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Original Article

In-Silico Study of Bioactive Compounds from *Moringa oleifera* Fruit as Anti Premature Senescence Agents in Cardiac Cells: A Study on the p53 Protein

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ABSTRACT

Background: Cellular senescence, characterized by irreversible cell cycle arrest, contributes significantly to the pathogenesis of cardiovascular diseases through mechanisms involving oxidative stress and activation of p53-mediated signaling. *Moringa oleifera*, widely recognized for its antioxidant properties, has demonstrated anti-aging effects; however, the specific bioactive compounds within its fruit and their mechanisms of action remain poorly understood. **Objective:** This study aimed to investigate the potential of *M. oleifera* fruit-derived compounds as anti-premature senescence agents targeting the p53 protein using in-silico molecular docking approaches. **Methods:** Bioactive compounds from *M. oleifera* fruit were screened via molecular docking against the human p53 protein, with Nutlin-3 used as a positive control. Binding affinities, hydrogen bonding, and hydrophobic interactions were analyzed to determine ligand–receptor interactions. **Results:** Niacin and oxalic acid exhibited stronger binding affinities (–5.90 and –6.00 kcal/mol, respectively) compared to Nutlin-3 (–5.64 kcal/mol). Niacin formed stable hydrogen bonds and hydrophobic interactions with key residues within the p53 active site, suggesting a capacity to modulate p53 activity. Oxalic acid demonstrated the highest binding affinity but lacked hydrogen bonding, indicating potential instability despite strong interaction. These findings support previous studies highlighting *M. oleifera*'s role in ROS suppression and p53 modulation, pointing to its therapeutic relevance in mitigating cellular aging. **Conclusion:** Niacin and oxalic acid from *M. oleifera* exhibit promising binding characteristics as modulators of the p53 pathway. Their anti-senescence potential warrants further validation through molecular dynamics simulations and biological assays. This study supports the development of natural compound-based therapeutics for age-related cardiac degeneration.

Keywords: *Moringa oleifera*, cellular senescence, p53 protein, in silico, molecular docking, antioxidant

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INTRODUCTION

Cellular senescence is a condition marked by irreversible cell cycle arrest, which can be triggered by various stressors, including biological aging, DNA damage, and elevated levels of reactive oxygen species (ROS) (Davalli et al. 2016). Senescence in cardiac cells has been linked to a variety of cardiovascular diseases (CVD), such as atherosclerosis (Wang & Bennett, 2012), valvular heart disease, cardiomyopathy, and arrhythmia. The initiation and maintenance of senescence in heart tissue contribute to the severity and progression of cardiac disorders[1].

Cellular senescence is a heterogeneous phenotype that depends on cell type and biological context and is defined by the irreversible loss of proliferative capacity[2]. It can be further categorized into several subtypes, including replicative senescence, oncogene-induced senescence, and stress-induced premature senescence[3]. Senescence is fundamentally different from quiescence, an adaptive response to nutrient signaling changes that leads to reversible cell cycle arrest (Marescal & Cheeseman, 2020). A hallmark of senescence is permanent cell cycle arrest in the G1/S or G2 phase, causing cells to exit the proliferative pool definitively[2].

The heart is a complex organ with four chambers, each with distinct morphology and function. Cardiac function declines with age, even in the absence of heart disease, due to the heart's inherent limitations in regenerative capacity[4]. During aging, cardiomyocytes undergo phenotypic changes resembling senescent cells, ultimately resulting in age-related myocardial dysfunction[5]. Aging myocardium experiences structural changes such as progressive cardiomyocyte hypertrophy, interstitial fibrosis, and inflammation, contributing to both diastolic and systolic dysfunction. The heart's limited regenerative ability makes elderly individuals more vulnerable to cardiac disorders[6].

