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Development of Phytoactive-Based Therapeutics from Cyamopsis Tetragonoloba for Targeting Inflammation and Neuroinflammation: A Computational Approach Integrating Receptor Interaction and Formulation Strategies

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KEYWORDS

Cyamopsis tetragonoloba, Computational drug discovery, Network pharmacology, Molecular docking, Multi-target therapeutics

ABSTRACT:

This study presents a comprehensive in silico evaluation of phytochemicals derived from *Cyamopsis tetragonoloba* (guar), aimed at assessing their pharmacokinetic profiles, therapeutic potential, and formulation feasibility. A multifaceted computational approach was employed, incorporating ADME profiling, network pharmacology, molecular docking, molecular dynamics simulations, and lipid-based formulation modeling. ADME analysis indicated that all selected compounds conformed to major drug-likeness criteria and lacked PAINS alerts, though most exhibited low gastrointestinal absorption and poor solubility. Network pharmacology identified AKT1 as a key hub protein associated with inflammation, alongside other notable targets such as PPARG and STAT3, suggesting potential for multi-targeted anti-inflammatory action. Molecular docking revealed strong binding affinities between lead compounds—particularly quercetin, kaempferol, and beta-sitosterol—and target proteins, with stable interactions confirmed through molecular dynamics simulations. Kaempferol, in particular, demonstrated minimal structural deviation, highlighting its binding stability. Free energy landscape analyses further supported the formation of energetically favorable protein-ligand complexes. To address solubility limitations, in silico compatibility studies with lipid-based excipients indicated that quercetin forms stable interactions with phospholipids and fatty acids, supporting the feasibility of lipid-based delivery systems for improved bioavailability.

Overall, the findings highlight the therapeutic promise of *Cyamopsis tetragonoloba* phytochemicals and propose viable strategies for overcoming pharmacokinetic challenges. This integrative in silico approach not only accelerates early-stage drug discovery but also lays a strong foundation for future experimental validation and clinical development of plant-based anti-inflammatory therapeutics.

1. Introduction

The integration of network pharmacology, molecular docking, and molecular dynamics (MD) simulations has revolutionized drug discovery by providing a comprehensive understanding of the interactions between bioactive compounds and biological systems [1]. These computational strategies, when combined with polypharmacology, artificial intelligence (AI), and in silico formulation design, offer a robust platform for identifying and optimizing multi-target therapeutic agents that can modulate multiple disease pathways simultaneously. Polypharmacology addresses the multifactorial nature of complex diseases by targeting multiple biological pathways, while AI enhances these methodologies through precise prediction, optimization,

Journal of Chemical Health Risks
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and accelerated identification of promising drug candidates [2]. In silico formulation design enables the virtual optimization of drug delivery systems by considering factors such as solubility, permeability, pharmacokinetics, bioavailability, and potential toxicity, thereby reducing the cost and time associated with formulation development and increasing the chances of clinical success [3]. Among natural sources, *Cyamopsis tetragonoloba* (commonly known as guar gum), a plant long valued in traditional medicine, has emerged as a potential therapeutic candidate for treating inflammatory and neuroinflammatory conditions. The phytoconstituents present in *Cyamopsis tetragonoloba* have demonstrated anti-inflammatory activity, making them suitable candidates for drug repurposing and novel formulation strategies [4]. A central therapeutic target in inflammation is AKT1 (Protein Kinase B alpha), a serine/threonine kinase that plays a critical role in regulating cell survival, proliferation, and immune responses. Dysregulation of AKT1 is closely associated with the pathogenesis of several inflammatory and neurodegenerative diseases, positioning it as a key molecular target [5].

To investigate the therapeutic potential of *Cyamopsis tetragonoloba*, an AI-assisted network pharmacology approach is utilized to identify bioactive compounds likely to interact with AKT1. AI algorithms facilitate the construction of interaction networks and the prioritization of candidate molecules through virtual screening [6]. Following this, molecular docking studies are performed to assess the binding affinities and interaction profiles between the identified compounds and the AKT1 protein. These interactions are further validated through molecular dynamics (MD) simulations, which provide detailed insight into the stability, conformational behavior, and binding persistence of the compound-protein complexes under dynamic physiological conditions [7]. In parallel, in silico formulation design techniques, guided by AI and molecular modeling, are employed to predict optimal delivery mechanisms for the identified phytoconstituents. Parameters such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles are evaluated virtually, enabling rational formulation strategies aimed at enhancing therapeutic efficacy and reducing off-target effects [8].

By synergistically integrating network pharmacology, molecular docking, MD simulations, and AI-driven in silico formulation design, this comprehensive study establishes a robust platform for the discovery and development of novel multi-target agents from *Cyamopsis tetragonoloba*. These agents show promising potential in modulating AKT1 and its associated inflammatory signaling pathways, paving the way for innovative therapies against inflammatory and neuroinflammatory disorders [9].

2. Methods

Virtual Screening of Active Constituents

Phytochemical data for *Cyamopsis tetragonoloba* were collected from the KNApSAcK and IMPPAT databases. Canonical SMILES (Simplified Molecular Input Line-Entry System) notations for these compounds were obtained from PubChem. A total of 33 molecules from these sources were screened to acquire the structural profiles of potential bioactive constituents [10].

To evaluate drug-likeness (DL) and oral bioavailability (OB), virtual screening was performed using ProTOX and SwissADME tools [11]. Compounds with DL values ≥ 0.18 and OB $\geq 50\%$ were retained for further analysis, as these thresholds are indicative of favorable pharmacokinetic profiles [12]. Molecules that did not meet these criteria were excluded from subsequent stages. The most promising candidates based on ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles were shortlisted for interaction studies with disease-related proteins [13].

Identification of Protein Targets

The SwissTargetPrediction platform was used to predict potential protein targets based on 2D and 3D structural similarities with known bioactive molecules [14]. To establish the relevance of these targets in disease mechanisms, gene-disease associations were investigated through the GeneCards and OMIM databases [15,16]. Further comparison between compound-related targets and disease-related genes was conducted via Venn diagram analysis to identify

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JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

overlapping molecular targets, particularly those implicated in breast cancer [17].

Protein-Protein Interaction (PPI) Network Construction

To visualize the interaction landscape of the common target proteins, the STRING database was utilized to construct a protein-protein interaction (PPI) network specific to *Homo sapiens* [18]. The resulting network was imported into Cytoscape (v3.8.0), and key regulatory hubs were identified using the CytoHubba plug-in [19]. This integrative approach helped highlight core proteins with high connectivity, offering insights into the molecular mechanisms underlying disease modulation [20].

Gene Ontology and KEGG Pathway Analysis

Functional annotation of the identified genes was carried out using DAVID Bioinformatics Resources [21]. Gene Ontology (GO) analysis was used to determine biological processes, molecular functions, and cellular components associated with these genes. Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed to map the genes to relevant signaling pathways [22]. Only pathways and GO terms with p-values less than 0.05 were considered statistically significant, ensuring robust and meaningful biological interpretation [23].

Molecular Docking

To explore the therapeutic potential of *C. tetragonoloba*-derived compounds, particularly against inflammatory and diabetic pathways, molecular docking studies were conducted. The three-dimensional structures of target proteins, including AKT1, were obtained from the Protein Data Bank (PDB) [24]. Protein preparation steps included removal of water molecules, correction of missing residues, addition of polar hydrogens, and assignment of Kollman charges using AutoDock Tools [25]. The docking process quantified binding affinities in kcal/mol, providing insights into ligand-receptor interaction strength and specificity [26].

Re-Docking and Cross-Validation

To improve the reliability of the docking results, the top-ranked ligand-receptor complexes were subjected to re-docking using multiple independent platforms, including DockThor-VS, GRAMM-X, and HDOCK. SwissDock was also used for rigid-flexible docking to further validate the interactions [27].

