table 1: Lipinski's Rule of Five

S. No	Compounds	Molecular weight	HB Acceptor	HB Donor	Liphophilicity	Molar Refractivity	Rule of five	GI Absorption
1	Syringic acid	198.17	2	1	5.20	80.80	1	HIGH
2	Quercetin	302.24	2	1	5.65	89.94	1	HIGH
3	Kaempferol	286.24	2	1	4.597	89.46	1	HIGH
4	Linalool	154.25	1	1	7.24	133.23	1	LOW
5	Citric acid	192.12	6	4	-1.42	35.12	0	HIGH

2. Target Prediction and Disease Gene Overlap

The SwissTargetPrediction tool was used to identify potential protein targets for each phytochemical. Concurrently, urolithiasis-related genes were retrieved from the GeneCards database using "urolithiasis" as the query keyword. Venn diagram analysis using the Venny tool identified overlapping targets between the phytochemicals and disease-associated genes, suggesting probable therapeutic intervention points.

3. Protein-Protein Interaction (PPI) Network and Hub Gene Identification

The intersecting genes were used to construct a PPI network using the STRING database. The resulting network was imported into Cytoscape software for visualization and topological analysis. The MCODE and CytoHubba plugins were utilized to identify key hub genes, including AKT1, BCL2L1, CASP3, and MMP9, based on degree centrality and clustering coefficients. These hub genes are involved in crucial biological processes such as apoptosis, oxidative stress, and inflammation, mechanisms known to be associated with urolithiasis.

These findings provide mechanistic insight into how *Kalanchoe pinnata* may exert anti-urolithiasis effects through the modulation of multiple gene targets and pathways, aligning with the polypharmacological behavior of phytochemicals.

