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# Bioinformatics Study of Arctigenin from *Jatropha curcas* as a KEAP1-Nrf2 Modulator for Antioxidant Activity

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## *ABSTRACT*

*Oxidative stress is a major contributor to metabolic disorders, making the Keap1–Nrf2 pathway a key therapeutic target for antioxidant interventions. This study aimed to evaluate the therapeutic potential of Arctigenin, a lignan from Jatropha curcas leaves, as a natural Keap1 antagonist using an in silico bioinformatics approach. Molecular docking with YASARA Structure was used to determine bond affinity and interaction dynamics, followed by 50-ns molecular dynamics simulations to assess the stability of the complex. ADMET and drug-likeness predictions were performed to evaluate pharmacokinetic properties and safety. Arctigenin demonstrated strong binding affinity (-8.73 kcal/mol) and formed six hydrogen bonds with key Kelch residues (Arg415, Ser508, Val604, Leu365, Ala510, Val463), along with stable hydrophobic and π interactions involving Arg415, Ala556, Leu557, and Tyr572. Molecular dynamics confirmed complex stability, indicated by low RMSD values for Cα and backbone (1.6–1.8 Å), minimal residue fluctuations, stable radius of gyration (17.7–18.1 Å), and consistent SASA. ADMET predictions showed excellent intestinal absorption (94.416%), low blood-brain barrier permeability, and favorable safety profile (AMES-negative, non-hepatotoxic). Overall, Arctigenin exhibits strong potential as a natural Keap1 inhibitor for the development of antioxidant, antihypertensive, or antidiabetic drugs.*

***Keywords:*** *Arctigenin, Keap1-Nrf2, molecular docking, molecular dynamics, antioxidant.*

## INTRODUCTION

Oxidative stress constitutes a significant contributor to the pathophysiology of a diverse array of chronic ailments, encompassing malignancies, diabetes mellitus, neurodegenerative conditions, and inflammatory disorders (Dash et al., 2025). Unlike conventional antioxidant mechanisms that rely on direct free radical scavenging, the human organism is equipped with intrinsic defense systems, notably the Keap1-Nrf2 signaling pathway, which acts as a master regulator of cytoprotective gene transcription (J. Wu et al., 2024), Under physiological circumstances, Keap1 engages with Nrf2, thereby facilitating its targeted proteasomal degradation (Tian et al., 2018). Nonetheless, in the presence of oxidative stress, these interactions are perturbed, allowing Nrf2 to translocate into the nucleus of the cell and activate the expression of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (L. Zhang et al., 2024). Consequently, the modulation of Keap1-Nrf2 interactions emerges as a pivotal approach for amplifying endogenous antioxidant defenses (Lu et al., 2016). However, the primary challenge and a critical science gap in current therapeutic development remains the identification of specific modulators capable of activating this pathway without excessively perturbing cellular redox homeostasis.

Various synthetic agents, including dimethyl fumarate (DMF) and oltipraz, have been developed to activate Nrf2 by inhibiting Keap1 (Matsumaru & Motohashi, 2021). Nonetheless,

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the application of these predominantly covalent inhibitors is constrained by adverse side effects, metabolic instability (Phillips et al., 2017), and non-specific electrophilic actions that may perturb the redox equilibrium (Li et al., 2025). Conversely, naturally occurring compounds such as flavonoids, terpenoids, and lignans present safer and more selective alternatives as non-covalent Keap1 inhibitors (Culletta et al., 2024), thereby enhancing antioxidant defenses without the significant toxicological risks associated with electrophilic stress.

One naturally occurring lignan that has been extensively investigated for its antioxidant properties is Arctigenin (Chen et al., 2024), which is present in various botanical species, including *Jatropha curcas* L. (Y. Wang et al., 2023). Previous in vitro and in vivo studies have demonstrated that Arctigenin exhibits robust antioxidant, anti-inflammatory, anticancer, and neuroprotective effects (N. Zhang et al., 2018), primarily through the modulation of gene expression associated with oxidative stress within the Keap1-Nrf2 signaling pathway (R. Wu et al., 2014). Numerous in vivo investigations have also indicated that Arctigenin can engage with the Kelch Keap1 domain, prevent the degradation of Nrf2, and augment the activity of cytoprotective enzymes (Yang et al., 2018). The selection of *Jatropha curcas* L. as the botanical source for this study highlights the underexplored potential of this plant's secondary metabolites for medical applications, reinforcing Arctigenin's significant potential as a natural modulator of Keap1.