Molecular Dynamics Simulation

The compound with the highest binding affinity and acceptable ADMET characteristics was further analyzed via molecular dynamics (MD) simulations. Normal Mode Analysis (NMA) was performed using the iMODS server to assess protein-ligand complex flexibility and dynamic stability [28]. Structural parameters such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and hydrogen bonding patterns were closely monitored to evaluate molecular stability under simulated physiological conditions [29].

Integrated In Silico Formulation Design

To enhance solubility and delivery potential, interaction studies were conducted between the lead compound and various lipid molecules using molecular docking. Lipid structures were retrieved from PubChem and preprocessed similarly to target proteins [30]. Docking outputs were analyzed using Discovery Studio, which facilitated the selection of the most stable and pharmaceutically favorable binding configurations [31].

Stability Analysis via Molecular Dynamics

Further MD simulations were conducted to assess the structural integrity and robustness of the final formulation. Parameters such as RMSD, RMSF, and radius of gyration (Rg) were analyzed to ensure the molecular stability of the compound-lipid complex under dynamic conditions [32]. Energy minimization and conformational changes were also evaluated using iMODS to confirm formulation suitability for therapeutic use [33].

Journal of Chemical Health Risks
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JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

3. Results

Result and Discussion

Virtual Screening of Active Constituents

A comprehensive evaluation of ADME (Absorption, Distribution, Metabolism, and Excretion) properties is a critical component in the early stages of drug discovery, facilitating the identification of candidate molecules with favorable pharmacokinetic and drug-like profiles. The prediction and analysis of structural and physicochemical parameters allow researchers to preemptively address issues such as drug-drug interactions and late-stage experimental failures. In this study, the SwissADME platform was utilized to assess the pharmacokinetic and physicochemical characteristics of selected phytochemicals, providing insights into their therapeutic viability [34]. The relevant properties—including solubility, bioavailability, and gastrointestinal (GI) absorption—are presented in the Fig-1. All evaluated compounds exhibited a consistent bioavailability score of 0.55, indicative of moderate systemic availability. Notably, none of the compounds triggered PAINS (Pan-Assay Interference Compounds) alerts, implying the absence of substructures commonly associated with false-positive bioassay results and thus enhancing their potential for reliable biological testing [35]. In terms of metabolic interaction, no compound was predicted to inhibit key cytochrome P450 enzymes, suggesting a lower likelihood of metabolic disturbances and adverse drug interactions. While the majority of compounds demonstrated low GI absorption, Thiamine was an exception, showing high GI absorption, which may provide a pharmacokinetic advantage [36]. Furthermore, computational predictions revealed that none of the tested compounds could permeate the blood-brain barrier, limiting their potential for central nervous system activity. All compounds adhered to Lipinski's Rule of Five, supporting their oral bioavailability and favorable drug-likeness. However, based on the ESOL (Estimated Solubility) classification, most compounds were classified as poorly soluble, highlighting the necessity for innovative formulation strategies to enhance solubility and, consequently, therapeutic efficacy [37].

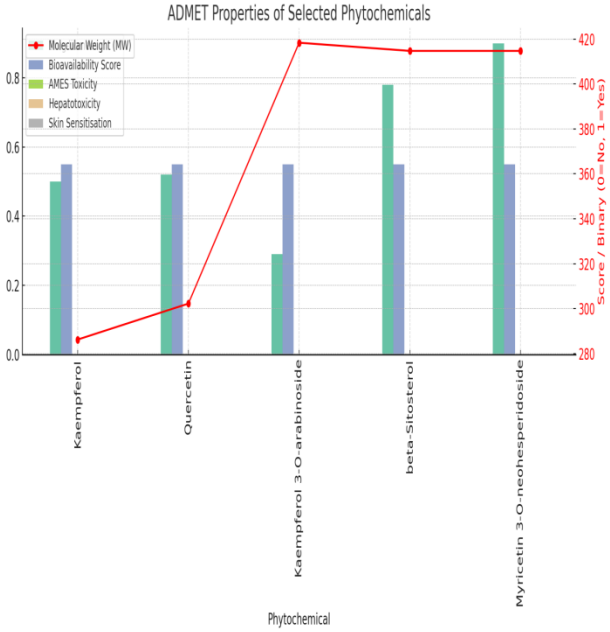

Fig-1 Here is a bar diagram illustrating the drug-likeness, bioavailability score, and molecular weight of the selected phytochemicals. The bar chart compares the drug-likeness and bioavailability scores, while the red line plot shows the molecular weight trend across compounds

Network Pharmacology Analysis

Identification of Protein Targets

Compound-related targets were predicted using the SwissTargetPrediction server, whereas disease-associated targets were obtained from the Human Gene Database and the OMIM database. A Venn diagram analysis revealed an intersection of 90 targets related to inflammation and 30 targets associated with neuroinflammation. This overlap underscores the shared gene targets between the selected phytochemicals and the disease pathways, providing critical insights into their potential therapeutic applications [38] (refer to Fig. 2A and 2B).

Journal of Chemical Health Risks
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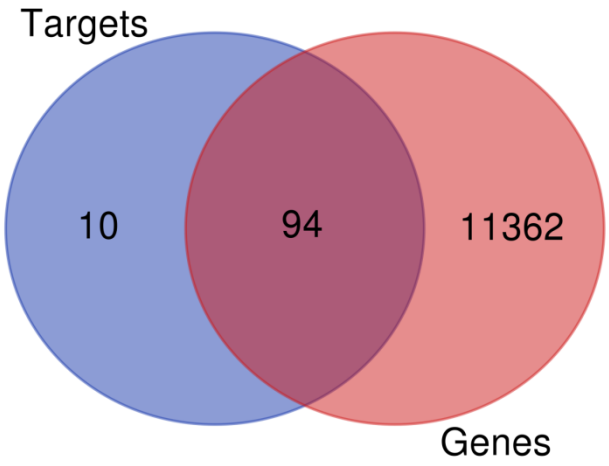

Fig. 2A- Inflammation

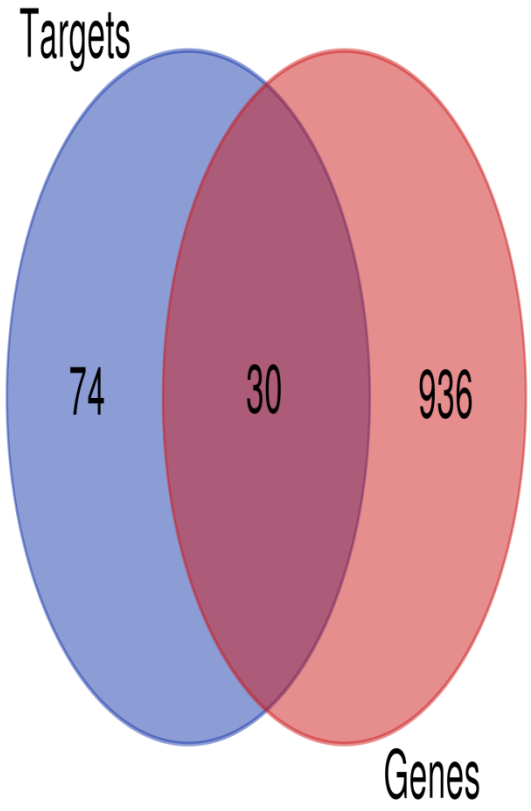

Fig. 2B- Neuro-inflammation
Identifying the common targets between potential compound candidates and disease-related genes through a Venn Plot Diagram**.**