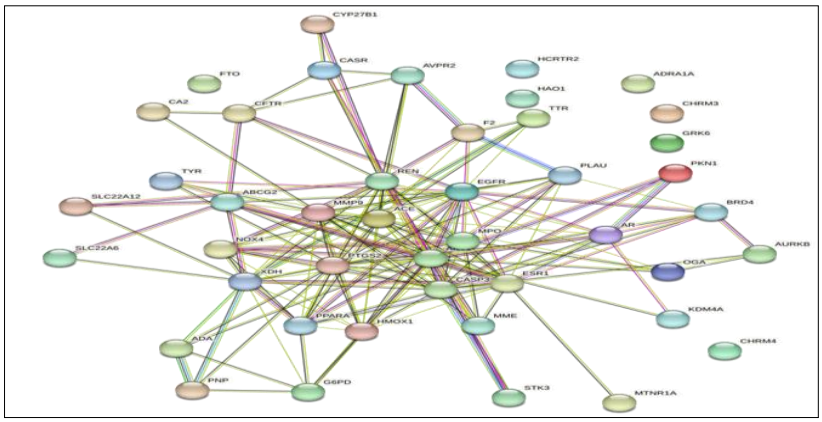


Fig 1: protein-protien interaction network

Common Genes Identification

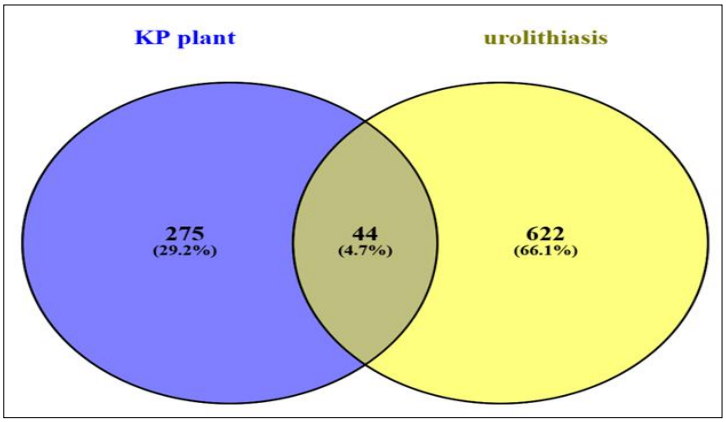


Fig 2: Venn diagram

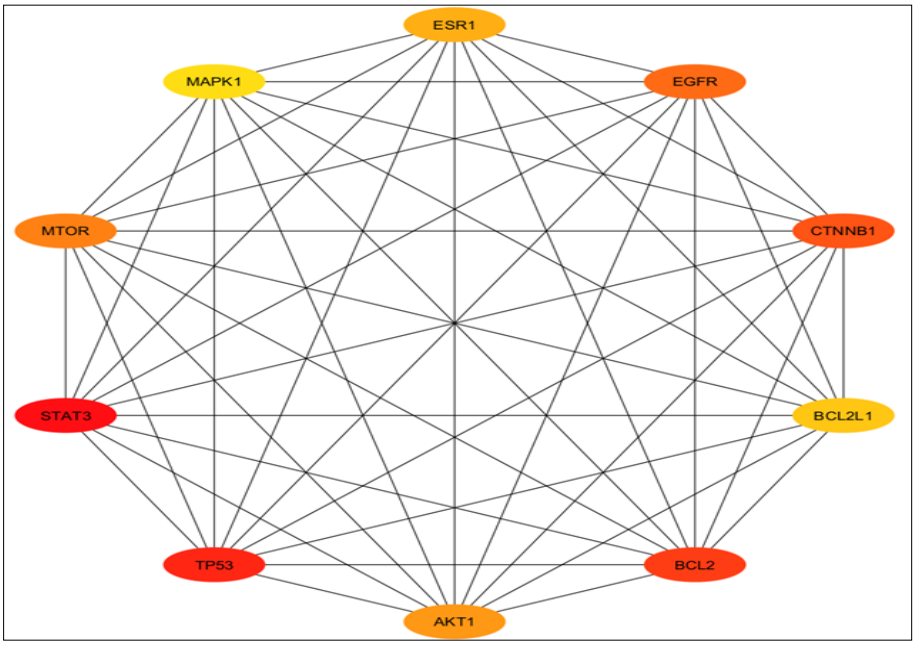


Fig 3: Hub genes

Functional Enrichment Analysis: GO and KEGG Pathways

To further elucidate the biological significance of the identified target genes, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using the Metascape platform. This helped categorize the genes according to their functional roles and biological pathways relevant to the pathophysiology of urolithiasis.

Gene Ontology (GO) Analysis

GO analysis classified the intersecting genes into three major categories: biological processes (BP), molecular functions (MF), and cellular components (CC).

- **Biological Processes (BP):** The majority of genes were involved in key processes, such as, these processes are known to play vital roles in kidney stone formation, where oxidative stress and apoptosis contribute to renal tubular injury and crystal retention.
- **Molecular Functions (MF):** Such functions imply the involvement of regulatory enzymes and signaling molecules that phytochemicals could modulate.
- **Cellular Components (CC):** These components are crucial for intracellular signaling and metabolic regulation during crystal nucleation and aggregation.

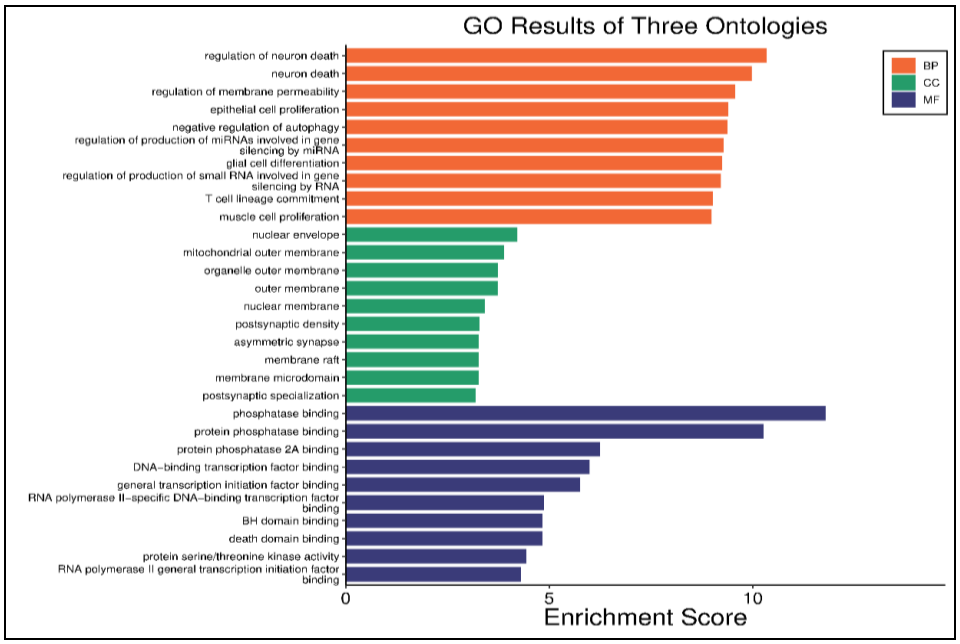


Fig 4: Gene ontology score

KEGG Pathway Enrichment Analysis

KEGG pathway enrichment revealed significant involvement in several disease- and stress-related pathways, these pathways are closely related to cell survival, inflammation, fibrosis, and oxidative stress, which are central to urolithiasis pathogenesis. the PI3K-AKT pathway,