Senescent cells in the heart and other tissues exhibit key markers indicative of their status. These cells typically show activation of the p53, p21, or p16-retinoblastoma (Rb) protein pathways, along with markers associated with DNA damage response (DDR), such as phosphorylated p38 MAPK and histone γH2AX[7]. The p53 and p16INK4a–RB pathways are central to the induction of cellular senescence[8]. Ataxia telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR) kinases increase p53 levels through phosphorylation and destabilization of murine double minute 2 (MDM2), an E3 ubiquitin ligase that targets p53 for proteasomal degradation (Meek, 2009).Additionally, ATM and ATR can activate p53 directly or indirectly through checkpoint kinases (CHKs) that phosphorylate p53. As a transcription factor, p53 induces the expression of CDK inhibitor p21WAF1/CIP1, which halts the

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cell cycle, allowing time for DNA repair. Persistent DNA damage signals, however, lead to sustained DDR and induction of p16INK4a expression. p16INK4a inhibits CDK4/6 activity, resulting in decreased phosphorylation of the RB protein. Hypophosphorylated RB binds to and inhibits E2F transcription factors, which are involved in DNA replication and cell cycle progression. Thus, the p16INK4a–RB pathway enforces stable cell cycle arrest[10]. Several epigenetic and transcriptional regulators are involved in modulating p16INK4a expression[11].

Moringa oleifera is widely consumed in Indonesia due to its affordability, accessibility, and perceived health benefits. Its leaves are rich in phenolic compounds such as alkaloids, tannins, saponins, and flavonoids, which exhibit antioxidant and anti-aging properties[12]. The plant has garnered increasing attention for its anti-aging potential. *M. oleifera*leaf extracts have shown anti-aging activity by enhancing oxidative stress resistance and nutrient-sensing signaling pathways[13], extending lifespan, and increasing stress tolerance in *Caenorhabditis elegans* models[14]. Furthermore, *M. oleifera* leaf extracts significantly reduced age-related neurodegeneration in treated aged rats[15], and topical creams based on *M. oleifera* leaves have been shown to restore skin vitality and reduce signs of skin aging[16].

However, the specific bioactive components. in *M. oleifera* fruit that contribute to its anti-aging effects remain unclear. Therefore, identifying and characterizing the key compounds responsible for these effects is crucial. A comprehensive understanding of how cellular senescence influences cardiovascular disease pathogenesis is essential for developing effective therapeutic strategies to counteract the detrimental impacts of aging and exploring potential therapeutic approaches targeting cellular senescence.

METHODS

Ligand Preparation (Virtual Screening)

The selection of bioactive compounds from *Moringa oleifera* fruit was performed using the Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/). The chemical structures and canonical SMILES of each active compound were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). PyRx v0.8 [17], a structure-based virtual screening tool that incorporates the Open Babel module for ligand processing and utilizes AutoDock Vina 1.12 [18]for molecular docking, was used in this study. The 3D structures of the ligands were loaded into PyRx, and energy minimization was performed using the conjugate gradient algorithm with the Universal Force Field (UFF). The minimized structures were subsequently converted into the PDBQT format. To evaluate the drug-likeness of the ligands, Lipinski's Rule of Five (Ro5) was assessed using the SWISS ADME web tool (http://www.swissadme.ch/). Lipinski's Ro5 consists of a set of criteria used to determine the feasibility of a compound as an orally active drug candidate based on its physicochemical properties (Lipinski et al., 2001).

Protein Preparation

The p53 protein with PDB ID **5ZXF** was utilized in this study [20]. This protein includes **chain A,** representing human p53, and comprises 87 amino acids derived from Homo sapiens. The overall crystal structure resolution is **1.25 Å**. Prior to docking, the native ligand molecules, non-interacting ions, and water molecules were removed using **BIOVIA Discovery Studio Client 2020** , to ensure accurate docking results.

Molecular Docking of Bioactive Compounds with Protein Target

Molecular docking was carried out using **PyRx 0.8**. Grid box coordinates were defined and adjusted as needed for the docking process. The software then computed and scored the docking interactions between ligands and the macromolecule. The purpose of docking was to determine the optimal binding between the protein (p53) and the ligand (bioactive compounds) using the **Vina Wizard** module in PyRx. Key parameters obtained from docking included **binding affinity** and **RMSD** values. The **Root Mean Square Deviation (RMSD)** is a crucial parameter used to measure the deviation of ligand atomic positions in docking predictions compared to a reference conformation. This value assists in assessing the accuracy and consistency of the predicted docking poses. A docking pose with the **lowest (most negative) binding energy** and **RMSD < 2 Å** is considered optimal[21].

