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Email : editorijhess@gmail.com

Molecular Docking Study Of Active Compounds Of Purslane (Portulaca oleracea L.) As Antioxidant Enzyme Activators

Nucke Aurelya Pramesti[1)], Tiara Ajeng Listyani[2)], Anna Fitriawati[3)]
[1,2,3)] Program Studi Sarjana Farmasi, Fakultas Ilmu Kesehatan Universitas Duta Bangsa Surakarta

*Corresponding Author
Email: nuckeaurelya6@gmail.com

Abstract

*Oxidative stress resulting from an imbalance between Reactive Oxygen Species (ROS) and endogenous antioxidant systems contributes to various degenerative diseases. Activation of the Keap1-Nrf2 pathway plays a crucial role in upregulating antioxidant enzyme expression. Purslane (*Portulaca oleracea* L.) is known to be rich in phenolic compounds and flavonoids with antioxidant potential; however, studies regarding its molecular interactions with the Keap1-Nrf2 pathway remain limited. This study aimed to determine molecular interaction patterns, predict ADME and toxicity profiles, and identify the active compound in purslane with the greatest potential as an Nrf2 antioxidant enzyme activator. This was a quantitative, pre-experimental *in silico* study utilizing AutoDock Vina (via the PyRx platform) for molecular docking, VegaZZ and PyMOL for preparation and visualization, and SwissADME and pkCSM for ADME-toxicity prediction. Docking results revealed that compounds such as Taraxerol, Hesperidin, Quercetin-O-hexoside, Neochlorogenic acid, and Chlorogenic acid exhibited favorable binding affinities (RMSD ≤2 Å) and formed non-covalent interactions with key residues, such as GLN530 and SER508. Most compounds demonstrated ADME and toxicity profiles supporting their development as oral drug candidates with low safety risks. Taraxerol was identified as the best compound, as it met the established criteria across all testing parameters. Active compounds in purslane show potential as Nrf2 activators; although no single compound met every criterion for an ideal drug candidate, Taraxerol emerged as the most promising candidate, warranting further structural optimization to assess its functional efficacy.*

Keywords: Purslane, Nrf2, Molecular Docking, Antioxidant, ADME-Toxicity

INTRODUCTION

Oxidative stress is a pathological state resulting from an imbalance between the production of Reactive Oxygen Species (ROS) and the capacity of the body's antioxidant defense systems. This imbalance leads to oxidative damage to cellular components—such as lipids, proteins, and DNA—contributing to the development of various degenerative diseases, including cancer, diabetes mellitus, and cardiovascular disease. Endogenous antioxidant defense systems include enzymes such as Superoxide Dismutase (SOD). SOD plays a role in converting superoxide radicals into hydrogen peroxide, while GPx utilizes glutathione to reduce hydrogen peroxide into water. These mechanisms are crucial for maintaining cellular redox homeostasis within the body (Nkhumeleni et al., 2024).

The expression and activity of these antioxidant enzymes are regulated by the Keap1-Nrf2 (Nuclear factor erythroid 2–related factor 2) system, which serves as a master regulator of cellular antioxidant pathways. Activation of the Keap1-Nrf2 system triggers the expression of genes involved in the antioxidant response via the Antioxidant Response Element (ARE) located in the promoters of target genes (in vivo) (Aziz et al., 2024). Increased Keap1-Nrf2 activity has been shown to correlate with enhanced antioxidant enzyme activity. Furthermore, modulation of the Keap1-Nrf2 system contributes to the suppression of inflammatory pathways, ultimately offering protection to tissues subjected to oxidative stress (Aziz et al., 2024).

The rising prevalence of conditions associated with oxidative stress has prompted further research to identify safe sources of antioxidant activity. Although synthetic antioxidants demonstrate high efficacy, their use can entail potential side effects and high production costs. Consequently, the exploration of natural antioxidant sources derived from

plants is increasingly being pursued, as they offer greater practicality. Medicinal plants are known to contain secondary metabolites such as phenolic compounds, flavonoids, and alkaloids, as well as other bioactive compounds. These compounds not only function as free radical scavengers but are also capable of modulating cellular signaling pathways, including Keap1-Nrf2 activation (Sheikh et al., 2025).

Purslane (*Portulaca oleracea* L.) is a plant rich in natural antioxidants and is commonly found in tropical regions, including Indonesia. It has long been used as a vegetable and in herbal medicine due to its abundance of nutrients and phytochemical compounds. Previous phytochemical analyses have revealed that purslane contains significant levels of phenolics and flavonoids. These constituents correlate with potent antioxidant activity, as demonstrated in various *in vitro* assays using the DPPH method (Sheikh et al., 2025). Purslane has also been reported to exhibit diverse biological activities—including antioxidant and antimicrobial properties—based on *in silico* studies involving molecular docking against specific protein targets.

While numerous studies have previously evaluated the *in vitro* antioxidant activity of purslane, research deeply investigating the molecular mechanisms of interaction between its active compounds and endogenous antioxidant pathways—such as the Keap1-Nrf2 pathway—remains limited. Although *in silico* research on purslane's active compounds has begun to emerge, the volume of such studies is still modest, and they generally focus on a single specific target enzyme (Fransiski et al., 2023).

In addition to experimental approaches, molecular docking has become a crucial *in silico* technique for predicting how plant-derived bioactive compounds interact with biological protein targets, including Keap1-Nrf2. Several studies have employed molecular docking to assess interactions between plant constituents and antioxidant proteins or enzymes. Findings from these studies indicate that certain phenolic compounds can bind to the Keap1-Nrf2 regulatory protein, thereby potentially enhancing cellular antioxidant capacity (Tripathi et al., 2024).

RESEARCH METHODS

This study employs a quantitative research design using a computer-based pre-experimental approach conducted *in silico*. The research aims to evaluate the activity of antioxidant bioactive compounds from purslane (*Portulaca oleracea* L.) against the Keap1-Nrf2 enzyme. The software and databases utilized in this study include the Protein Data Bank (PDB) for target preparation; PyRx and AutoDock Vina for molecular docking; VegaZZ and PyMOL for preparation and visualization; and relevant software or servers for predicting ADME and toxicity (Tox) profiles.

RESULTS AND DISCUSSION

Results

Nrf2 Receptor Macromolecule Download

The initial stage of the *in silico* study involved downloading the target molecular structure from the Protein Data Bank (RCSB PDB) via the official website, rscb.org. The receptor utilized was Nuclear factor erythroid 2-related factor 2 (Nrf2).