Despite the extensive documentation of the pharmacological efficacy of Arctigenin, research specifically exploring its atomic-level interaction with Keap1 via bioinformatics methodologies remains sparse. The majority of existing studies focus on general biological outcomes rather than elucidating the specific molecular binding mechanisms and ligand affinity at the active site of the Keap1 Kelch domain. Indeed, in silico methodologies such as molecular docking and molecular dynamics can facilitate a comprehensive understanding of interaction mechanisms, critical residues, complex stability, and selective inhibition capabilities (Ravikumar et al., 2023). This atomic-level analysis holds significant urgency for evaluating the efficacy of Arctigenin as a competitive, non-covalent inhibitor of Keap1, providing a rational framework that endorses further empirical validation.

The objective of this study is to elucidate the molecular interaction between Arctigenin, derived from the leaves of *Jatropha curcas* L., and the Keap1 protein using a bioinformatics approach. By employing molecular docking techniques, this investigation evaluated bond affinity, the nature of non-covalent interactions, and the key residues within the Kelch Keap1 domain that are instrumental in Nrf2 binding. The anticipated outcomes of this investigation are expected to elucidate the mechanistic role of Arctigenin as a specific non-covalent modulator of Keap1 and to facilitate the development of antioxidant phytopharmaceutical candidates by activating the Nrf2 signaling pathway. This research constitutes the first comprehensive systematic evaluation specifically investigating Arctigenin sourced from the leaves of *Jatropha curcas* L. in relation to the Kelch Keap1 domain (6SP4) using an integrative approach that combines docking, molecular dynamics, and ADMET analysis within a unified bioinformatics framework.

## MATERIALS AND METHODS

### Materials

Arctigenin, a secondary metabolite belonging to the lignan class, is derived from the foliage of *Jatropha curcas* L. (Y. Wang et al., 2023) and was utilized as a ligand, with its three-dimensional structure procured from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The target receptor under investigation is Keap1 (PDB

ID: 6SP4), which was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/pdb/).

**Computational Platform and Equipment**

The primary computational platform utilized for both molecular docking and molecular dynamics simulations was YASARA Structure version 25.9.17. The selection of YASARA was predicated on its capacity to provide a unified and continuous simulation environment. Unlike multi-software pipelines where topology data or partial charges may be compromised during file format conversions, YASARA integrates docking algorithms with its proprietary dynamics engine using a consistent AMBER14 force field. This integration ensures strict thermodynamic consistency from the initial docking phase through the molecular dynamics trajectory, thereby minimizing computational artifacts and enhancing the reliability of the predicted binding poses. BIOVIA Discovery Studio Visualizer 2025 was employed complementarily for 2D/3D interaction visualization and initial geometry refinement.

**Ligand and Receptor Preparation**

The three-dimensional structure of Arctigenin obtained from PubChem served as the ligand, whereas data about Keap1 (6SP4) were downloaded in PDB format. Receptor preparation processes undertaken in YASARA encompassed the elimination of water molecules, endogenous ligands, and non-standard residues, as well as the incorporation of hydrogen atoms and the rearrangement of protonation states and protein chains. The geometry and partial charges were refined utilizing BIOVIA Visualizer 2025, with additional parameters being retained in accordance with established protocols (W. Wang et al., 2019).

**Molecular Docking Validation**

Validation was executed by redocking against the endogenous ligand of Keap1 (6SP4), followed by a comparative analysis with its crystalline conformation to ascertain the accuracy of the model. The spatial grid box was delineated based on the coordinates of the bond pocket, measuring 23.81 × 23.81 × 23.81 Å. The validation process employed YASARA 25.9.17 with the dock_run.mcr macro (100 iterations) and the AMBER14 force field (Patel et al., 2019). incorporating a 5 Å area coverage of the native ligand. Following validation, virtual screening was conducted using dock_run.mcr and AutoDock Vina to generate sequenced binding configurations based on their affinity and interaction profiles.