where AKT1 acts as a major node, regulates survival of renal epithelial cells during oxidative damage. MMP9, enriched in the extracellular matrix (ECM) remodeling pathway, may also play a role in crystal adhesion and tissue injury.

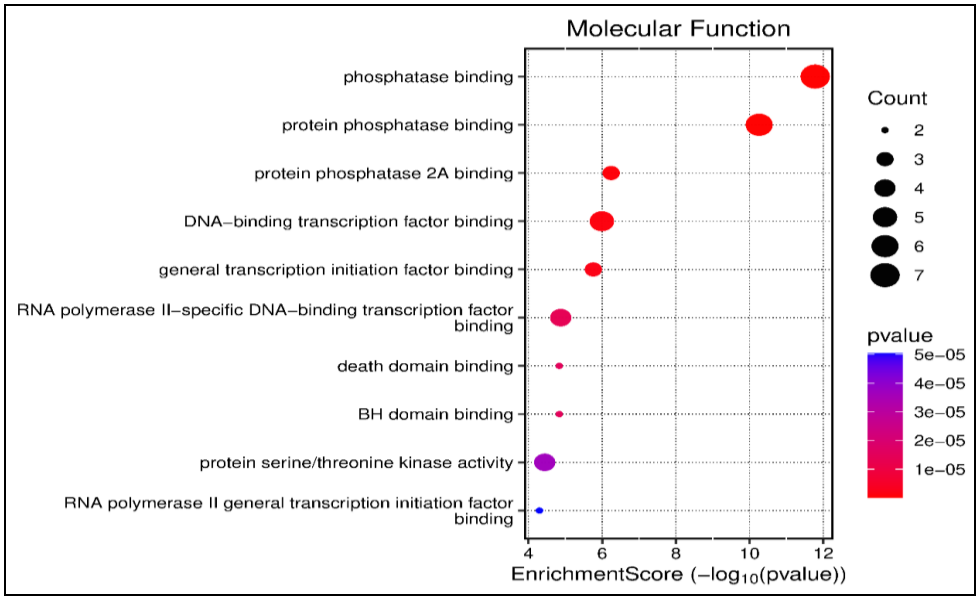


Fig 5: KEGG Pathway Enrichment score

Interpretation

The enrichment analysis supports the multi-target nature of *Kalanchoe pinnata* phytochemicals, particularly Quercetin, Kaempferol, and Syringic acid, in modulating several urolithiasis-relevant biological processes and pathways. The findings strongly align with docking and PPI results, reinforcing the hypothesis that these compounds exert their anti-urolithiasis effects through anti-inflammatory, anti-apoptotic, and antioxidant mechanisms.

Molecular Docking Analysis

Molecular docking was performed to validate interactions between key phytochemicals and hub proteins. The following results were observed:

- **Quercetin:** AKT1 (−9.67 kcal/mol), CASP3 (−8.35 kcal/mol)
- **Kaempferol:** BCL2L1 (−8.11 kcal/mol), MMP9 (−9.12 kcal/mol)
- **Syringic acid:** AKT1 (−8.61 kcal/mol), CASP3 (−7.99 kcal/mol)
- **Linalool and Citric acid:** showed weaker or even positive docking scores, suggesting low specificity or weak binding.