Visualization and Docking Analysis

Docking visualization was performed to assess whether the ligand successfully binds to the protein, to identify the specific amino acid residues involved, and to determine the types of chemical interactions formed between the ligand and the protein. This analysis was conducted usi**ng** BIOVIA Discovery Studio (Figure 1**).**

Drug-Likeness and Toxicity Prediction

This step aimed to assess the oral bioavailability potential of the ligands based on Lipinski's Rule of Five. Furthermore, the toxicity profiles of the bioactive compounds were predicted using the Pharmacokinetics Properties platform (*https://biosig.lab.uq.edu.au/pkcsm/*).

RESULT AND DISCUSSION

Ligand Screening and Optimization

The bioactive compounds (ligands) derived from *Moringa oleifera* fruit were analyzed using the **SWISS ADME** online platform. This analysis aimed to evaluate the oral drug-likeness potential of each compound based on **Lipinski's Rule of Five** parameters. The results are presented in Table 1.

As shown in Table 1, all bioactive compounds demonstrated zero violations of Lipinski's Rule of Five, indicating that they meet the basic criteria to be considered as potential oral drug candidates. Ascorbic acid (vitamin C) has a LogP of 4.22 (within the acceptable range), 4 hydrogen donors, and 6 hydrogen acceptors. It is widely known for its strong antioxidant activity and good water solubility. Choline has a LogP of -1.86 (hydrophilic) and a very low molecular weight (104.17 g/mol), suggesting rapid absorption potential. It plays an essential role in nerve function. Indole-3-acetic acid and indole-3-acetonitrile are two low-molecular-weight compounds with ideal H-donor/acceptor profiles. These indole derivatives are reported to exhibit various biological activities, including anticancer and anti-inflammatory effects [22]. Niacin (Vitamin B3), with a low molecular weight and a LogP of 0.32, shows strong oral bioavailability potential. It is well known for its role in reducing cholesterol and preventing cardiovascular disease [23]. Oxalic acid has a negative LogP (-0.79) and appropriate hydrogen bonding characteristics; however, its high hydrophilicity may limit its membrane permeability. Riboflavin (Vitamin B2), despite its relatively high molecular weight (376.36 g/mol), does not violate Lipinski's rules. However, due to the presence of multiple polar groups, it may have low oral bioavailability and could require specific transporters for absorption [24]. Thiamine (Vitamin B1) possesses good water solubility, a moderate molecular weight, and a balanced hydrogen bond distribution, making it a favorable candidate. Pyridoxine (Vitamin B6) has only 1 hydrogen donor and 6 hydrogen acceptors, an ideal configuration for a potential drug molecule.

According to Lipinski's Rule of Five, an ideal compound for oral absorption should have a molecular weight (MW) of less than 500 g/mol, a LogP value (a measure of lipophilicity) less than 5, hydrogen bond donors (H-donors) (typically OH and NH groups) of five or fewer, and hydrogen bond acceptors (H-acceptors) (typically N and O atoms) of ten or fewer. The violation count refers to the number of Lipinski