**Molecular Docking between Receptors and Ligands**

Docking procedures using YASARA 25.9.17 were conducted after optimizing both the ligand and receptor. The ligand SDF file was converted via the smi2sdf application.mcr. Simulations were performed within a grid box that was validated using AutoDock Vina alongside the AMBER14 force field. Multiple docking poses were generated, with the conformation exhibiting the lowest Binding Free Energy (BFE) selected as the most stable interaction. BIOVIA Visualizer was employed to elucidate hydrogen bonds, hydrophobic interactions, and other non-covalent interactions, in addition to sequencing results based on affinity.

**Molecular Dynamics Simulation**

Molecular dynamics simulations were executed using YASARA Dynamics version 25.9.17 to evaluate the stability of the Keap1-Arctigenin complex. The initial configuration was protonated at a pH of 7.4, followed by energy minimization utilizing the AMBER14 force field. The system was simulated within a 0.9% NaCl solution, conforming to a periodic boundary condition of 5 Å. NVT and NPT ensembles were conducted for 100 ps at 1 bar and 310 K, respectively, succeeded by a 50 ns production simulation with a 1.5 fs time step. Trajectory analysis was performed employing md_analyze.mcr to compute RMSD, RMSF, Rg, SASA, and the total number of hydrogen bonds.

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**ADMET Prediction and Lipinski's Rule of Five**

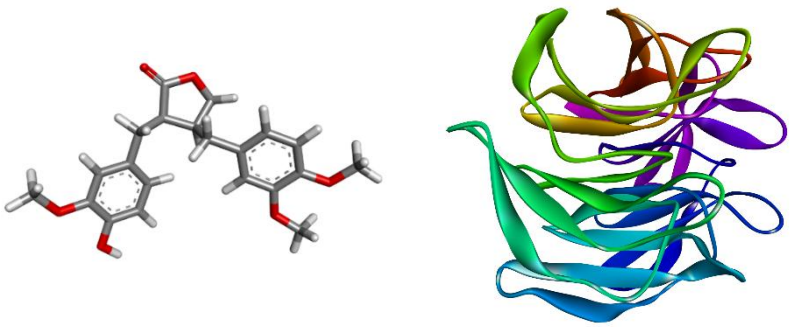
The ADMET profile of Arctigenin was assessed utilizing SwissADME (http://www.swissadme.ch/). The parameters analyzed included Lipinski's rule, gastrointestinal absorption, blood-brain barrier permeability, and CYP450 enzyme inhibition, to evaluate drug-likeness, bioavailability, and metabolic potential (Lipinski, 2004; Daina et al., 2017). The structures of the compounds were included in SMILES format to derive pharmacokinetic and toxicological predictions.

## RESULTS AND DISCUSSION

**Ligand and Receptor**

The three-dimensional conformation of the Arctigenin compound (Figure 1) was successfully retrieved from the PubChem database and optimized for subsequent docking analysis. This molecular entity features a lignan backbone, characterized by phenolic groups that serve as both potential hydrogen bond donors and acceptors, thereby contributing to its antioxidant efficacy and ligand binding affinity.

Simultaneously, the configuration of the Keap1 receptor (PDB ID: 6SP4) underwent refinement through the removal of water molecules, non-standard residues, and endogenous ligands, followed by the addition of hydrogen atoms to ensure the stability of the protonation state. The refinement methodology, supported by BIOVIA Discovery Studio 2025 and YASARA Structure, produced a precisely delineated binding pocket within the Kelch domain, where pivotal amino acid residues, such as Arg415, Ser508, and Tyr334, were significantly exposed. These residues play a crucial role in ligand recognition and electrostatic stabilization (J. Wu et al., 2025). The optimized structures of Arctigenin and Keap1 were subsequently employed in a molecular docking analysis to assess the binding affinity and orientation of ligand-receptor interactions.



Structure of Arctigenin Structure of Keap1

**Figure 1. Three-dimensional structure of Arctigenin from PubChem and the refined Keap1 receptor structure (PDB ID: 6SP4).**

**Molecular Docking Validation**

The docking methodology was validated through a redocking assessment using native ligands derived from the Keap1 receptor (PDB ID: 6SP4). The analysis of the superimposition between the native ligands and the redocking counterparts demonstrated a high degree of positional congruence within the binding pocket, with a root-mean-square deviation (RMSD) of 1.42 Å, thereby affirming the validity of the docking protocol as it remained below the threshold of 2 Å. The dimensions of the grid box, configured to encompass a 5 Å radius surrounding the native ligand, were shown to accommodate the ligand binding site with

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maximal stability effectively. Consequently, the docking protocol used has been validated and is suitable for examining the interaction dynamics of Arctigenin with the Keap1 receptor.