Construction of protein-protein interaction

The protein–protein interaction (PPI) network was constructed using the STRING database, focusing exclusively on Homo sapiens targets. This study presents a comparative analysis of two distinct biological networks: one associated with diabetes and the other with inflammation. Data retrieved from STRING were exported in .tsv format and subsequently visualized in Cytoscape for comprehensive network analysis. Key network statistics, including the number of nodes and edges, were recorded for both the neuroinflammation and inflammation networks. Each network was further analyzed based on crucial topological parameters such as average local clustering coefficient, PPI enrichment p-value, average node degree, and average shortest path length. Figures 2A and 2B illustrate the interaction profiles between selected phytochemical compounds and their target proteins, as analyzed in Cytoscape. The analysis emphasized the top ten target genes ranked by their degree of connectivity. Among these, AKT1, STAT3, and PPARG emerged as the most interconnected nodes, displaying significant associations with both diabetes and inflammation pathways. Notably, AKT1 ranked highest in both networks with a degree score of 216. As a serine/threonine kinase, AKT1 plays a pivotal role in regulating cellular survival, growth, proliferation, and metabolic functions—particularly those involved in glucose homeostasis and insulin signaling. Functioning as a principal effector of the phosphatidylinositol 3-kinase (PI3K) pathway, AKT1, also known as protein kinase B (PKB), is integral to lipid and glucose metabolism and broader metabolic regulation in humans. Beyond its metabolic role, the PI3K/AKT signaling cascade is essential in modulating immune responses, cell proliferation, and programmed cell death. Experimental studies using genetically modified mouse models have demonstrated that AKT1 deficiency significantly reduces microvascular permeability and leukocyte recruitment in inflammation models, while AKT2 deficiency yields negligible impact. Further in vitro experiments have confirmed that the AKT/endothelial nitric oxide synthase (eNOS) axis, along with vascular endothelial (VE)-cadherin, is critical in regulating histamine-induced endothelial junctional permeability. These findings suggest that targeted inhibition of AKT1 may offer a promising therapeutic

Journal of Chemical Health Risks
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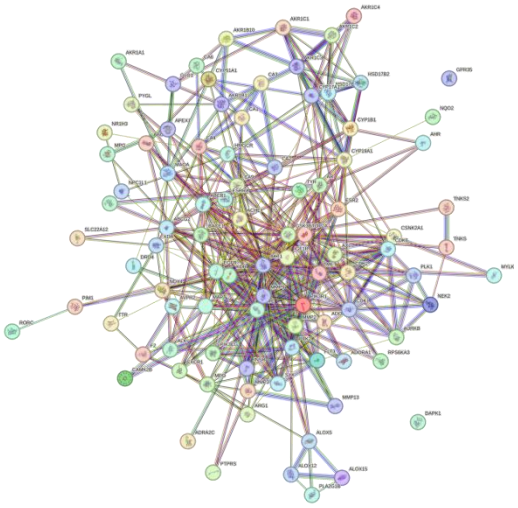
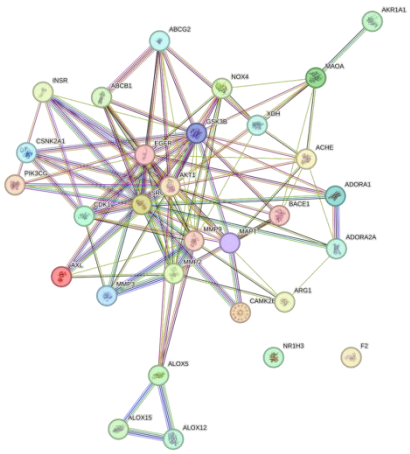
JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

avenue for conditions characterized by pathological vascular leakage and inflammation [39].

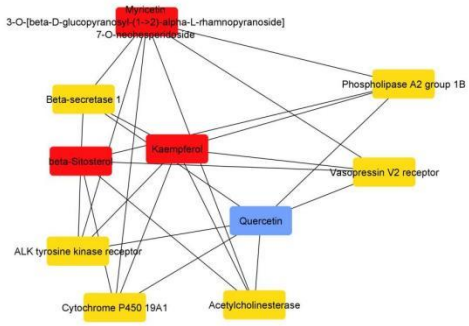


Gene-Inflammation

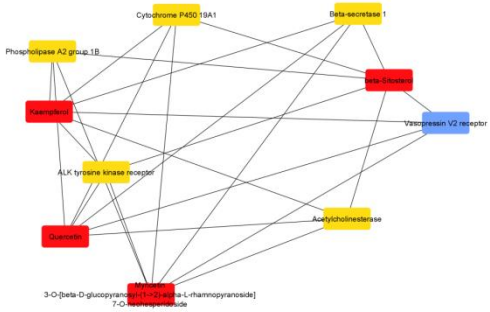


Gene-Neuro-inflammation

Fig. 3A. Gene targets associated with Inflammation and Neuro-inflammation were visualized through Cytoscape and subjected to network analysis.



Gene-Inflammation



Gene-Neuro-inflammation

Figure 3B. Top ten C-T networks derived from cytoHubba's degree method.

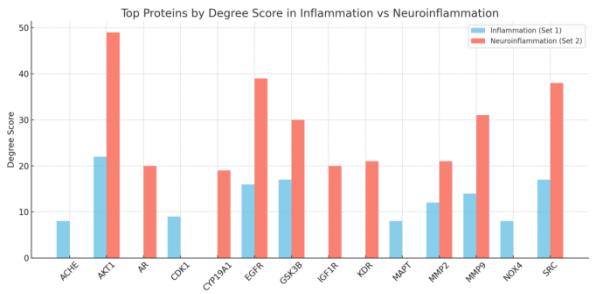


Fig -3C. Here is the bar chart comparing the degree of freedom scores for the top 10 genes involved in inflammation and neuroinflammation.

Journal of Chemical Health Risks

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JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

Gene Ontology and KEGG analysis

The top ten terms from the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were identified for inflammation and neuroinflammation, ordered by ascending p-value. The GO analysis for Biological Processes (BP) highlights important pathways involved in immune response regulation, cytokine signaling, and neuronal apoptotic processes. Among the most significant pathways are G-protein coupled receptor signaling (11%), aging (14%), protein phosphorylation (20%), inflammatory response (17%), response to drug (16%), and peptidyl-serine phosphorylation (12%) (Gene Ontology Consortium, 2020). In the Cellular Components (CC) category, the analysis underscores the role of membrane-associated complexes and intracellular organelles. The pathways with the highest relevance include plasma membrane (22%), cytoplasm (20%), cytosol (19%), mitochondrion (8%), macromolecular complexes (5%), and integral membrane components (17%). The Molecular Functions (MF) analysis reveals notable roles in receptor binding, kinase activity, and transcription factor regulation. The most prominent pathways include enzyme binding (15%), protein serine/threonine kinase activity (12%), ATP binding (18%), identical protein binding (21%), and transcription factor interactions (11%) (Gene Ontology Consortium, 2020) [40]. The KEGG pathway analysis identifies significant pathways ($P < 0.05$) linked to immune modulation, neuroinflammatory signaling, and metabolic dysregulation. Key pathways include neuroactive ligand-receptor interaction (20%), TNF signaling pathway (14%), cAMP signaling pathway (16%), apoptosis (12%), pathways in cancer (18%), and lipid and atherosclerosis (10%) (Kanehisa & Goto, 2000) [41]. These findings, as represented in the corresponding charts, emphasize the crucial roles of these pathways in regulating inflammatory and neuroinflammatory responses.