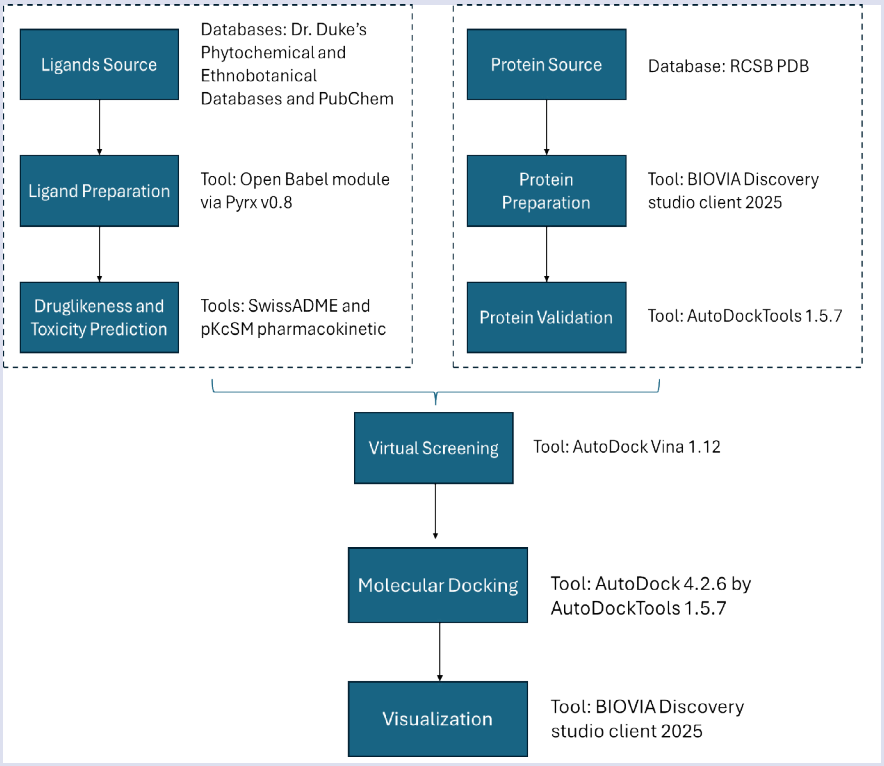


Figure 1. Workflow Chart

Table 1. Predicted physicochemical properties of bioactive compounds in *M. oleifera* fruits

Compounds	MW (g/mol)	LogP	H-donor	H-acceptor	Violation
Ascorbic Acid	176.12	4.22	4	6	0
Choline	104.17	-1.86	1	1	0
Indole-3-Acetic-Acid	185.26	1.30	2	3	0
Indole-3-Acetonenitrile	156.18	0.10	1	1	0
Niacin	123.11	0.32	1	3	0
Oxalic Acid	90.03	-0.79	2	4	0
Riboflavin	376.36	-0.19	5	8	0
Thiamine	265.35	0.53	2	3	0
Pyridoxine	231.14	0.17	1	6	0

criteria not met; the fewer the violations, the better the compound's oral drug-likeness [22]. Based on these parameters, all compounds listed in Table 1 exhibit strong potential as oral drug candidates, as none of them violate any of the fundamental pharmacokinetic criteria. These compounds also include several essential vitamins and bioactive metabolites that have been extensively studied or clinically used, further supporting the validity of the findings. However, it is important to note that Lipinski's Rule primarily predicts oral bioavailability and does not encompass pharmacodynamic properties, toxicity, or specific molecular interactions. Therefore, additional assessments, including ADMET profiling and in vitro/in vivo biological assays, are necessary to confirm their therapeutic potential. Virtual screening of the nine selected compounds was conducted using AutoDock Vina integrated in PyRx. Based on binding energy values and interaction profiles, two compounds with binding affinities lower than −5.6 kcal/mol were selected for further analysis. The binding energies of all nine compounds are presented in Table 1.

Protein Preparation and Optimization

The three-dimensional structure of the p53 protein was obtained from the Protein Data Bank using PDB ID: 5ZXF. The initial visualization of the unprepared protein is presented in Figure 2A, illustrating the native 3D conformation of p53, which still includes solvent molecules such as water. The p53 protein displays a multidomain architecture, predominantly composed of β-sheet secondary structures, interspersed with several α-helical segments and loop regions connecting these structural elements. For molecular docking analysis, a rigorous protein preparation step is essential to optimize computational performance and improve the reliability of docking results. The preparation process included: removal of water molecules, elimination of non-interacting ions, and selection of chain A (auth N) as the primary domain for ligand interaction. The prepared structure of p53 is shown in Figure 2B, depicting a cleaned and simplified model comprising only the functional domain (chain A), free of non-essential components. This refined structure was then used for the docking analysis, focusing on potential active or binding sites relevant to ligand interaction.