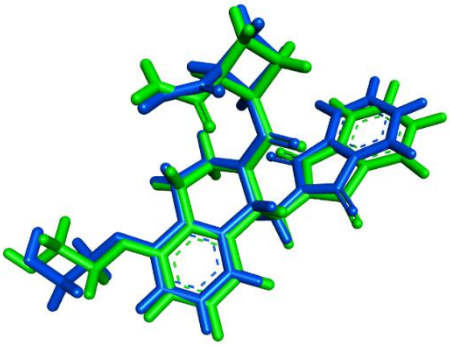

Figure 2. Overlay of the native LQK ligand (depicted in blue) and the results of redocking (represented in green) within the binding site of the Keap1 receptor.

**Molecular Docking Analysis between Receptor and Ligand**

The findings from the molecular docking analysis indicate that the compound Arctigenin exhibits a significant binding affinity towards the Keap1 receptor, characterized by a Binding Free Energy value of -8.73 kcal/mol. Structurally, the Kelch domain of Keap1 consists of five distinct sub-pockets (P1 to P5) that accommodate the ETGE motif of Nrf2. The interaction profile of Arctigenin demonstrates that it selectively occupies the highly basic central pockets, specifically P4 and P5. The predominant interactions are mediated through the formation of six hydrogen bonds with residues ARG415, SER508, TYR334, and VAL463. Importantly, the absence of covalent linkage to cysteine residues confirms that Arctigenin functions as a classical non-covalent inhibitor. These residues constitute critical components of the Kelch domain involved in Nrf2 interaction (Yurchenko et al., 2023). The observed low binding energy value and the specific occupation of the P4/P5 pockets indicate a high degree of complex stability, suggesting that Arctigenin has the potential to competitively inhibit the interactions between Keap1 and Nrf2, thereby functioning as a safe, non-covalent antioxidant agent

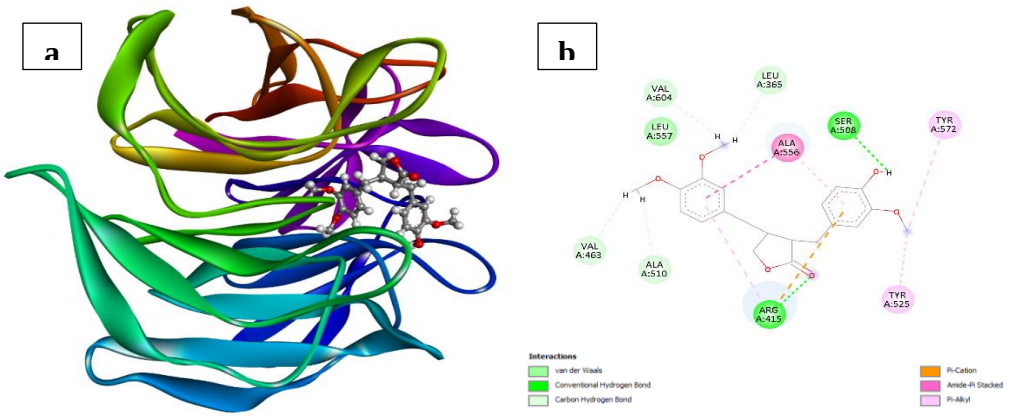


**Figure 3. Molecular docking visualization of Keap1 (PDB ID: 6SP4) with Arctigenin: (a) 3D binding conformation, and (b) 2D interaction profile with key amino acid residues.**