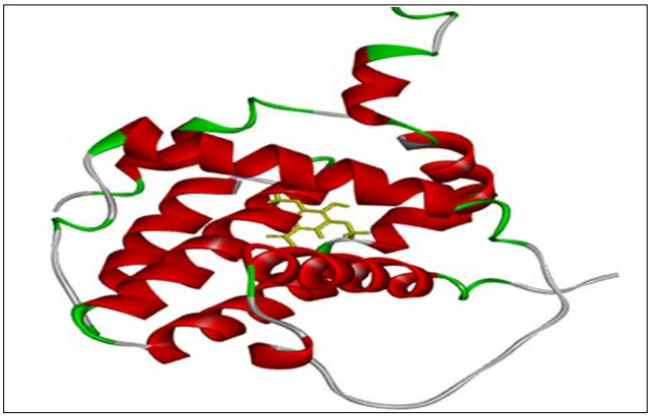
Tools & Methods Used

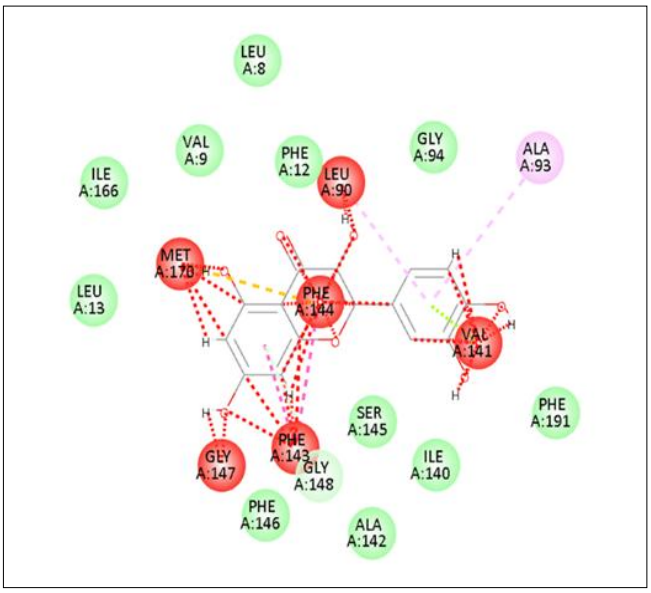
- **Docking Software:** AutoDock Vina, SwissDock
- **Visualization:** BIOVIA Discovery Studio
- **Ligand & Protein Sources:** PubChem and RCSB PDB
- **Docking Strategy:** Blind docking with defined grid box; exhaustiveness adjusted
- **Validation:** Binding site prediction and pharmacophore analysis

Table 2: Molecular docking score

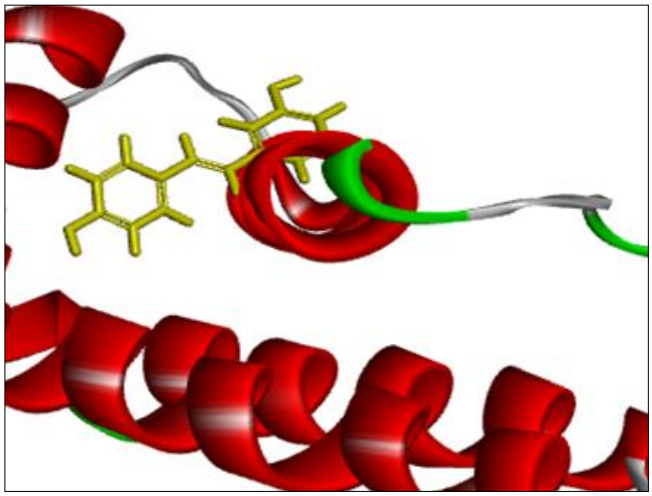
PHYTOCHEMICALS	AKT1	BCL2L1	CASP3	MMP9
Syringic acid	-8.61	-7.54	-7.99	-6.71
Kaempferol	-7.21	-8.11	-7.31	-9.12
Quercetin	-9.67	-8.67	-8.35	-6.99
Linalool	-8.72	-8.34	-9.65	-7.12
Citric acid	-8.41	-7.34	-5.60	-6.89
	GENES			

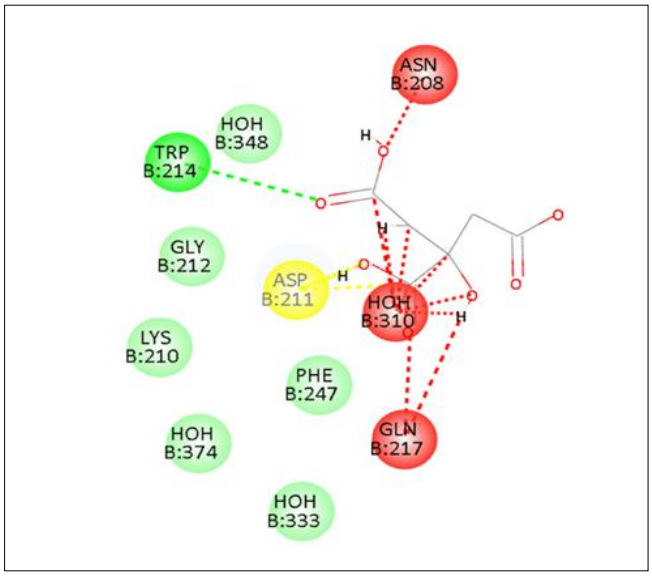
The study analyzed the binding affinity of phytochemicals to proteins, with Quercetin and Kaempferol showing the lowest docking scores, suggesting strong interactions with key proteins involved in urolithiasis. Proteins like AKT1, CASP3, BCL2L1, and MMP9 showed high-affinity interactions with phytochemicals, suggesting they may serve as central molecular targets in anti-urolithiasis therapy. Linalool and Citric acid showed weaker interactions, indicating lower therapeutic relevance. Several phytochemicals showed good binding with multiple proteins, supporting their multi-target potential in treating complex diseases like urolithiasis.