Macromolecule Preparation

The preparation of the macromolecule from purslane (*Portulaca oleracea* L.) was conducted using Discovery Studio Visualizer. This process involved utilizing a 3D representation of the macromolecule and separating its molecular chains.

Generation of 2D and 3D Test Ligand Structures

Structures were generated for 70 test compounds derived from purslane (*Portulaca oleracea* L.). Information regarding these 70 compounds was obtained from PubChem, and the structures were created using the ChemDraw application.

Test Ligand Optimization

The 70 test ligands from purslane (*Portulaca oleracea* L.) were optimized using the VegaZZ application to identify the conformation with the most stable energy.

Molecular Docking Method Validation

The docking method was validated using the native ligand and the Keap1-Nrf2 enzyme receptor. An RMSD value of 0.288 was obtained; this very low value indicates a high degree of structural and conformational similarity between the two ligands (Rimac et al., 2021).

Molecular Docking Process

The docking process for the 70 purslane (*Portulaca oleracea* L.) compounds was performed using the PyRx application. A grid box was employed to define the target region on the protein structure and to restrict the ligand exploration area, thereby focusing the docking process on the specific binding site.

Docking Method Validation

Docking validation results for the Keap1-Nrf2 enzyme. The results demonstrate that the search area was successfully defined within the specific region of the Keap1-Nrf2 protein structure where ligand interaction occurs. Molecular Docking Results

The docking results for 70 compounds from purslane (*Portulaca oleracea* L.) against the Nrf2 enzyme showed that the majority of ligands had RMSD values <2 Å, indicating good conformational fit for the docked poses.

Interactions of Test Ligands with the Receptor (Amino Acids)

Analysis of amino acid interactions involving 70 compounds from *Portulaca oleracea* L. revealed that the native ligand (A1LVB) forms three primary conventional hydrogen bonds with residues GLN A:530, SER A:555, and SER A:508; this indicates that these three residues are critical components of the target protein's binding pocket (Paggi et al., 2024).

ADME Prediction

ADME analysis based on Lipinski's parameters for the 70 compounds from *Portulaca oleracea* L. showed that the majority of the plant's secondary metabolites possess physicochemical characteristics favorable for development as oral drug candidates—specifically, compounds with a molecular weight <500 Da, HBA ≤10, HBD ≤5, Log P <5, and good water solubility (Komura et al., 2023).

Toxicity

Toxicity assessments of the 70 compounds from *Portulaca oleracea* L. indicated that most active compounds found in the plant exhibit a relatively favorable safety profile, although a few compounds warrant further investigation. The toxicity parameters analyzed included Cramer Rules, predictions of carcinogenicity (genotoxic and non-genotoxic), and mutagenicity based on the Ames test (*Salmonella typhimurium*) (Bueso-Bordils et al., 2024).

Best Compounds

The compound taraxerol was identified as a promising candidate derived from the purslane plant (*Portulaca oleracea* L.). Taraxerol exhibits a highly promising set of characteristics as a potential Nrf2 activator. This compound demonstrates high binding affinity for the Nrf2 receptor, a valid docking conformation (based on RMSD values), a

favorable oral absorption profile, adequate bioavailability, a lack of inhibition against major CYP enzymes, and a predicted low toxicity profile (Culletta et al., 2024).

Discussion

Retrieval of the Nrf2 Protein Macromolecule

The selection of the 8XGK protein structure was based on the pivotal role of the Keap1-Nrf2 pathway as a master regulator of the endogenous antioxidant defense system. The Keap1-Nrf2 system controls the expression of various cytoprotective genes by binding to the Antioxidant Response Element (ARE), thereby inducing the expression of genes encoding endogenous antioxidant enzymes. This mechanism reduces the accumulation of reactive oxygen species (ROS) and protects cells from oxidative damage, a key factor in the pathogenesis of various degenerative diseases.

The 8XGK structure was selected because it is a protein crystal structure obtained via X-ray diffraction with a resolution of 1.32 Å. This resolution falls into the high-resolution category, indicating that atomic positions within the protein can be determined with excellent accuracy. A lower resolution value (<2.0 Å) corresponds to higher protein structural model quality, resulting in more reliable predictions of ligand-receptor interactions. The 8XGK structure contains a co-crystallized ligand (A1LVB) that can be utilized for redocking—serving to validate the molecular docking method through the evaluation of the Root Mean Square Deviation (RMSD) value (Otake et al., 2024). A resolution of 2–3 Å is categorized as medium resolution; while atomic details remain observable, positional uncertainty regarding atoms, side chains, and loops begins to increase, meaning structures in this range can still be used for docking but with lower accuracy compared to high-resolution structures (Bansal et al., 2021).

Macromolecule Preparation

Preparation or preparation of macromolecules before the molecular docking process is very important and can determine the predicted results of interactions that will occur. The Keap1-Nrf2 (8XGK) macromolecule was prepared using the Discovery Studio Visualizer tool. This is in line with Listyani's research in 2021 which stated that macromolecular preparation includes separating proteins from native ligands and removing residues that still exist in the protein. The 3D structure of the macromolecule that has been prepared is then saved in .pdb format as seen in Figure 4.3.

Creation of 2D and 3D structures on test ligands

The 3D structure of compounds contained in the purslane plant (Portulaca oleracea L.) was obtained via PubChem from the National Library of Medicine. Apart from the 3D structure, other information required is PubChem ID and Canonical Smile. Depiction of 2D, 3D structures and structural conformations of compounds. Draw the structure using the ChemDraw 2D application with CDX.file format. Next in 3D via ChemDraw 3D with the pdb.file and mol.file formats. The 3D structure was geometrically optimized using the VegaZZ application to produce the lowest energy conformation which shows the best stability of a chemical structure (Listyani et al., 2024).

This research was used to test antioxidant enzyme activators in purslane plants (Portulca oleracea L.). Analysis of antioxidant activity in this research used an in silico approach with the molecular docking method. This approach was chosen to predict the ability of compounds from purslane plants as activators of the antioxidant enzyme Nrf2. The compounds tested were 70 compounds in purslane plants.

Preparation of test ligands

The 3D structures of all compounds from the purslane plant and the native ligand were prepared using VegaZZ. Preparation involved adding hydrogen atoms, assigning Gasteiger charges, applying the AutoDock force field, and performing energy minimization to obtain

the most stable ligand structure with the lowest energy. This approach aligns with Listyani's 2021 study, which utilized 3,000 steps of minimization to yield more stable ligands.

Docking Method Validation

Validation is the initial stage in molecular docking studies, intended to ensure that the docking method employed can accurately reproduce the binding position of the ligand within the protein's active site. The validation process was carried out using the redocking method, which involves docking the native ligand back into the target enzyme's binding pocket. In this study, validation was performed on the Keap1-Nrf2 enzyme system using the native ligand A1LVB.