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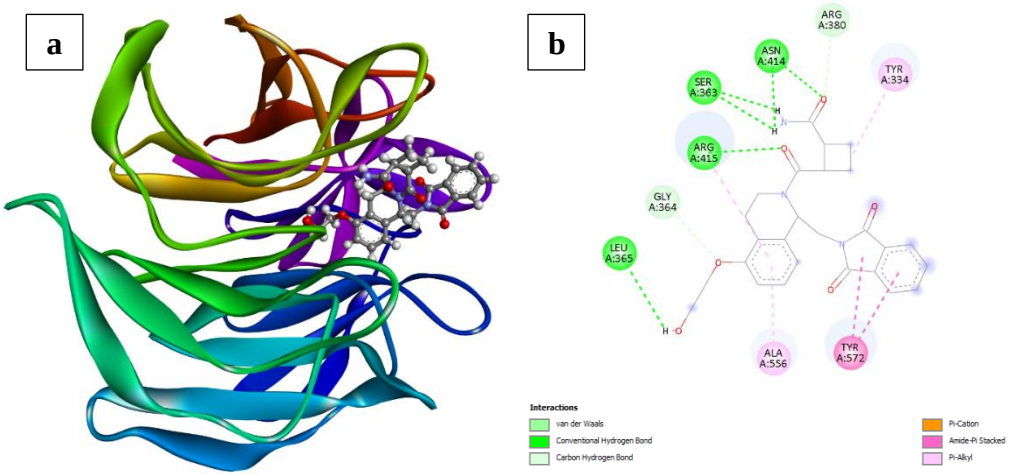


**Figure 4. Interaction of Keap1 (PDB ID: 6SP4) with its native ligand LQK: (a) 3D binding conformation, and (b) 2D interaction map with key amino acid residues.**

The findings presented in Table 1 indicate that the endogenous ligand LQK exhibits the most favorable binding energy (-11.67 kcal/mol), indicating its superior affinity towards Keap1. This observation aligns with the interaction dynamics illustrated in Table 2, wherein LQK establishes six hydrogen bonds with critical residues ASN414, ARG415, SER363, LEU365, GLY364, and ARG380, in addition to robust π–π and π–alkyl interactions with the aromatic residues TYR334 and TYR572. The substantial presence of hydrogen bonds coupled with aromatic interactions elucidates the rationale behind LQK's pronounced binding energy.

**Table 1. Binding energy comparison of Arctigenin, native ligand (LQK), and reference compound Astaxanthin toward the Keap1 receptor.**

| PubChem ID | Molecular Formula | Compound name | Binding energy (kcal/mol) |
|---|---|---|---|
| 64981 | $C_{21}H_{24}O_6$ | Arctigenin | -8.73 |
| 146048119 | $C_{26}H_{27}N_3O_6$ | LQK (native ligand) | -11.67 |
| 5281224 | $C_{40}H_{52}O_4$ | Astaxanthin® | -8.55 |

The compound Arctigenin demonstrates a competitive binding energy (-8.73 kcal/mol) and manifests an interaction profile that significantly reinforces the stability of the complex. Notably, six hydrogen bonds were identified with the pivotal residues ARG415, SER508, VAL604, LEU365, ALA510, and VAL463, complemented by substantial π–cation and π–alkyl interactions involving ARG415, ALA556, LEU557, and TYR572. The engagement of hotspot residues such as ARG415 and TYR572, which also serve as critical interaction sites for LQK, suggests that Arctigenin occupies and stabilizes the same binding pocket through a mechanism of biological relevance.

In contrast, Astaxanthin® demonstrates a diminished binding affinity (-8.55 kcal/mol) and engages in the formation of a singular hydrogen bond (VAL418). The remaining interactions consist predominantly of hydrophobic/π interactions with ARG415, ALA556, ILE559, TYR525, and TYR572, which, while contributing to the overall stability, do not rival the synergistic effect of hydrogen bonds and aromatic interactions exhibited by both LQK and Arctigenin. This finding is strongly supported by recent pharmacophore modeling and molecular dynamics studies of Keap1 inhibitors, which emphasize that the synergistic

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combination of strong hydrogen bonding at core basic residues (such as ARG415 and SER508) coupled with π-π or π-alkyl stacking at aromatic residues (such as TYR334 and TYR572) is absolutely essential for achieving high-affinity, competitive inhibition of the Kelch domain (Alzain et al., 2023;Singh et al., 2025).