Prior to performing molecular docking on the p53 protein, it is necessary to define the grid box parameters in AutoDock Vina. This step is essential for guiding the docking algorithm to explore the ligand-binding region, including the active site, binding pocket, and surrounding areas that may facilitate molecular interactions between bioactive compounds from *Moringa oleifera* and the p53 protein. The

grid box settings used in this study for the docking simulation are presented in Table 2.

Drug-Likeness and Toxicity Prediction

Drug-likeness prediction was conducted to evaluate the potential of each compound as an oral drug candidate based on multiple **pharmacokinetic screening rules** and **bioavailability scores**. Each rule is based on specific physicochemical parameters: **Lipinski's Rule of Five** considers molecular weight, lipophilicity (LogP), hydrogen bond donors, and hydrogen bond acceptors. **Ghose's Rule** includes criteria such as molecular weight (160–480 Da), LogP (−0.4 to 5.6), and total number of atoms (20–70). **Veber's Rule** emphasizes molecular flexibility and polarity, with criteria of ≤10 rotatable bonds and polar surface area (PSA) ≤140 Å². **Egan's Rule** uses PSA ≤132 Å² and LogP ≤5.88 to predict intestinal absorption. **Muegge's Rule** assesses drug-likeness based on molecular weight (200–600 Da) and LogP (−2 to 5). The bioavailability score reflects the probability that a compound will be orally bioavailable in humans. The prediction results are summarized in **Table 3**.

The results presented in Table 3 show that all compounds satisfied Lipinski's Rule of Five, indicating strong potential for oral bioavailability. Notably, only thiamine and pyridoxine passed all five drug-likeness filters, demonstrating an ideal pharmacokinetic profile. In contrast, riboflavin failed all rules except Lipinski, suggesting that it is unlikely to be absorbed orally without the assistance of active transport mechanisms. Niacin and oxalic acid exhibited the highest predicted bioavailability scores (0.85), despite failing three of the five rules. This implies that predicted bioavailability is not solely determined by rule-based filters, but also influenced by other factors such as small molecular weight and balanced polarity. The majority of compounds exhibited moderate bioavailability scores, ranging from 0.55 to 0.85.

Among these rules, Lipinski's Rule remains the most widely used classical approach for evaluating oral drug-likeness, as it focuses on simple parameters that reflect a compound's ability to passively diffuse across intestinal membranes[19]. Other filters such as Veber, Egan, and Ghose consider molecular flexibility, polarity, and optimal molecular size, which are important for systemic absorption and distribution [25]. For instance, although thiamine and pyridoxine have moderate predicted bioavailability scores (0.55–0.56), they passed all five filters, suggesting suitability for pharmaceutical optimization and advanced formulation development.

Bioavailability scores were predicted using the SWISS ADME platform, which estimates the probability of intestinal absorption in humans. The highest scores were observed for niacin and oxalic acid, likely due to their low molecular weight, moderate polarity, and independence from active transport. This finding is consistent with previous research by Daina et al. (2017), which emphasized that the combination of drug-likeness prediction and bioavailability scores provided by SWISS ADME offers an effective strategy for early-stage drug candidate screening[26].

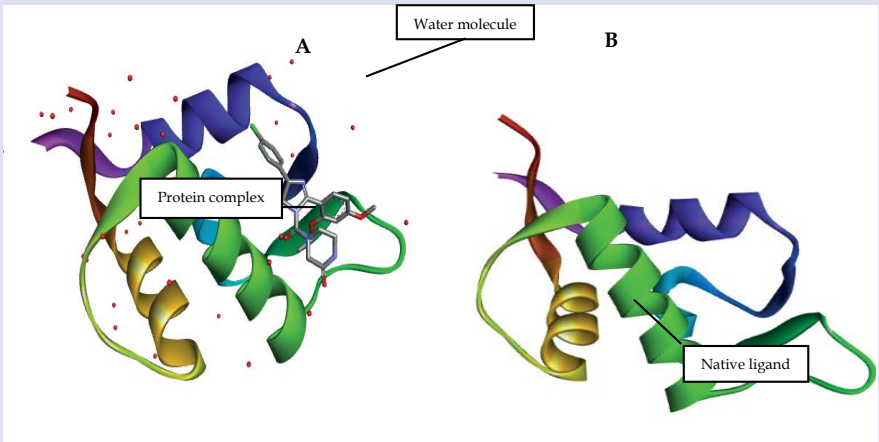


Figure 2. 3D dimensional structure of the p53 protein. (A) Unprepared structure, including water molecules and non-essential components. (B) Prepared structure after removal of water, ions, and selesction of chain A (auth N) for docking analysis.