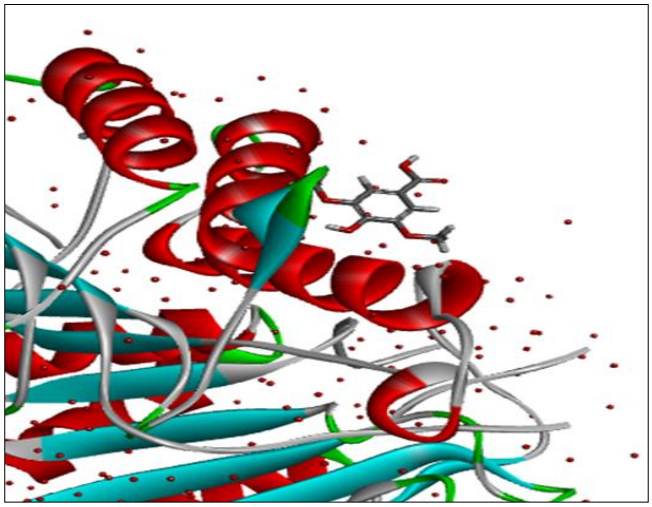


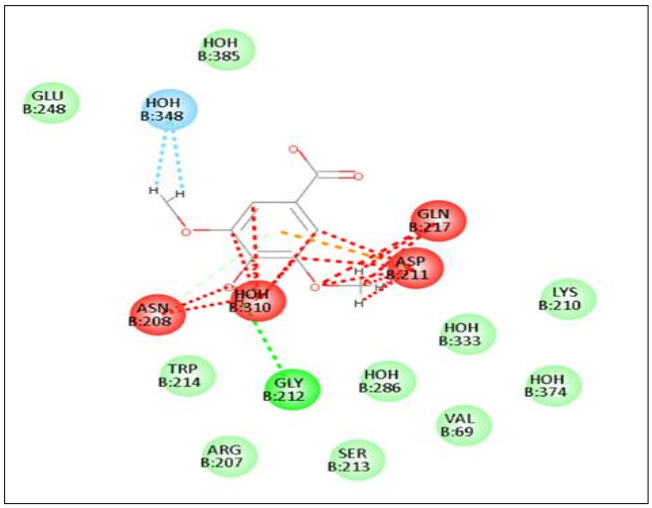
Kaempferol Complex with AKT1: 2D interaction diagram of Kaempferol docked with AKT1 showing hydrogen bonds and key interacting residues.





Quecertin complex with CASP3: 2D interaction plot of Quercetin with CASP3 showing amino acid contacts and ligand positioning within the binding site





Syringic acid complex with MMP9: 2D interaction diagram of Syringic acid docked with MMP9 showing interaction with catalytic site residues.

Visualization and Interpretation

A heatmap of docking scores was generated to visualize the interaction strength between phytochemicals and protein targets. The results support the network pharmacology findings, showing that Quercetin and Kaempferol have high binding potential to key targets implicated in urolithiasis. These interactions suggest inhibition of oxidative stress, modulation of apoptosis, and reduction of inflammation in renal tissues.

Conclusion of *in silico* Findings

The combined network pharmacology and molecular docking analysis demonstrated that *Kalanchoe pinnata* possesses promising multi-target anti-urolithiasis potential. Key phytochemicals like Quercetin and Kaempferol emerged as strong candidates with favorable pharmacokinetic properties and significant binding affinities. These *In silico* findings provide a rational basis for future *in vitro* and *in vivo* validation studies, potentially leading to the development of phytopharmaceutical interventions for urolithiasis.

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