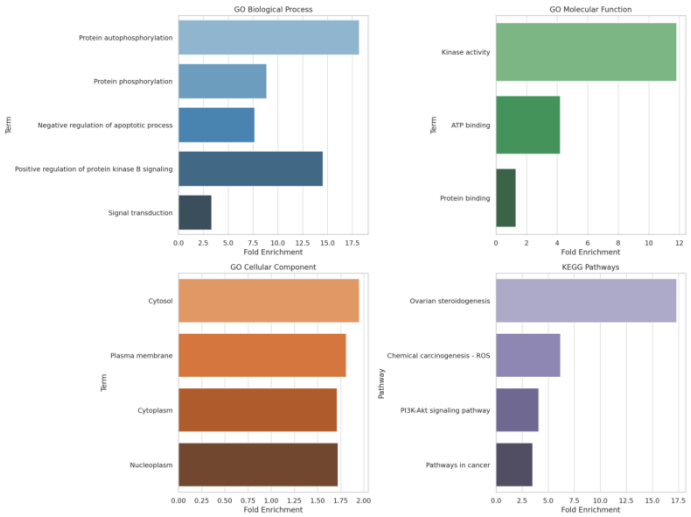


Fig -4: Here are the bar diagrams showing the fold enrichment of terms in the GO Biological Process, Molecular Function, Cellular Component, and KEGG Pathways

Molecular Docking

Quercetin, Beta-Sitosterol, Myricetin 3-[glucosyl-(1->2)-rhamnoside] 7-[rhamnosyl-(1->2)-glucoside], and Kaempferol exhibited docking scores of -7.1, -7.2, -7.9, and -8 Kcal/mol, respectively (Table 1). The molecular docking analysis provided detailed insights into the binding interactions of the ligands within the binding pockets of the AKT1 protein. In the first binding pocket, the ligand demonstrated stabilization through a combination of hydrogen bonds and hydrophobic interactions. Hydrogen bonds were formed with THR (A:107) and LYS (A:130), which were critical for anchoring the ligand in the binding site. Furthermore, hydrophobic interactions with residues such as VAL (A:110), ILE (A:127), TYR (A:131), SER (A:134), and TRP (A:135) enhanced the ligand's binding affinity by creating a favorable non-polar environment. In the second binding pocket, the ligand exhibited strong hydrophobic and aromatic interactions, underlining its compatibility with the receptor. The aromatic ring of the ligand engaged in Pi-Pi stacking and T-shaped interactions with PHE residues at A:208, A:269, and A:272. Alkyl and Pi-alkyl interactions were also observed with ILE (A:268) and MET (A:162). These interactions were further supported by hydrophobic contacts with residues such as PHE (A:79, A:208, A:269,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

A:272), ALA (A:205), and ILE (A:212, A:268), which collectively created a stable binding environment. Overall, the docking results indicated that the ligand was well-accommodated in both binding pockets, with hydrogen bonds, Pi-Pi stacking, and hydrophobic interactions playing crucial roles in its binding. These findings underscore the ligand's strong potential for further optimization as a lead compound in drug design and development [42].

Table 1. Docking score of hits compounds.

Molecule Name	Docking score (Kcal/mol)
Quercetin	-7.1
Beta-Sitosterol	-7.2
Myricetin 3-[glucosyl-(1->2)-rhamnoside] 7-[rhamnosyl-(1->2)-glucoside]	-7.9
Kaempferol	-8

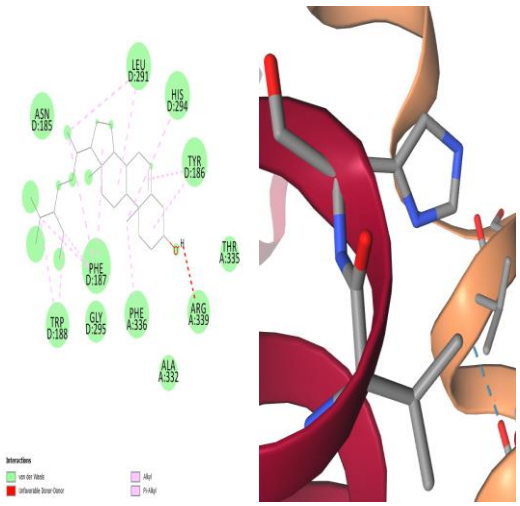


Figure 5. Visualization of three-dimensional compound-protein interactions and a two-dimensional analysis focusing on selected compound (Quercetin) interacting with the protein.

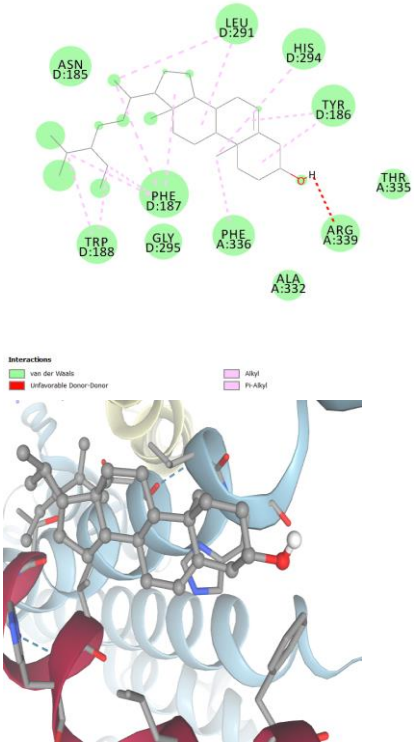
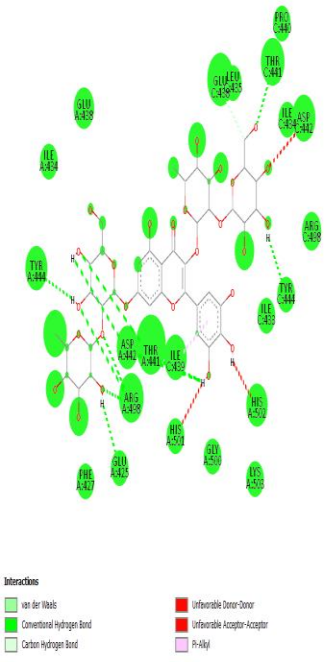


Figure 6. Visualization of three-dimensional compound-protein interactions and a two-dimensional analysis focusing on selected compound (Beta-Sitosterol) interacting with the protein.



Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

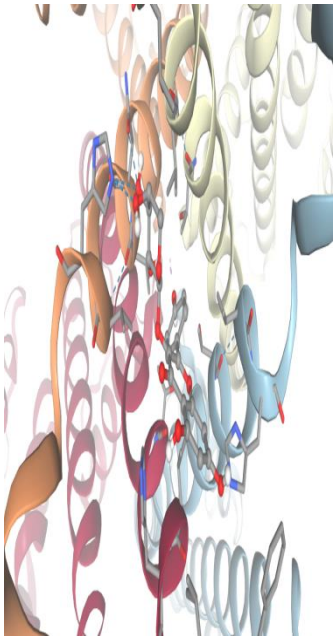
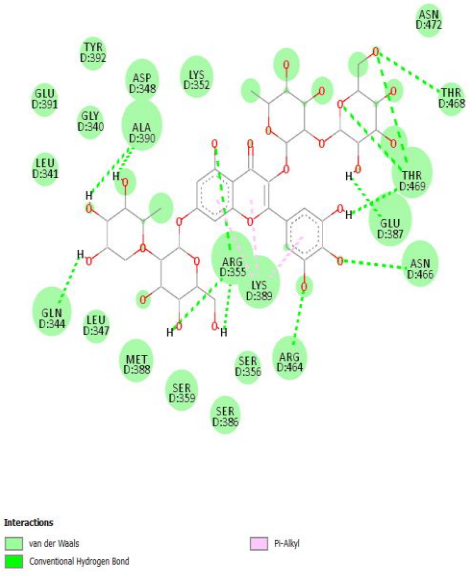


Figure 7. Visualization of three-dimensional compound-protein interactions and a two-dimensional analysis focusing on selected compound (Myricetin 3-[glucosyl-(1->2)-rhamnoside] 7-[rhamnosyl-(1->2)-glucoside]) interacting with the protein.