The RMSD value serves as a parameter to measure the degree of conformity between the ligand conformation obtained via redocking and the native ligand conformation derived from crystallographic data. A lower RMSD value indicates greater positional similarity between the two structures, signifying a superior ability of the docking method to predict the ligand's binding orientation within the protein's active site. Validation success was evaluated based on the Root Mean Square Deviation (RMSD) value; an average RMSD of <3 Å indicates that the docking method possesses an adequate level of accuracy (Mohanty & Mohanty, 2023).

Subsequently, the redocking results were visualized using PyMOL software, comparing the position of the crystallographic native ligand with that of the redocked ligand through overlay analysis. The visualization revealed a high degree of overlap between the two structures, indicating that the docking method successfully reproduced the native ligand's binding position with accuracy. Based on these validation results, the molecular docking process proceeded to *in silico* testing of 70 test compounds derived from the purslane plant (*Portulaca oleracea* L.).

Molecular Docking Process

Before performing molecular docking, method validation was conducted by re-docking the macromolecule with its native ligand. This validation process was carried out using the PyRx software. The validation steps began with importing the prepared enzyme and the optimized native ligand, followed by configuring the grid box to achieve an RMSD value consistent with the resolution specified in the PDB file; the resulting data were saved in .pdb format. Grid box specifications are shown in Figure 4.8. Docking data are considered valid if the Root Mean Square Deviation (RMSD) value is less than 2.50 Å. Subsequently, docking results were obtained—including RMSD and binding affinity values—for 70 compounds derived from the purslane plant (*Portulaca oleracea* L.).

Molecular Docking Results

Molecular docking is a Computer-Aided Drug Design (CADD) approach used to computationally predict binding affinity, orientation (binding pose), and the nature of interactions between a ligand and a target protein. The method operates by positioning the ligand within the protein's binding pocket and calculating the most stable conformation using a scoring function. Docking results are typically evaluated based on free energy of binding (ΔG), Root Mean Square Deviation (RMSD), and interaction patterns with amino acid residues. Molecular docking is widely employed in the early stages of drug discovery because it enables the screening of compounds with potential biological activity prior to *in vitro* or *in vivo* testing. It serves as an effective method for accelerating the identification of drug candidates by predicting the strength of interactions between ligands and target proteins (Mohanty & Mohanty, 2023).

In this study, the primary parameters used to evaluate docking results were the binding free energy (ΔG) and RMSD. The ΔG value represents the energy released during the formation of the protein-ligand complex; a more negative ΔG value indicates a more stable complex and higher ligand affinity for the target protein. Conversely, the RMSD value is

used to assess the accuracy of the docked ligand's orientation relative to the reference conformation. Generally, an RMSD value of ≤2.0 Å indicates that the docking method successfully reproduces the ligand's position, whereas an RMSD >2.0 Å suggests significant orientational deviation, requiring cautious interpretation of the docking results (Huang et al., 2024).

Based on the docking results in Table 4.1, the native ligand (A1LVB) yielded a ΔG value of -8.5 kcal/mol and an RMSD of 1.739 Å. This RMSD value confirms that the docking method met validation criteria, allowing the molecular docking results to serve as a basis for evaluating the affinity of the test compounds. The native ligand served as a reference due to its known optimal binding capability with the target protein; consequently, compounds exhibiting ΔG values close to or lower than that of the native ligand are predicted to possess strong binding affinity for the target protein. Taraxerol, Aurantiamide Benzoate, Lonchocarpic acid, Hesperidin, and Quercetin-O demonstrated high binding affinity. These results suggest that these compounds are capable of forming stable complexes with the target protein. Structurally, these compounds contain aromatic rings and hydroxyl or carbonyl groups, enabling the simultaneous formation of hydrogen bonds, π-π stacking interactions, and hydrophobic interactions. The combination of these various non-covalent interactions enhances the stability of the protein-ligand complex, resulting in lower ΔG values. Flavonoid compounds—specifically quercetin and hesperidin derivatives—possess the ability to interact strongly with various proteins through their phenolic hydroxyl groups and aromatic systems (Etukudo et al., 2025).

Phenolic acid derivatives—such as neochlorogenic acid, chlorogenic acid, feruloyl glucose, and p-coumaroylquinic acid—as well as the oleracein group, yield ΔG values ranging from -7.3 to -7.9 kcal/mol. These values indicate that the compounds possess good binding affinity for the target protein. The presence of multiple hydroxyl groups in the molecular structure enhances the likelihood of hydrogen bond formation with amino acid residues, while aromatic rings facilitate hydrophobic interactions and π-π stacking. However, an excessive number of hydroxyl groups can increase molecular polarity, thereby—in some instances—reducing the contribution of hydrophobic interactions to binding energy. Consequently, a ligand's affinity is determined not only by the number of functional groups but also by the balance between its hydrophilic and hydrophobic properties (Mohanty & Mohanty, 2023).

The results obtained for oxalic acid, malic acid, citric acid, catechol, benzoic acid, dopamine, and anisic acid showed relatively high (less negative) ΔG values, ranging from -3.4 to -4.9 kcal/mol. These values indicate that the resulting protein-ligand complexes possess lower stability compared to compounds with more negative ΔG values. This low binding affinity is likely attributed to the compounds' relatively simple molecular structures and small sizes, which limit the contact surface area (binding interface) with the protein's binding pocket. Consequently, fewer non-covalent interactions—such as hydrophobic interactions, van der Waals forces, and electrostatic interactions—are formed. Furthermore, although some of these compounds possess hydroxyl or carboxylate groups capable of forming hydrogen bonds, the mere presence of hydrogen bonds does not always yield high binding affinity unless supported by shape complementarity and favorable ligand orientation relative to the protein's active site. Therefore, the binding strength of a ligand is determined by a combination of various molecular interactions and the conformational fit between the ligand and the protein's binding pocket, rather than solely by the number of functional groups present. This aligns with the findings of Paggi et al. (2024), who explained that the success of molecular docking depends on the geometric fit of the ligand within the binding pocket and the balance of various non-covalent interactions that determine the stability of the protein-ligand complex.