**Table 2. Types of interactions and amino acid residues in the Keap1 receptor (PDB ID: 6SP4) involved in the binding of Arctigenin, the native ligand (LQK), and the comparator Astaxanthin.**

| Receptor | Compounds | Interacting Amino Acids in the Receptors |
|---|---|---|
| 6SP4 | Arctigenin | Hydrogen Bonds: ARG415, SER508, VAL604, LEU365, ALA510, VAL463<br>Other residues: ARG415 (π-cation, π-alkyl), ALA556 (π-alkyl), LEU557 (amide–π stacked), TYR572 (π-alkyl) |
| | LQK | Hydrogen Bonds: ASN414, ARG415, SER363, LEU365, GLY364, ARG380<br>Other residues: TYR572 (π–π stacked, π-alkyl), TYR334 (π–π stacked, π-alkyl), ARG415 (π-alkyl), ALA556 (π-alkyl) |
| | Astaxanthin® | Hydrogen Bonds: VAL418<br>Other residues: ARG415 (alkyl), ALA556 (alkyl), ILE559 (alkyl), TYR525 (π-alkyl), TYR572 (π-alkyl) |

**ADMET Predictions and Lipinski's Rule of Five**

The outcomes of ADMET predictions suggest that Arctigenin possesses a favorable pharmacokinetic profile characterized by substantial intestinal absorption (94.416%), is not categorized as a P-glycoprotein substrate, and exhibits limited permeability to the blood-brain barrier (LogBB –0.582; LogPS –3.01), thereby indicating a selective distribution devoid of neurotoxic consequences. Regarding metabolic processes, Arctigenin is not a substrate for CYP2D6 but is a substrate for CYP3A4, signifying that it undergoes standard metabolism through the predominant enzymatic pathway. The total clearance rate of 0.214 log mL/min/kg reflects effective elimination, while the negative findings in AMES and hepatotoxicity assays suggest a favorable toxicological safety profile.

**Table 3. Assessment of the drug-likeness of Arctigenin, the native ligand (LQK), and Astaxanthin compounds in accordance with the parameters delineated by Lipinski's Rule of Five.**

| Rule of Five parameters | Ligands | | |
|---|---|---|---|
| | Arctigenin | LQK | Astaxanthin® |
| Molecular Weight < 500 D | 372 g/mol | 477.5 g/mol | 596.84 g/mol |
| H-Donor < 5 | 1 | 2 | 2 |
| H-Acceptor < 10 | 6 | 6 | 4 |
| Log P < 5 | 2.99 | 1.29 | 7.4 |
| Molar Refractivity 40–130 | 99.55 | 124.4 | 195.98 |
| Meets | **Yes** | **Yes** | No |

Based on the parameters delineated by Lipinski's Rule of Five, both the compound Arctigenin and the endogenous ligand LQK satisfy all the requisite criteria for oral pharmacological applicability. In contrast, Astaxanthin exhibits a significant deviation,

specifically a markedly elevated LogP value (7.4) and a molar refraction that exceeds the permissible maximum (195.98). Arctigenin is characterized by a relatively low molecular weight (372 g/mol), a LogP of 2.99, and an optimal quantity of hydrogen bond donors and acceptors (1 and 6), which collectively suggest an advantageous permeability and solubility profile conducive to membrane diffusion. LQK, as an endogenous ligand, similarly adheres to all Lipinski's guidelines, albeit with a greater molecular weight (477.5 g/mol), indicative of increased structural complexity. In contrast, Astaxanthin, which fails to comply with the Lipinski criteria, may exhibit diminished oral bioavailability attributable to excessive lipophilicity and substantial molecular dimensions. In summary, these findings substantiate the notion that Arctigenin possesses favorable drug-like attributes, rendering it a viable candidate for oral pharmacological development with enhanced bioavailability and pharmacokinetic stability when compared to the reference ligands.

Despite meeting Lipinski's criteria, evaluating the feasibility of Arctigenin as a "real" therapeutic drug requires addressing its physicochemical limitations. Arctigenin exhibits a LogP value of 2.99, indicating moderate lipophilicity. While this facilitates cell membrane permeation, its relative polarity and poor aqueous solubility may restrict optimal gastrointestinal absorption and systemic bioavailability in a clinical setting. Therefore, for future translational research and pharmacological development, it is highly recommended to formulate Arctigenin using advanced drug delivery systems. Developing Arctigenin into nano-emulsions, liposomes, or solid lipid nanoparticles (SLNs) could significantly enhance its solubility, protect it from premature metabolic degradation, and ensure targeted delivery, thereby bridging the gap between in silico discovery and practical clinical application.