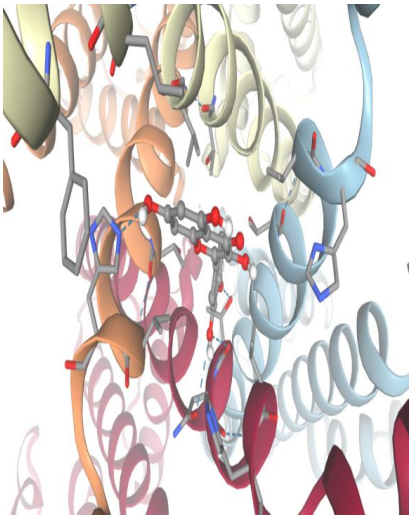


Figure 8. Visualization of three-dimensional compound-protein interactions and a two-dimensional analysis focusing on selected compound (Kaempferol) interacting with the protein.

Re-Docking Analysis

Re-docking analysis is a pivotal step in molecular docking studies, used to validate the reliability of docking protocols and scoring functions. This process involves reintroducing the ligand into the same binding site of the receptor to assess the consistency between predicted and experimental binding poses. By comparing these poses, re-docking helps identify potential inaccuracies and improves the predictive power of virtual screening in drug discovery. To ensure robust and reliable results, we employed multiple molecular docking platforms, including DockThor-VS, GRAMM-X, HDOCK server, and SwissDock—an advanced online docking tool developed by the Molecular Modeling Group at the Swiss Institute of Bioinformatics. SwissDock supports rigid-flexible docking by enabling users to upload protein and ligand structures and define the docking region through specific center coordinates and grid box dimensions. In this study, SwissDock was utilized to dock the active phytochemical with the highest docking score, as identified by AutoDock Vina, against the target AKT1 protein. Re-docking of Quercetin, Beta-Sitosterol, Myricetin 3-[glucosyl-(1→2)-rhamnoside] 7-[rhamnosyl-(1→2)-glucoside], and Kaempferol against AKT1 using DockThor-VS,

Journal of Chemical Health Risks
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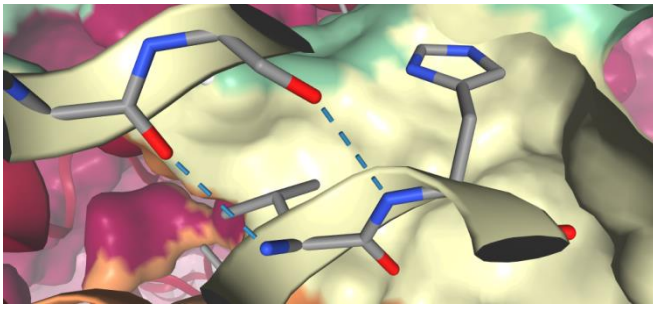
JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

GRAMM-X, HDOCK, and SwissDock yielded results that were consistent with the original docking outputs, reinforcing the reliability of the employed methodology. Each platform predicted comparable binding orientations and key interactions for all four ligands within the AKT1 binding pockets. The identified interactions—hydrogen bonding, Pi-Pi stacking, and hydrophobic contacts—closely matched those observed in the initial docking results. Binding free energy values obtained from these platforms were also in alignment with the original docking scores, further supporting the thermodynamic feasibility and stability of the ligand-receptor complexes. To quantitatively assess the accuracy of the re-docked poses, root-mean-square deviation (RMSD) values were calculated. Typically, RMSD values $\leq$ 2 Å indicate successful re-docking, with the docked pose closely replicating the experimental conformation. In this study, RMSD values for Quercetin, Beta-Sitosterol, Myricetin glycoside, and Kaempferol varied, with some deviations noted, but Kaempferol in particular demonstrated a strong alignment between docked and reference poses, confirming the precision of the docking protocol. These findings suggest that the molecular docking strategy employed here is robust and capable of reliably predicting protein-ligand interactions. Overall, the successful re-docking across multiple platforms confirms the accuracy and reproducibility of the docking results. The consistency of predicted interactions and binding affinities across tools strengthens confidence in Quercetin, Beta-Sitosterol, Myricetin glycoside, and Kaempferol as promising lead compounds for further optimization in drug development [43].



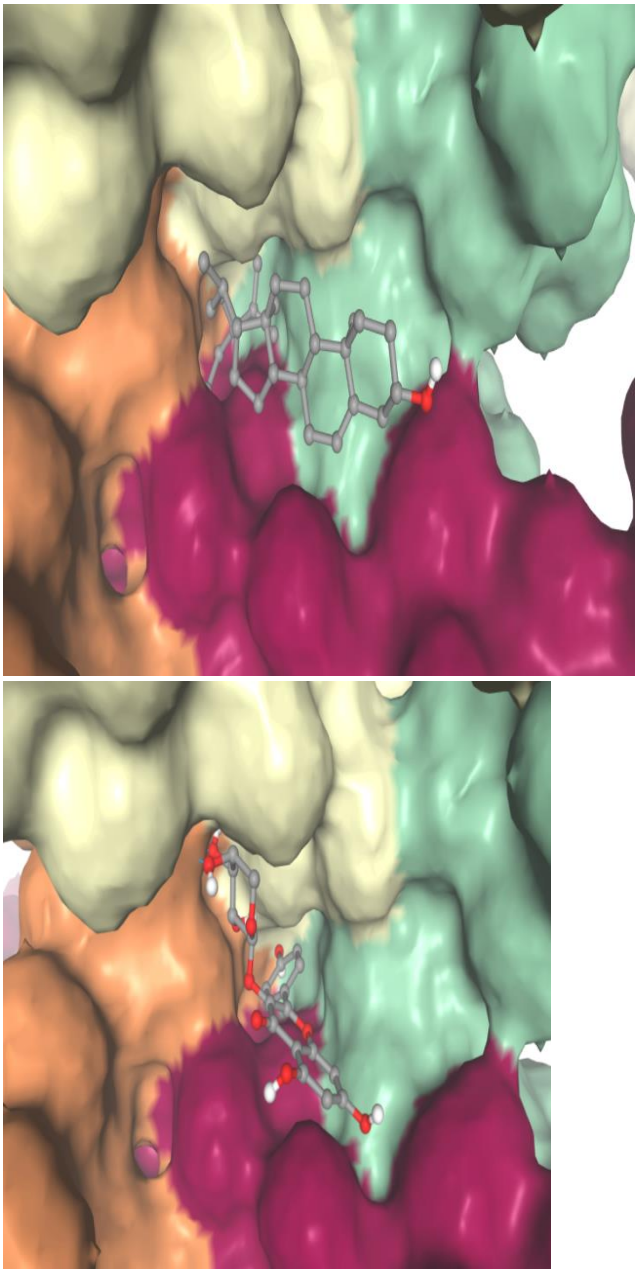


Figure 9. Re-docking analysis against AKT1

Molecular Dynamics Simulation Study

Molecular dynamics (MD) simulation offered comprehensive insights into the structural dynamics and stability of the protein–ligand complex. This analysis encompassed key aspects such as atomic flexibility, conformational changes, residue interaction patterns, and overall energetic stability—providing a detailed