Table 2. Gridbox parameters for p53 protein

Protein	Grid Box Dimenssion			Grid Box Center Coordinates (Å)		
	Center X	Center Y	Center X	X	Y	Z
p53	40	46	40	8,584	-19,462	-5,682

Table 3. Drug-likeness prediction of bioactive compounds from *M.oleifera* fruit

Compounds	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Ascorbic Acid	Yes	No	Yes	Yes	No	0.56
Choline	Yes	No	Yes	Yes	No	0.55
Indole-3-Acetic-Acid	Yes	Yes	Yes	Yes	No	0.55
Indole-3-Acetonenitrile	Yes	No	Yes	Yes	No	0.55
Niacin	Yes	No	Yes	Yes	No	0.85
Oxalic Acid	Yes	No	Yes	Yes	No	0.85
Riboflavin	Yes	No	No	No	No	0.55
Thiamine	Yes	Yes	Ya	Yes	Yes	0.55
Pyridoxine	Yes	Yes	Ya	Yes	Yes	0.56

Toxicity prediction was carried out to estimate the potential lethality of the selected compounds using the LD_{50} (lethal dose 50%) value, which refers to the dose required to cause death in 50% of test animals. LD_{50} values are expressed in mol/kg and subsequently converted to g/kg to reflect practical relevance. A higher LD_{50} value indicates a lower toxicity risk. The results are presented in Table 4.

According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), all tested compounds fall into **Toxicity Class 6** (LD_{50} oral > 2000 mg/kg), which denotes **"relatively non-toxic"** or **"practically non-toxic"** substances (OECD, 2001). Most compounds are natural plant-based metabolites or essential vitamins that are widely recognized as safe. For instance, **ascorbic acid (vitamin C)** exhibits a very high LD_{50} (>11 g/kg in rats), reflecting low acute toxicity even at high doses (American College of Toxicology, 2005). **Riboflavin** and **thiamine**, both water-soluble B vitamins, also have high LD_{50} values and are excreted through urine when consumed in excess, confirming their oral safety profile[27].

Interestingly, no direct correlation was observed between molecular weight and toxicity. For example, **riboflavin** has a relatively high molecular weight (376.36 g/mol) but remains safe with an LD_{50} of 816.70 g/kg. In contrast, **oxalic acid**, with a low molecular weight (90.03 g/mol), presents a lower LD_{50}, although still within the safe range. This indicates that **chemical structure and metabolic pathway** play a more significant role in toxicity than molecular weight alone. From a drug development perspective, these findings support the safety of *M. oleifera* fruit-derived compounds for oral use. The minimal predicted toxicity highlights their suitability for further development as dietary supplements or phytopharmaceutical candidates with a favorable safety margin.

Molecular Docking of Protein and Ligands

Molecular docking was initially validated by redocking the native ligand, **Nutlin-3**, into the binding site of the **p53 protein**. This validation step aimed to ensure that the docking protocol and parameters used in this study were reliable and capable of accurately reproducing the native binding pose of the ligand as observed in the crystallographic structure. The accuracy of the redocking process was evaluated based on the **Root Mean Square Deviation (RMSD)** between the docked pose and the original crystallographic position. An RMSD value of **less than 2.0 Å** indicates that the docking procedure is valid and acceptable for further docking studies. This threshold ensures that the predicted orientation and position of the ligand within the active site are sufficiently close to the experimentally determined structure. The successful validation of Nutlin-3 docking thus provides a strong foundation for the subsequent docking analysis of the selected **bioactive compounds from** *M.oleifera*. Moreover, the binding affinity obtained from the native ligand docking (−5.9 kcal/mol) was used as a benchmark for selecting candidate ligands with equal or stronger binding potential. A visual representation of the redocking result, including the superimposition of the docked and crystallographic ligand poses, is provided in **Figure 3** demonstrating the high degree of structural similarity.