**Molecular Dynamics Simulation**

The analysis of surface area metrics reveals that the SurfVdW, SurfMol, and SurfAcc of the Keap1-Arctigenin complex exhibit stability over the 50 ns simulation duration, with no significant fluctuations. The constancy of these parameters implies an absence of substantial alterations in residue exposure or protein conformation, thereby suggesting a stable configuration of the complex. The Radius of Gyration (Rg) remains consistently within the range of ±17.7-18.1 Å, characterized by only minor oscillations, thereby corroborating that the Keap1 structure in complex with Arctigenin maintains a compact form without any appreciable expansion or contraction, which aligns with prior investigations into Keap1-ligand interactions (Y. Zhang et al., 2024).

The RMSD values for Cα and the backbone are maintained at 1.6–1.8 Å, while the all-atom RMSD stabilizes at approximately 2.0–2.1 Å, indicative of rapid equilibration and sustained structural integrity, consistent with findings from other analyses of Keap1 inhibitors (Singh et al., 2025). Furthermore, RMSF values for the majority of residues are observed to reside within the range of 0.5-1.5 Å, with elevated fluctuations noted exclusively in flexible loops. Critical binding residues (ARG415, SER508, VAL604, LEU365, ALA510, VAL463, ALA556, LEU557, TYR572) demonstrate minimal fluctuation, reflecting a highly stable binding environment.

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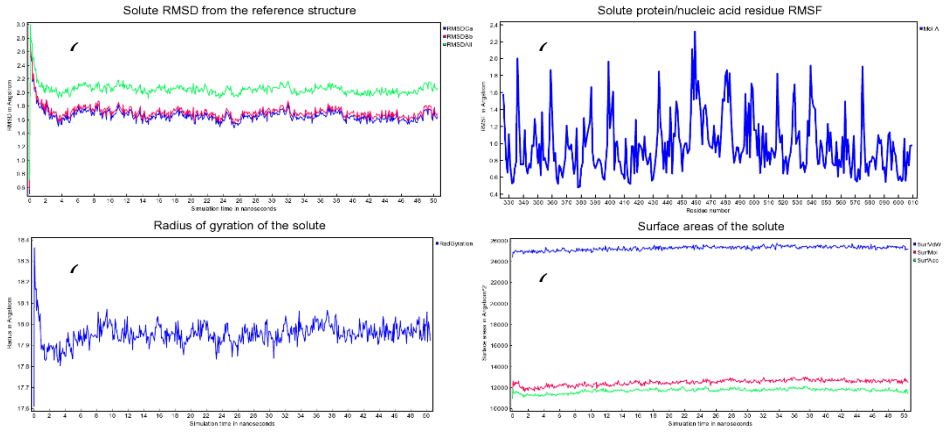


**Figure 5. illustrates the profiles of RMSD (a), RMSF (b), Rg (c), and SASA (d) for the Keap1-Arctigenin complex throughout a 50 ns molecular dynamics simulation.**

These observations are congruent with trends documented in investigations concerning natural Keap1-Nrf2 inhibitors, wherein stable RMSD, Rg, and SASA profiles denote ligands that uphold the integrity of the Kelch domain (Alzain et al., 2023). Furthermore, research focused on lignan-based modulators provides additional evidence supporting their capacity to bind stably within the Keap1 binding pocket (Hassanein et al., 2024). Consequently, the observed stability in SASA, Rg, RMSD, and RMSF values substantiates the assertion that Arctigenin displays considerable dynamic stability and substantial potential as a Keap1 inhibitor.

## CONCLUSION

In silico analysis demonstrated that Arctigenin has strong potential as a natural Keap1 inhibitor. Molecular docking showed a favorable binding affinity (-8.73 kcal/mol) supported by six hydrogen bonds and stable hydrophobic/π interactions with key Kelch-domain residues such as ARG415, SER508, LEU365, VAL463, ALA556, LEU557, and TYR572. Molecular dynamics simulations for 50 ns confirmed complex stability through low RMSD (<2 Å), minimal RMSF fluctuations, stable Rg (17.8-18.0 Å), and consistent SASA values. ADMET and Lipinski evaluations revealed excellent absorption, non-toxicity, and good drug-likeness characteristics. These results align with findings from previous studies on polyphenolic Keap1 inhibitors, reinforcing that Arctigenin interacts through a biologically relevant mechanism capable of modulating the Keap1–Nrf2 pathway. Overall, Arctigenin is a promising candidate for further development as a natural antioxidant agent targeting Nrf2 activation.

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