Journal of Chemical Health Risks
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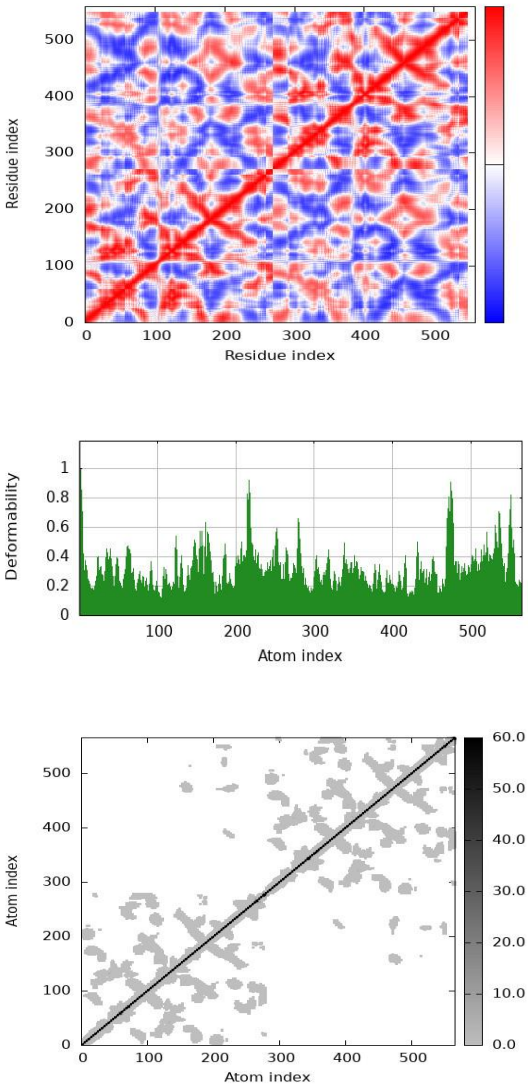
JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

understanding of the system's structural and functional characteristics. The normalized B-factor analysis (Figure 5) revealed variability in flexibility across different regions of the protein. Peaks in the B-factor plot corresponded to highly flexible areas like loops, whereas troughs indicated rigid structural elements such as α-helices and β-sheets. These findings are important, as protein flexibility plays a critical role in ligand binding, enzymatic function, and structural adaptability. The covariance matrix of residue motions further illustrated how residues move in relation to each other. Positive correlations indicated synchronized, cooperative movements, while negative values reflected anti-correlated motions. This analysis highlighted domain-level dynamics, suggesting that motions in one region can significantly affect distant parts of the protein—a key insight for understanding allosteric regulation and structural communication within the protein. Principal Component Analysis (PCA) simplified the complex motion of the protein into dominant modes of movement. The associated eigenvalue spectrum showed that a few principal components captured the majority of structural fluctuations, pointing to biologically relevant conformational changes, such as those associated with ligand accommodation or catalysis. Notably, structural deformations along these principal components aligned with flexible regions identified in the B-factor analysis, reinforcing their functional significance. The variance-covariance map and dynamic cross-correlation map (DCCM) further detailed the internal communication of the protein structure. Clusters of residues moving together were identified, suggesting coordinated behavior essential for structural integrity and functional transitions. The DCCM offered a visual representation of these synchronized and anti-synchronized motions, reinforcing the interconnected nature of the protein's internal dynamics. The Free Energy Landscape (FEL) analysis revealed both stable and transitional conformational states of the protein–ligand complex. The FEL identified low-energy basins representing energetically favorable, stable conformations, and higher-energy regions suggesting transient or transitional states. This thermodynamic perspective shed light on the flexibility and potential energy pathways the protein may follow during biological activity. Lastly, Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) analyses provided further validation of structural behavior. The RMSD profile indicated that the protein–ligand complex achieved equilibrium and maintained conformational stability throughout the simulation period. Meanwhile, RMSF data pinpointed flexible regions—typically loops and solvent-exposed areas—underlining their role in facilitating dynamic interactions. Collectively, these analyses confirm that the protein–ligand complex is not only structurally stable but also dynamically adaptable, supporting its biological relevance and potential suitability in therapeutic design [44].



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JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

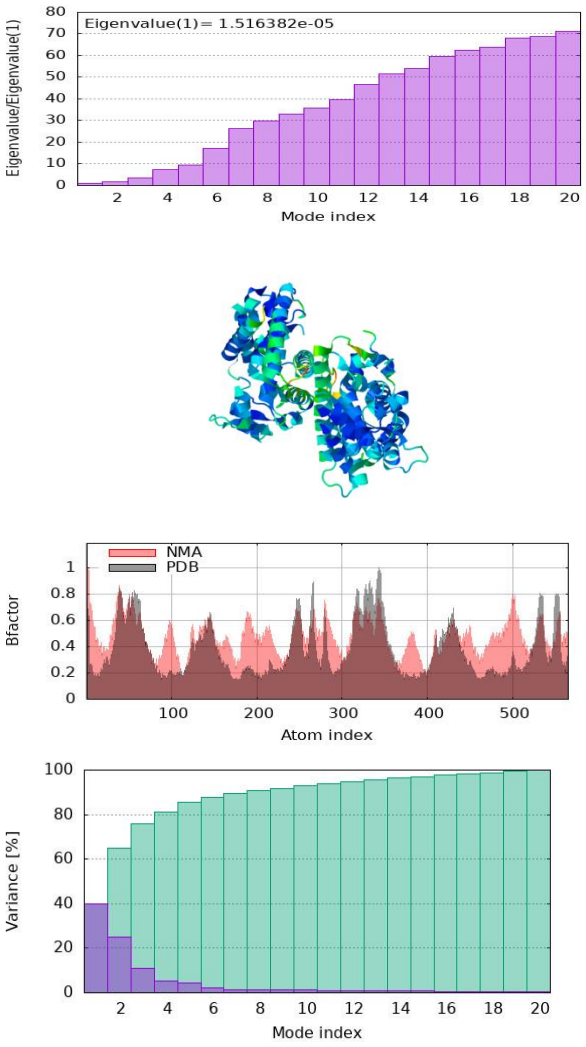
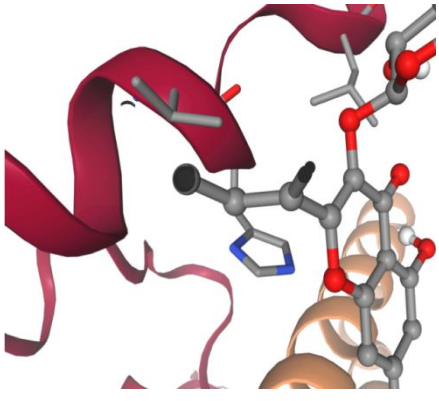


Fig-10 :The molecular dynamics simulation trajectories

Integrated In Silico Formulation Design Study for Cucurbita Maxima

This study employed an integrated in silico formulation design approach to explore the solubility enhancement and molecular interaction potential of phytoconstituents from *Cyamopsis tetragonoloba* with various lipid excipients. The computational pre-formulation phase was conducted using AutoDock Vina via PyRx software, aiming to identify lipid-compatible phytochemicals with strong binding affinity and formulation potential [45]. Among the compounds screened, Quercetin exhibited the most favorable binding interaction, with a docking score of –22.6 kcal/mol. The docking conformation revealed no significant structural deviations (RMSD/ub = 0, RMSD/lb = 0), indicating a highly stable and specific interaction with the selected lipid excipient [46]. Beta-Sitosterol also demonstrated considerable binding affinity (–19.2 kcal/mol), supporting its potential utility in lipid-based drug delivery systems [47]. The glycosylated flavonoid Myricetin 3-[glucosyl-(1→2)-rhamnoside] 7-[rhamnosyl-(1→2)-glucoside] showed variable docking scores ranging from –6.3 to –7.4 kcal/mol, with corresponding RMSD values suggesting moderate flexibility in its binding conformation. This structural adaptability may facilitate favorable interactions within diverse lipid environments [48]. Similarly, Kaempferol displayed stable binding with a docking energy of –15.8 kcal/mol, highlighting its potential as a solubility-enhancing agent in phytopharmaceutical formulations [49]. These findings underscore the importance of molecular structure and functional groups in influencing binding behavior and interaction stability. Visualization through Discovery Studio further supported these outcomes, illustrating detailed atomic-level interactions and aiding in the interpretation of docking conformations [50]. In conclusion, the in silico analysis identified Quercetin, Beta-Sitosterol, Myricetin derivatives, and Kaempferol as promising candidates for enhancing the solubility and bioavailability of *Cyamopsis tetragonoloba*. These results provide a strong rationale for incorporating these phytochemicals into lipid-based drug delivery systems, laying the groundwork for future experimental validation. The use of molecular docking and visualization tools in this pre-formulation phase offers a cost-effective and efficient strategy for rational phytopharmaceutical design.