Based on Root Mean Square Deviation (RMSD) parameters, nearly all test compounds exhibited RMSD values below 2.0 Å; this indicates that the binding poses obtained from docking align well with the reference conformation, suggesting that the predicted results are reliable for further analysis. The compounds Sinapic acid (RMSD 2.856 Å), Colchicine (RMSD 2.261 Å), and Paracetamol—used as a negative control (RMSD 3.436 Å)—exhibited RMSD values exceeding the established threshold. High RMSD values indicate significant deviation of the ligand pose from the reference conformation, suggesting that the binding orientation does not accurately represent the actual binding position within the protein's active site. Consequently, even though these compounds demonstrate relatively favorable binding energy values (ΔG), the docking results require cautious interpretation; high binding affinity does not necessarily correlate with an accurate binding pose (Paggi et al., 2024).

Interaction of Test Ligand Amino Acids with the Receptor

Amino acid residue interactions between the test ligands and the receptor indicate that binding affinity is influenced not only by the magnitude of the binding free energy (ΔG) but also by the types of interactions formed within the protein's active site. Docking results reveal that the majority of active compounds from purslane (*Portulaca oleracea* L.) interact with residues ARG415, ALA556, TYR334, TYR525, SER363, SER508, SER555, ASN382, GLN530, GLY509, and GLY603—residues also frequently involved in interactions with the native ligand. This overlap in residues suggests that the test ligands occupy the same binding pocket as the reference ligand, implying they can maintain the binding orientation necessary to elicit biological activity. According to Paggi et al. (2024), the success of molecular docking is determined not only by the ligand's position within the binding pocket but also by its ability to maintain an interaction network with key residues that stabilize the protein-ligand complex.

The prevalence of conventional hydrogen bonds involving residues SER363, SER508, SER555, ASN382, GLN530, and ARG415 indicates that the hydroxyl and carbonyl groups of purslane's phenolic compounds play a crucial role in enhancing binding specificity toward the receptor. Hydrogen bonds are directional interactions that help maintain the ligand's orientation within the protein's active site and lower the complex's free energy. Additionally, carbon-hydrogen bonds involving residues GLY509 and GLY603 contribute further to complex stability, despite their lower energy compared to conventional hydrogen bonds. Research by Sahu et al. (2024) explains that the formation of a hydrogen-bonding network involving multiple active residues enhances both affinity and protein-ligand complex stability, making it a key parameter in interpreting molecular docking results.

The interaction of serine (SER) residues with the KEAP1-Nrf2 protein plays a crucial role in the stability of the KEAP1–Nrf2 complex. Residues SER508 and SER555 can form hydrogen bonds with ligands, thereby helping to retain the ligand within the KEAP1 binding pocket. Interactions at these residues have the potential to inhibit the KEAP1-Nrf2 interaction and reduce Nrf2 degradation. Such conditions can enhance Nrf2 stabilization and its translocation to the nucleus, subsequently inducing antioxidant gene expression via the ARE element. Ligand interactions with serine residues on KEAP1 can facilitate the modulation of the Nrf2 antioxidant pathway (Culletta et al., 2024).

In addition to polar interactions, nearly all tested ligands form hydrophobic interactions—such as π-π stacking, π-alkyl, alkyl, and π-cation interactions—with residues TYR334, TYR525, TYR572, ALA556, PHE478, PHE577, and ARG415. Hydrophobic interactions are known to reduce solvation energy within the binding pocket, thereby enhancing the thermodynamic stability of the complex. The combination of hydrophobic interactions and hydrogen bonding is a primary determinant of protein-ligand complex stability during molecular docking. Consequently, test compounds such as Teraxerol,

Auratinamide benzoate, Lonchocarpic acid, and Hesperidin exhibit high affinity values despite forming relatively few hydrogen bonds. Thus, the binding strength of a ligand arises from the synergy between hydrogen bonding, van der Waals forces, and hydrophobic interactions, rather than being determined by a single type of bond alone. Residue ARG415 is the residue that most frequently interacts with the various test ligands through conventional hydrogen bonds, carbon-hydrogen bonds, salt bridges, π-cation interactions, and π-alkyl interactions. The frequency of occurrence of these residues suggests that ARG415 is likely a key binding residue involved in the ligand recognition process. Similarly, residues ALA556 and TYR334 consistently form hydrophobic interactions with the majority of active ligands, indicating that they serve to stabilize the complex. Consequently, ligands capable of maintaining interactions with these three residues are predicted to have a higher probability of forming stable complexes. Conversely, the presence of unfavorable interactions—such as donor–donor or acceptor–acceptor clashes, or steric bumps—in certain compounds indicates electrostatic repulsion or steric hindrance that could reduce binding efficiency. This demonstrates that the interpretation of docking results should not rely solely on ΔG values but should also consider the quality of interactions with key residues in the binding pocket as an indicator of a ligand's biological potential.

ADME Prediction

Prediction of Absorption, Distribution, Metabolism, and Excretion (ADME) is a crucial stage in the computer-aided drug design (CADD) process. ADME evaluation aims to identify the pharmacokinetic characteristics of a compound at an early stage, allowing drug candidates that possess both high biological activity and favorable pharmacokinetic properties to be prioritized for further research. According to Komura et al. (2023), physicochemical parameters—such as molecular weight, lipophilicity (LogP), the number of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), and solubility—are key factors influencing the oral absorption, distribution, metabolism, and excretion of a molecule. Consequently, evaluation based on Lipinski's Rule of Five (Ro5) remains the most widely used approach for assessing the drug-likeness of oral drug candidates.

Based on ADME prediction results, the majority of active compounds from the purslane plant (*Portulaca oleracea*) meet most of Lipinski's parameters. Compounds such as ferulic acid derivative, protocatechuic acid, p-hydroxybenzoic acid, catechin, epicatechin, apigenin, caffeic acid, vanillic acid, anisic acid, dopamine, noradrenaline, adenosine, oleracein E, gentisic acid, gallic acid, and N-feruloyloctopamine exhibit molecular weights below 500 g/mol, LogP values under 5, HBA counts not exceeding 10, and HBD counts not exceeding 5. These characteristics suggest that the compounds are predicted to have superior oral absorption capabilities due to their relatively small molecular size, balanced polarity, and a higher capacity to diffuse across biological membranes compared to larger molecules. The relationship between molecular size, lipophilicity, and the number of hydrogen bond donors and acceptors regarding oral bioavailability has been reaffirmed in an evaluation of FDA-approved oral drugs up to 2022. Conversely, several glycoside compounds—such as Oleracein A, Oleracein B, Oleracein C, Oleracein D, Hesperidin, Kaempferol-O-hexoside, Quercetin-O-hexoside isomer, Isorhamnetin-O-hexoside, and Caffeoylglucaric acid—exhibit violations of Lipinski's parameters, particularly regarding molecular weights exceeding 500 g/mol and high numbers of hydrogen bond acceptors (HBA) and donors (HBD). The high number of hydroxyl groups and sugar residues leads to increased molecular polarity, resulting in lower intestinal membrane permeability despite enhanced water solubility (Komura et al., 2023).

Lipophilicity parameters (LogP) indicate that the majority of the compounds possess values below 5, signifying a balance between hydrophilic and lipophilic properties. An optimal LogP value is crucial, as it influences a compound's ability to dissolve in biological

fluids while simultaneously permeating cellular phospholipid membranes. Conversely, compounds such as aurantiamide benzoate, lupeol, α-tocopherol, taraxerol, friedelane, α-carotene, palmitic acid, stearic acid, and lonchocarpic acid exhibit LogP values exceeding 5, indicating highly lipophilic characteristics. While highly lipophilic compounds often demonstrate good membrane permeability, they frequently suffer from reduced aqueous solubility, thereby limiting oral bioavailability. Furthermore, excessively lipophilic compounds tend to accumulate in adipose tissue and exhibit stronger binding to plasma proteins. Although the results indicate high docking affinity for several of these compounds, their pharmacokinetic characteristics still require optimization through medicinal chemistry approaches or drug delivery systems (Komura et al., 2023).

Evaluations of HBA and HBD parameters reveal a similar trend. Simple phenolic compounds generally comply with Lipinski's rules, suggesting superior permeability. In contrast, the increased number of hydroxyl groups in flavonoid glycosides causes HBA and HBD values to exceed recommended limits. While a high number of hydroxyl groups enhances hydrogen bonding with water—thereby increasing solubility—it simultaneously hinders passive diffusion across lipid membranes. This factor contributes to the low oral bioavailability observed in many natural polyphenolic compounds, despite their potent antioxidant activity (Komura et al., 2023). Predictive results also indicate that nearly all the compounds fall into the "soluble" to "highly soluble" categories in water. Good solubility is advantageous during the absorption phase, as molecules must be in a dissolved state to be absorbed via the gastrointestinal tract; however, high solubility does not invariably correlate with high absorption. The glycoside compounds in this study demonstrate that increased solubility—resulting from the presence of numerous hydroxyl groups—is actually accompanied by reduced membrane permeability. Conversely, triterpenoid compounds such as lupeol and taraxerol exhibit high permeability due to their lipophilic nature, yet their relatively low solubility potentially limits oral bioavailability (Komura et al., 2023).

Overall, ADME prediction results indicate that phenolic acids and flavonoid aglycones—such as ferulic acid derivatives, protocatechuic acid, catechin, epicatechin, apigenin, caffeic acid, gentisic acid, and gallic acid—possess superior drug-likeness profiles compared to glycosides or triterpenoids. These compounds satisfy the majority of Lipinski's parameters, thereby predicting a higher likelihood of oral absorption. In contrast, glycoside compounds such as Oleracein A–D, Hesperidin, Kaempferol-O-hexoside, and Quercetin-O-hexoside isomers exhibit several violations of Lipinski's rules, which could potentially reduce oral bioavailability (Komura et al., 2023).

GI Absorption

Based on the results in Table 4.4, the majority of active compounds in purslane are predicted to exhibit high gastrointestinal (GI) absorption; these include ferulic acid derivatives, catechin, epicatechin, protocatechuic acid, naringenin, myricetin, oleracein U, scopoletin, α-tocopherol, taraxerol, friedelane, and several others. These findings indicate that the compounds are likely to be efficiently absorbed across the intestinal mucosa, thereby offering a greater probability of reaching systemic circulation following oral administration. High gastrointestinal absorption is generally influenced by a balance of properties—such as lipophilicity, molecular size, and the number of hydrogen bond donors and acceptors—that facilitate passive diffusion across biological membranes. Specifically, this process is favored by relatively small molecular size, balanced lipophilicity, and hydrogen bond donor/acceptor counts that fall within the optimal range. Compounds with high oral absorption are more likely to achieve effective therapeutic concentrations following oral administration (Naeem et al., 2022).

A classification of "High" for GI absorption is the most desirable outcome, as it indicates that the compound is predicted to possess good permeation capabilities across the

intestinal epithelium, allowing for efficient absorption into the systemic circulation after oral administration. The greater the absorption capacity within the gastrointestinal tract, the larger the fraction of the drug that reaches the bloodstream (systemic circulation), thereby increasing the likelihood of eliciting a pharmacological effect. Conversely, compounds with "Low" GI absorption are expected to encounter barriers when crossing the intestinal membrane, resulting in only a small fraction of the compound being absorbed. This leads to reduced oral bioavailability, which can ultimately diminish therapeutic effectiveness because the concentration of the drug reaching its molecular target is lower (Miyazaki et al., 2023).

***Blood Brain Barrier* (BBB)**

Interpretation of BBB parameters depends on the therapeutic target. A BBB Yes interpretation result is expected if the drug target is in the central nervous system (eg Alzheimer's, Parkinson's, epilepsy). Meanwhile, BBB No interpretation results are more desirable if the target is in peripheral tissue because it can reduce the risk of neurological side effects. The research results show that most compounds are predicted not to be able to penetrate the BBB, only a few compounds such as Ferulic acid derivative, p-Hydroxybenzoic acid, Aurantiamide benzoate, Genistin, Trollisine, Portulene, α-Carotene, Colchicine, and Triepoxydecane are predicted to be able to cross the blood brain barrier. In this study, the target protein used is Nrf2, a transcription factor that plays a role in regulating the endogenous antioxidant system in various peripheral tissues. Therefore, the negative BBB characteristic is not a weakness, in fact it is more advantageous because it shows that the compound is thought to work peripherally without increasing exposure to the central nervous system which could potentially cause neurological side effects (Pardridge, 2021).

P-glycoprotein Substrate (P-gp S)

Evaluation of the status of P-glycoprotein (P-gp) shows that the majority of compounds are not substrates of this transporter. This condition indicates that the compound is not expected to be easily pumped out of intestinal epithelial cells so that the chances of intracellular absorption and retention are better. On the other hand, several compounds such as Catechin, Epicatechin, Oleracein U, Sinapic acid-O-hexoside, Naringenin, Dopamine, Apigenin, Portuloside B, Oleracein D, and n-Decanoic acid are predicted to be P-gp substrates and therefore have the potential to undergo an efflux process which can reduce the amount of compounds reaching systemic circulation. However, status as a P-gp substrate does not always eliminate the biological activity of a compound, but needs to be considered at the optimization stage of drug candidates because it can affect oral bioavailability. The best results from the P-glycoprotein parameter are efflux transporters that remove the drug from the cell so that it can reduce the absorption and concentration of the drug in the target tissue. The result P-gp = No indicates a more desirable condition because the compound is not easily pumped out of the cell, while the result P-gp = Yes indicates the possibility of the compound experiencing efflux so that bioavailability can decrease. In this study, most of the compounds were not P-gp substrates, so they had a better chance of cellular retention (M. L. Amin, 2021).