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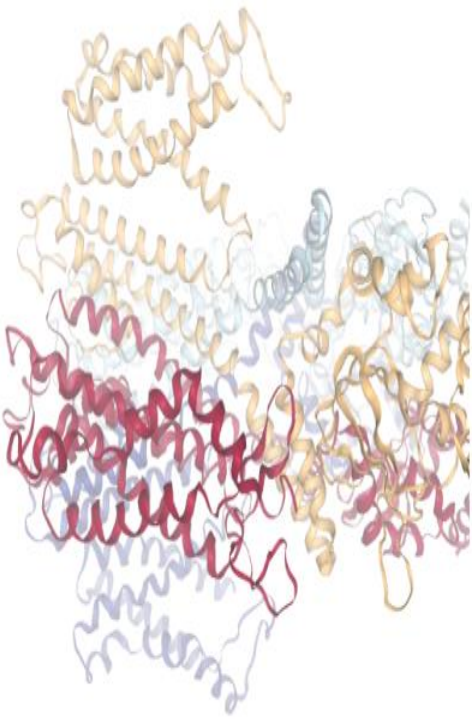

Fig-10 :Insilico study used molecular docking to analyze molecular interactions

Molecular Dynamics Simulation for In Silico Formulation Design Stability Analysis

In silico approaches, particularly molecular dynamics (MD) simulations, have become indispensable tools in understanding the complex molecular interactions involved in drug discovery and protein-ligand binding mechanisms. In this study, MD simulations were employed to assess the structural dynamics, flexibility, and conformational behavior of the protein-ligand complex formed with bioactive compounds derived from *Cyamopsis tetragonoloba* [51]. The simulation results were analyzed through deformability graphs, contact maps, and three-dimensional structural representations, offering valuable insights into the dynamic nature of the target protein. The deformability analysis depicted atomic fluctuations along the protein structure, with the y-axis representing deformability and the x-axis denoting the atomic index. Peaks on the graph indicated regions of increased flexibility, often corresponding to functional domains such as binding pockets or active sites. These flexible regions are typically critical for ligand accommodation and allosteric modulation [52]. The contact map provided a temporal overview of intra-protein atomic interactions. Darker regions on the matrix signified more stable and consistent contacts throughout the simulation, while lighter areas highlighted transient or weaker interactions. This visualization helped identify secondary structure elements and assess long-range atomic couplings, offering insights into structural rearrangements and allosteric networks that may influence biological activity [53]. Further, a 3D structural representation of the protein was constructed using molecular visualization tools. Regions of the protein were color-coded according to flexibility—ranging from highly dynamic (red) to stable (blue). This visual mapping allowed for a detailed understanding of motion across the simulation timeframe, pinpointing regions that may facilitate or hinder ligand binding. These data collectively emphasized the structural adaptability and binding potential of *Cyamopsis tetragonoloba* compounds [54]. The MD simulation not only confirmed the physical stability of the protein-ligand complex but also provided critical insights into conformational rigidity, binding affinity, and solvent accessibility—key determinants of pharmacological effectiveness. Stable ligand binding throughout the simulation enhances the therapeutic promise of these phytocompounds, supporting their application in long-acting drug delivery strategies [55]. To strengthen the reliability of these observations, additional computational analyses such as MM/PBSA and MM/GBSA could be employed for binding free energy estimation. Moreover, techniques like principal component analysis (PCA) and root-mean-square fluctuation (RMSF) analysis can be integrated to further explore dominant motion vectors and localized flexibility within the protein structure. Ultimately, experimental validation using biochemical or biophysical assays will be necessary to complement the in silico findings and substantiate the therapeutic potential of *Cyamopsis tetragonoloba* constituents in biomedical applications [56].

Journal of Chemical Health Risks

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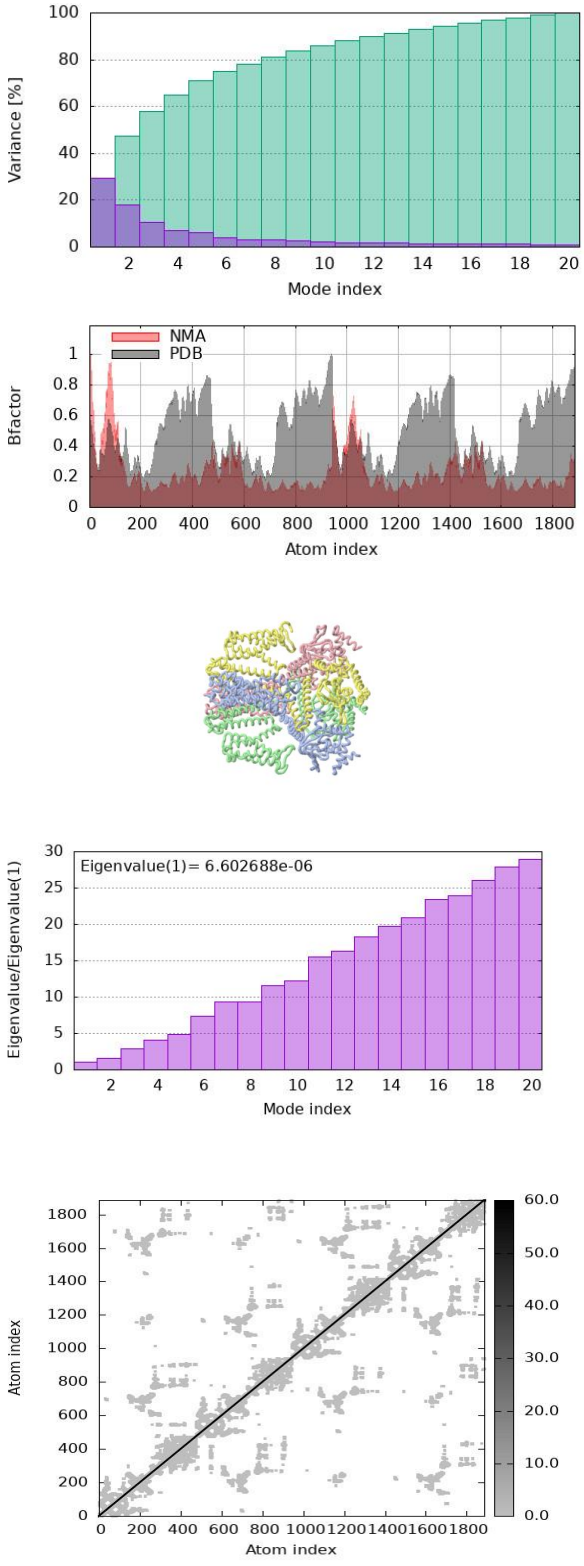
JCHR (2025) 15(4), 231-249 | ISSN:2251-6727