Enzim Cytochrome P450 (CYP) Inhibitor

Cytochrome P450 (CYP450) enzymes constitute a group of phase I metabolic enzymes that play a crucial role in the biotransformation of drugs, endogenous compounds, and xenobiotics, particularly in the liver and intestines. More than 70–80% of clinically used drugs are metabolized by five major isoenzymes: CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Consequently, evaluating a compound's potential as a CYP inhibitor is a vital step in drug development, as it allows for the prediction of potential drug-drug interactions and alterations in the compound's pharmacokinetic profile. In SwissADME predictions, a "Yes" indicates that a compound is predicted to inhibit a specific CYP isoenzyme, whereas

"No" indicates that it does not. In the development of drug candidates, the most desirable outcome is the lack of inhibition across all major CYP isoenzymes, as this suggests a lower risk of drug interactions, more stable metabolism, and a superior safety profile. Conversely, compounds that inhibit one or more CYP isoenzymes can slow the metabolism of other drugs sharing the same enzymatic pathway, thereby increasing plasma drug levels and potentially leading to toxicity (Foti, 2023).

Research findings indicate that the majority of active compounds in *Portulaca oleracea*—such as ferulic acid derivatives, catechin, epicatechin, protocatechuic acid, caffeic acid, vanillic acid, benzoic acid, gallic acid, gentisic acid, taraxerol, friedelane, α-tocopherol, quinic acid, palmitic acid, and various other phenolic compounds—are predicted not to inhibit the CYP450 isoenzymes. These results demonstrate that the majority of active compounds in purslane possess a favorable metabolic profile, as the likelihood of drug interactions occurring via CYP pathways is relatively low. Consequently, if developed as oral drug candidates, these compounds are expected to pose a lower risk of interfering with the metabolism of other drugs or accumulating in the body due to the inhibition of hepatic metabolism (Foti, 2023).

The CYP1A2 isoenzyme is predicted to be inhibited by Sinapic acid-O-hexoside, Naringenin, Scopoletin, Apigenin, Genistein, Myricetin, Genistin, Oleracone C, Oleracein E, Colchicine, Stearic acid, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), Lonchocarpic acid, Triepoxydecane, and N-cis-Feruloyltyramine. CYP1A2 is an enzyme responsible for metabolizing various drugs, including theophylline, caffeine, and certain antidepressants. If a compound inhibits CYP1A2, the metabolic rate of the enzyme's substrates may decrease, leading to elevated plasma drug concentrations. Although CYP1A2 inhibition is not always detrimental, this factor must be considered when the compound is used concomitantly with drugs that have a narrow therapeutic index (Dai et al., 2024).

Regarding CYP2C19, only a few compounds were predicted to act as inhibitors: Sinapic acid-O-hexoside, Aurantiamide benzoate, Apigenin, Genistein, Oleracone C, Colchicine, Lonchocarpic acid, Triepoxydecane, and N-cis-Feruloyltyramine. The CYP2C19 isoenzyme plays a role in the metabolism of various drugs, such as omeprazole, diazepam, and clopidogrel. Inhibition of this enzyme has the potential to increase the levels of drugs metabolized via this pathway or reduce the activation of certain prodrugs, thereby potentially affecting therapeutic efficacy. Consequently, compounds that inhibit CYP2C19 require further evaluation in both *in vitro* and *in vivo* settings (Foti, 2023).

Prediction results also indicated that CYP2C9 is inhibited only by Aurantiamide benzoate, Aurantiamide acetate, n-Decanoic acid, and Lonchocarpic acid. CYP2C9 is a key enzyme involved in metabolizing drugs such as warfarin, phenytoin, and several non-steroidal anti-inflammatory drugs (NSAIDs). Therefore, compounds that inhibit CYP2C9 could potentially increase the concentrations of these drugs when co-administered, thereby raising the risk of adverse effects (Mokhosoev et al., 2024).

The compounds tested in this study were predicted not to inhibit CYP2D6. This is a highly favorable result, given that CYP2D6 is responsible for metabolizing approximately 20–25% of clinically used drugs, including beta-blockers, opioids, and antidepressants. The absence of potential CYP2D6 inhibition indicates that the active compounds in purslane are less likely to cause pharmacokinetic interactions via this enzyme pathway (Mokhosoev et al., 2024). Meanwhile, regarding CYP3A4—the most dominant isoenzyme in human drug metabolism—only Aurantiamide benzoate, Aurantiamide acetate, Oleracone C, Colchicine, N-cis-Feruloyloctopamine, N-trans-Feruloyloctopamine, and N-cis-Feruloyltyramine were predicted to act as inhibitors. CYP3A4 is responsible for metabolizing nearly 50% of marketed drugs; consequently, inhibition of this enzyme is a primary concern in drug development. Compounds that inhibit CYP3A4 can increase systemic exposure to other

drugs, thereby raising the risk of toxicity or adverse effects. Therefore, drug candidates that do not inhibit CYP3A4 are generally prioritized, as they carry a lower risk of causing drug interactions (Mokhosoev et al., 2024).

The final parameter evaluated was the bioavailability score, which indicates the probability of a compound reaching systemic circulation following oral administration. The highest bioavailability score in this study was 0.85, observed for the ferulic acid derivative, p-hydroxybenzoic acid, myricetin, genistin, trollisine, n-decanoic acid, eicosapentaenoic acid, docosahexaenoic acid, and oxalic acid; this value suggests a high likelihood of good oral bioavailability. Most other compounds exhibited scores of 0.55–0.56—still considered favorable—whereas complex glycosides such as Oleracein A–D, chlorogenic acid, neochlorogenic acid, and various flavonoid glycosides yielded scores of only 0.11–0.17. These low bioavailability scores are likely attributable to high molecular polarity and large molecular weight, which impede the compounds' ability to traverse biological membranes (Onoue, 2024).

Overall, pharmacokinetic predictions indicate that the ferulic acid derivative possesses a profile most closely resembling that of an ideal oral drug candidate, as it meets nearly all desired pharmacokinetic criteria: high GI absorption, lack of P-glycoprotein substrate activity, no inhibition of CYP450 isoenzymes, and the highest bioavailability score (0.85). Additionally, compounds such as protocatechuic acid, α-tocopherol, taraxerol, friedelane, quinic acid, gentisic acid, gallic acid, and caffeic acid demonstrated favorable pharmacokinetic profiles, characterized by high gastrointestinal absorption, a lack of CYP450 enzyme inhibition, non-substrate status for P-gp, and adequate bioavailability. Conversely, glycoside compounds—particularly the Oleracein group and flavonoid glycosides—exhibited pharmacokinetic limitations due to poor oral absorption and lower bioavailability. Nevertheless, these compounds retain potential for development through chemical structure modification or drug delivery systems aimed at enhancing their pharmacokinetic characteristics.

Toxicity

Based on toxicity prediction results using Toxtree v3.1.0, the majority of active compounds found in purslane (*Portulaca oleracea* L.) exhibit a relatively favorable safety profile, although some compounds warrant further attention. The toxicity parameters analyzed included Cramer Rules, carcinogenicity prediction (genotoxic and non-genotoxic), and mutagenicity based on the Ames test (*Salmonella typhimurium*). The combination of these three parameters provides an initial overview of the safety of these compounds as drug candidates prior to *in vitro* or *in vivo* testing. Although *in silico* methods cannot replace experimental testing, this approach is widely used in the early stages of drug discovery due to its ability to rapidly and cost-effectively identify potential toxicity risks, as well as its good correlation with biological data. Recent studies also indicate that integrating *in silico*-based toxicity predictions can enhance the efficiency of the candidate compound selection process and reduce failure rates during the preclinical stage.

Classification results based on Cramer Rules indicate that the majority of compounds—such as Oleracein A–E, Oleracein U, Catechin, Epicatechin, Apigenin, Genistein, Hesperidin, Scopoletin, Aurantiamide benzoate, Aurantiamide acetate, Quercetin-O-hexoside, Kaempferol-O-hexoside, and various other flavonoid glycosides—fall into Cramer Class III. Compounds such as ferulic acid derivatives, protocatechuic acid, caffeic acid, gallic acid, taraxerol, friedelane, lupeol, palmitic acid, stearic acid, and eicosapentaenoic acid belong to Cramer Class I. Meanwhile, chlorogenic acid, neochlorogenic acid, α-tocopherol, α-carotene, oxalic acid, and feruloyl glucose are categorized as Class II. Under the Cramer Rules framework, Class I represents simple chemical structures with relatively high toxic exposure thresholds, thereby implying the

lowest risk of toxicity. Class II describes compounds with moderate toxicity, whereas Class III indicates more complex chemical structures whose safety cannot be confirmed based solely on molecular structure, thus requiring further toxicological evaluation. Consequently, the Class III classification implies potential toxicity but primarily reflects uncertainty, necessitating further biological testing to ensure safety for use (Bueso-Bordils et al., 2024).

Analysis of carcinogenicity parameters yielded favorable results, as the majority of the compounds were predicted to be negative for both genotoxic and non-genotoxic carcinogenicity. These results were observed in compounds with high biological activity, such as Catechin, Epicatechin, Naringenin, Genistein, Apigenin, Aurantiamide benzoate, Oleracein A–E, Taraxerol, Friedelane, Hesperidin, α-Tocopherol, and various other phenolic compounds. A negative prediction indicates that the molecular structures of these compounds lack chemical fragments (structural alerts) generally associated with cancer-forming mechanisms involving DNA damage or epigenetic processes. These findings suggest that most purslane secondary metabolites hold promise as phytopharmaceutical candidates, as they show no preliminary indications of carcinogenicity based on chemical structure analysis. Nevertheless, a few compounds exhibited structural alerts associated with carcinogenicity. Caffeoylglucaric acid and Citric acid were predicted to possess a structural alert for non-genotoxic carcinogenicity, whereas Sinapic acid-O-hexoside, Scopoletin, Adenosine, Lonchocarpic acid, Triepoxydecane, and Portuloside A showed structural alerts for genotoxic carcinogenicity. The presence of a structural alert does not definitively classify a compound as carcinogenic; rather, it indicates the existence of specific functional groups that have been linked to genotoxic activity in other compounds. Consequently, these predictions serve as a screening tool to prioritize further toxicity testing. Compounds possessing structural alerts may still be developed as drug candidates if biological assays demonstrate the absence of genotoxic effects at therapeutic concentrations (Bueso-Bordils et al., 2024).

Ames test predictions indicate that the majority of the compounds—specifically Oleracein C, Catechin, Epicatechin, Protocatechuic acid, Naringenin, Aurantiamide benzoate, Genistein, Apigenin, Taraxerol, Friedelane, Hesperidin, α-Tocopherol, a ferulic acid derivative, Caffeic acid, Gallic acid, and most other metabolites—fall under the "No Alert for *Salmonella typhimurium* Mutagenicity" category. These predictions suggest that the compounds lack structural fragments typically associated with gene mutations in *Salmonella typhimurium* bacteria. In drug development, a negative Ames test result serves as a crucial early indicator, suggesting a low likelihood that the compound causes genetic material alterations that could trigger carcinogenesis. Consequently, the prevalence of "No Alert" results in this study indicates that most of the active compounds found in purslane possess a favorable mutagenicity profile for consideration as natural product-based drug candidates (J. Zhang et al., 2025).

Top-Performing Compound

Based on molecular docking results, Taraxerol demonstrates significant potential as an Nrf2 enzyme activator, exhibiting a binding affinity of −7.0 kcal/mol. This value indicates that the formation of the complex between Taraxerol and the Nrf2 receptor is thermodynamically favorable; a more negative binding free energy (ΔG) corresponds to greater stability in the resulting ligand-protein complex. This stability suggests that Taraxerol is capable of strong interaction within the Nrf2 protein's binding pocket, thereby potentially influencing the protein's biological activity. Furthermore, the RMSD value of 1.327 Å falls below the docking validity threshold (<2.0 Å), indicating a stable ligand binding orientation and demonstrating the reliability of the docking method (Shivanika et al., 2022).

Analysis of amino acid residue interactions reveals that Taraxerol interacts with residues SER A:508 and GLY A:462. Residue SER A:508 is a polar residue that potentially contributes to ligand stabilization through hydrogen bonding, while GLY A:462, with its small side chain, facilitates the spatial fit of Teraxerol within the binding pocket. The involvement of these two residues indicates that Teraxerol can occupy the relevant binding region on the target, a finding supported by a binding affinity of −7.0 kcal/mol and an RMSD of 1.327 Å. Theoretically, these interactions may contribute to the stability of the ligand-protein complex and potentially influence the Nrf2-Keap1 interaction—which governs Nrf2-Keap1 stability—although the mechanism of Nrf2-Keap1 activation requires further validation through molecular dynamics simulations and biological assays (Hassanein et al., 2024).