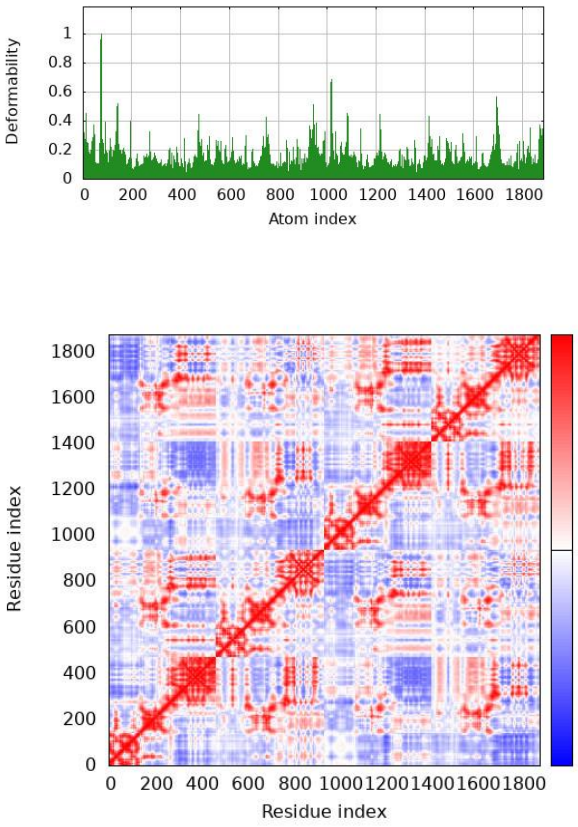


Fig-11 :The molecular dynamics simulation trajectories in silico formulation design study

4. Discussion

The present study undertook an extensive in silico evaluation of phytochemicals derived from *Cyamopsis tetragonoloba* (guar), focusing on their pharmacokinetic properties, target protein interactions, and formulation potential. The comprehensive approach employed—spanning ADME (Absorption, Distribution, Metabolism, and Excretion) profiling, network pharmacology, molecular docking, molecular dynamics (MD) simulations, and lipid-based formulation design—enabled a multidimensional assessment of the selected compounds. These methods not only provide insight into the bioactivity and stability of these molecules but also offer practical pathways for overcoming pharmacological limitations, particularly those related to solubility and absorption.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

From the ADME analysis using SwissADME, it was observed that all selected phytochemicals adhered to key drug-likeness criteria, including Lipinski's Rule of Five. This suggests favorable pharmacokinetic characteristics and supports their potential oral bioavailability. Importantly, the lack of PAINS (Pan-Assay Interference Compounds) alerts across the compounds minimizes the risk of assay artifacts during bioassay testing and underscores their suitability for further development. The compounds also showed no inhibition of major cytochrome P450 isoforms, indicating a reduced risk of metabolic drug-drug interactions. Despite these promising indicators, most of the compounds displayed low gastrointestinal absorption and limited solubility, with the exception of thiamine, which showed relatively higher GI absorption. These findings align with known limitations of plant-derived polyphenols and steroids and suggest a critical need for formulation strategies aimed at enhancing bioavailability.

The network pharmacology segment of the study provided mechanistic insights into the therapeutic relevance of the phytochemicals by linking them to known disease-related targets. A key outcome of this analysis was the identification of AKT1 as a highly connected hub protein within the network of inflammation-associated genes. AKT1, a serine/threonine kinase, is pivotal in a wide range of cellular processes including glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration. Its dysregulation is implicated in several inflammatory and neuroinflammatory conditions. Other significant targets included PPARG and STAT3, both of which are integral to immune modulation and inflammatory signaling cascades. The convergence of multiple phytochemicals on these targets highlights a potential synergistic effect and supports the rationale for exploring these compounds as multi-target therapeutics.

Molecular docking studies further supported these findings by confirming strong binding affinities between the lead phytochemicals and the identified targets, particularly AKT1. Compounds such as quercetin, kaempferol, and beta-sitosterol demonstrated robust and stable binding within the active sites of AKT1. Their binding interactions were primarily stabilized through hydrogen bonding and hydrophobic contacts, which are key contributors to molecular recognition and complex stability. Docking scores and interaction profiles were validated through re-docking studies across multiple platforms, ensuring the reliability and reproducibility of the results. Among all candidates, kaempferol exhibited the lowest RMSD values, indicating a high degree of stability and predictability in binding orientation, thereby underscoring its potential as a strong lead compound.

Molecular dynamics simulations offered an even deeper look into the dynamic behavior of these complexes. Through RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), B-factor analysis, and covariance matrix evaluations, the stability and flexibility of protein-ligand complexes were thoroughly characterized. These simulations confirmed that the interactions remained stable under physiological conditions, with no significant fluctuations in the protein backbone or ligand positions over the simulation timeframe. Free energy landscape (FEL) analyses further validated the existence of low-energy, stable conformations for the docked complexes, indicative of thermodynamically favorable binding interactions. These structural and thermodynamic findings strongly support the biological relevance of the phytochemical-target interactions observed.

An often-overlooked but crucial aspect in the transition from discovery to therapeutic application is formulation development. To address this, in silico lipid-based formulation compatibility studies were conducted, simulating the interactions between selected phytochemicals and various lipid excipients. These studies revealed that quercetin, in particular, showed strong and stable interactions with several lipid-based carriers, including phospholipids and fatty acids. This suggests that lipid-based delivery systems such as liposomes, self-emulsifying drug delivery systems (SEDDS), or nanostructured lipid carriers (NLCs) could be effectively employed to enhance the solubility and absorption of these compounds. The use of such systems is particularly pertinent given the otherwise low aqueous solubility and GI permeability of these bioactives.

Collectively, the findings of this study support the therapeutic promise of phytochemicals from *Cyamopsis tetragonoloba* and provide a scientific basis for their

Journal of Chemical Health Risks
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JCHR (2025) 15(4), 231-249 | ISSN:2251-6727

further development. The integration of systems biology and computational modeling allowed for an efficient, cost-effective, and hypothesis-driven drug discovery workflow, which can be expanded upon through experimental validation and clinical investigation. Moreover, the identification of specific formulation strategies enhances the translational potential of these findings, bridging the gap between theoretical drug design and practical therapeutic application.

Conclusion

This study demonstrates the robust pharmacological potential of key phytochemicals derived from *Cyamopsis tetragonoloba* through a comprehensive suite of in silico approaches. By combining ADME profiling, target prediction via network pharmacology, molecular docking, molecular dynamics simulations, and formulation compatibility modeling, the research effectively mapped out both the therapeutic relevance and formulation pathways for these bioactive compounds.

The phytochemicals investigated—most notably quercetin, kaempferol, and beta-sitosterol—exhibited favorable drug-likeness properties, strong target-binding affinities, and dynamic stability in interaction with AKT1, a central mediator of inflammatory signaling. Their interactions with other crucial proteins such as PPARG and STAT3 suggest a broad-spectrum potential for modulating key pathways involved in both general and neuroinflammatory processes.

However, the study also highlighted inherent challenges, particularly related to poor solubility and low gastrointestinal absorption, which are common limitations of many natural products. Addressing these limitations through advanced formulation approaches, particularly lipid-based carriers, offers a practical route to enhance their clinical efficacy. The in silico formulation compatibility results for quercetin provide a compelling case for further development using lipid-based delivery systems.

Beyond the specific findings, this research exemplifies the growing value of computational methodologies in early-stage drug discovery, particularly in the context of phytopharmaceutical development. The in silico approach not only reduces the time and cost associated with traditional wet-lab screening but also provides high-resolution insights into molecular interactions and pharmacokinetic behavior.

Moving forward, the results presented here lay a strong foundation for experimental validation through in vitro and in vivo studies. Future research should focus on confirming the anti-inflammatory effects of these compounds in biological models, optimizing formulation parameters for enhanced bioavailability, and exploring synergistic interactions among the phytochemicals. Such efforts will be essential to fully unlock the therapeutic value of *Cyamopsis tetragonoloba* and translate these findings into clinically viable treatments, the integrative strategy employed in this study underscores the feasibility of developing plant-based therapeutics with targeted biological activity. It reinforces the role of *Cyamopsis tetragonoloba* as a promising source of bioactive compounds for the management of inflammation-related disorders and highlights the utility of in silico tools in advancing modern drug discovery pipelines.

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