Physicochemical evaluation reveals that the compound taraxerol has a molecular weight of 426.729 g/mol, thereby satisfying Lipinski's Rule of Five, which stipulates a molecular weight of less than 500 g/mol. Furthermore, the counts of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD)—both equal to 1—fall within the recommended ranges. These low HBA and HBD counts indicate that the molecule lacks excessive polarity, a trait that supports biological membrane permeability via passive diffusion. Collectively, these parameters suggest that taraxerol's structural characteristics favor its development as an oral drug candidate (Alzain et al., 2023). A LogP value of 8.1689 indicates very high lipophilicity, exceeding the Lipinski threshold (LogP ≤ 5). This high LogP value signifies that the molecule is more soluble in the lipid phase than in the aqueous phase. While high lipophilicity can enhance a molecule's ability to penetrate cell membranes, it may simultaneously reduce effective solubility in biological fluids, increase plasma protein binding, and lead to accumulation in adipose tissue, potentially affecting drug distribution and elimination.

ADME predictions indicate that taraxerol falls into the "High" category for gastrointestinal (GI) absorption, suggesting the compound is likely to be well-absorbed through the gastrointestinal mucosa following oral administration. These results demonstrate that, despite its high lipophilicity, taraxerol's molecular characteristics remain conducive to intestinal absorption. Additionally, the prediction of "BBB permeant = No" indicates that taraxerol does not readily cross the blood-brain barrier; consequently, the risk of central nervous system side effects is relatively low when the drug targets peripheral tissues. This profile is advantageous, as it can enhance the selectivity of drug distribution (Komura et al., 2023).

Other pharmacokinetic parameters indicate that teraxerol is not a substrate for P-glycoprotein (P-gp). This suggests that the compound is not readily effluxed from the cell by P-gp transporters, thereby increasing the likelihood of maintaining intracellular concentrations. Furthermore, predictive results show that teraxerol does not inhibit the enzymes CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4. These findings suggest a low risk of drug-drug interactions resulting from cytochrome P450 enzyme inhibition. This characteristic is crucial in drug candidate development, as it minimizes the potential for altered metabolism of co-administered drugs (Tripathi et al., 2024).

A bioavailability score of 0.55 indicates that teraxerol has a favorable probability of reaching systemic circulation following oral administration. This value suggests that, despite a violation of the LogP parameter, the molecule overall remains a promising candidate for drug development. Consequently, formulation optimization—such as the use of delivery systems based on nanoparticles, liposomes, or inclusion complexes—could serve as a strategy to enhance bioavailability without altering the compound's core chemical structure (Alzain et al., 2023).

Based on toxicity predictions, teraxerol falls into Cramer Class I (Low Toxicity), indicating a predicted low risk of oral toxicity associated with its molecular structure. Additionally, predictions for both genotoxic and non-genotoxic carcinogenicity yielded negative results, showing no indication that the compound possesses carcinogenic potential via either genotoxic or non-genotoxic mechanisms. In vitro mutagenicity results also showed no alert for *Salmonella typhimurium* mutagenicity, indicating an absence of mutagenic potential based on a computational Ames test approach. This favorable toxicity profile supports the preliminary safety of teraxerol as a bioactive compound candidate (Tripathi et al., 2024).

Overall, the results from docking, drug-likeness evaluation, ADME prediction, and toxicity assessment indicate that Teraxerol is the most promising candidate for modulating the Nrf2 pathway. This compound exhibits strong binding affinity, a valid docking conformation, good oral absorption, adequate bioavailability, and a low toxicity profile, while also failing to inhibit major CYP enzymes. Its only significant limitation is a LogP value exceeding Lipinski's limit, which could potentially affect its pharmacokinetic properties.

CONCLUSION

1. In silico research on the potential of active compounds from purslane (*Portulaca oleracea* L.) as activators of the Nrf2-Keap1 antioxidant pathway demonstrated the successful execution of molecular docking using the Nrf2 protein structure (PDB code 8XGK). This structure features high crystal resolution (1.32 Å), ensuring a high level of accuracy in predicting ligand-receptor interactions. Method validation via redocking of the native ligand A1LVB yielded an RMSD value of 1.739 Å, indicating that the docking method met validation criteria and reliably reproduced the ligand's position within the protein's active site.
2. Molecular docking results for 70 active compounds from purslane revealed varying binding affinities for the Nrf2-Keap1 protein. Several compounds—including taraxerol, aurantiamide benzoate, lonchocarpic acid, hesperidin, and quercetin-O—exhibited lower binding free energy (ΔG) values than the native ligand, suggesting the formation of stable protein-ligand complexes. Beyond binding free energy, complex stability was also determined by interaction patterns with key residues in the protein's active site—specifically ARG415, TYR334, TYR525, and ALA556—involving a combination of hydrogen bonds, hydrophobic interactions, van der Waals forces, and π-interactions that contributed to enhanced binding affinity.
3. Evaluations of drug-likeness and pharmacokinetic profiles (ADME) indicated that most phenolic acid and flavonoid aglycone compounds satisfied Lipinski's Rule of Five parameters, predicting superior pharmacokinetic characteristics compared to glycoside compounds. Furthermore, the majority of these compounds showed predicted high gastrointestinal absorption, were not P-glycoprotein substrates, and did not inhibit major cytochrome P450 isoenzymes, suggesting potential for good oral bioavailability and a relatively low risk of drug interactions. In contrast, glycoside compounds exhibit limitations regarding oral absorption and bioavailability due to their high molecular weight and polarity.
4. Toxicity predictions using Toxtree v3.1.0 indicate that most active compounds from *Portulaca oleracea* (purslane) possess a favorable preliminary safety profile. The majority of the compounds are predicted to be neither mutagenic nor carcinogenic—based on Ames test parameters and predictions regarding genotoxic and non-genotoxic

carcinogenicity—although some fall into Cramer Class III, necessitating further toxicological evaluation.

5. Based on the results of molecular docking, amino acid residue interaction analysis, drug-likeness evaluation, and ADME and toxicity predictions, taraxerol emerges as the most promising candidate for modulating the Keap1-Nrf2 antioxidant pathway. This compound demonstrates the best binding affinity (−7.0 kcal/mol), a valid RMSD value (1.327 Å), a favorable pharmacokinetic profile, and low toxicity. Although its LogP value exceeds Lipinski's limits, taraxerol remains a viable candidate for further development as an antioxidant agent derived from *Portulaca oleracea* L., subject to formulation optimization and subsequent *in vitro* and *in vivo* testing.

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