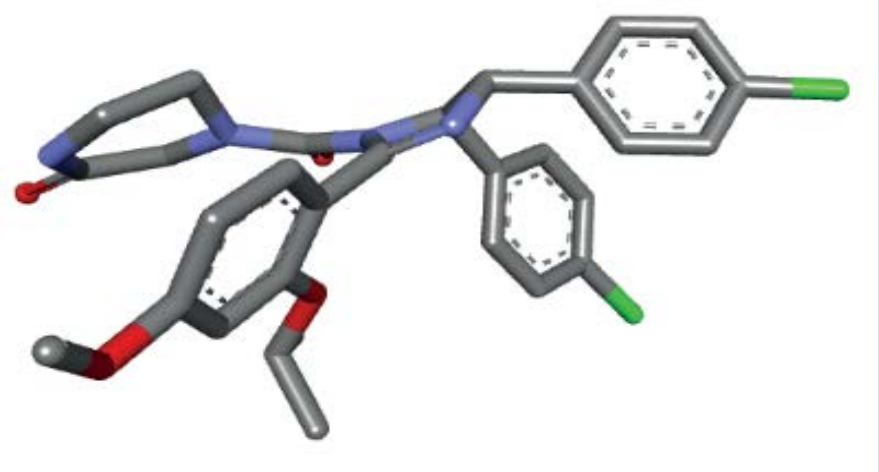


Figure 3. Native ligand of p53 protein

Table 4. Toxicity prediction of bioactive compounds from *M. oleifera*

Senyawa	Dosis Letal (LD50)		BM (g/mol)	Kelas	Klasifikasi
	mol/kg	g/kg			
Ascorbic Acid	1.063	187.22	176.12	6	Relatively non-toxic
Choline	1.939	201.99	104.17	6	Relatively non-toxic
Indole-3-Acetic-Acid	2.104	389.79	185.26	6	Relatively non-toxic
Indole-3-Acetonenitrile	2.339	365.31	156.18	6	Relatively non-toxic
Niacin	2.240	275.77	123.11	6	Relatively non-toxic
Oxalic Acid	1.680	151.25	90.03	6	Relatively non-toxic
Riboflavin	2.170	816.70	376.36	6	Relatively non-toxic
Thiamine	2.672	709.02	265.35	6	Relatively non-toxic
Pyridoxine	2.227	514.75	231.14	6	Relatively non-toxic

The results in Table 5 indicate a binding affinity of Nutlin-3 is -5.9 kcal/mol, suggesting that the interaction between the ligand and the protein is thermodynamically stable. A binding affinity of -5.9 falls within the category of moderate binding—while not extremely strong, it is biologically significant and relevant. The RMSD value reflects the spatial deviation between the redocked ligand position and the original ligand position in the crystal structure. An RMSD value of 0 indicates that the re-docked ligand precisely overlaps with its original position in the crystal structure. This result is ideal and confirms that the docking protocol employed is highly accurate and valid for analyzing the interaction of bioactive compounds from *Moringa oleifera* with the p53 protein. The docking results between p53 and Nutlin-3 validate the reliability of the method used. Therefore, the parameters applied in this docking study are considered appropriate and can be confidently used for virtual screening of other ligands. Subsequently, molecular docking analysis was conducted between the p53 protein and bioactive compounds derived from *M. oleifera*. Based on the phytochemical analysis using the Phytochem database website, nine bioactive compounds were identified, including ascorbic acid, indole-3-acetic acid, oxalic acid, indole acetonitrile, choline, niacin (vitamin B3), pyridoxine (vitamin B6), riboflavin (vitamin B2), and thiamine (vitamin B1). These nine bioactive compounds were subjected to docking analysis using the PyRx RunVina application, and the results are presented.

Based on Table 6, molecular docking analysis revealed that all nine bioactive compounds derived from *Moringa oleifera* fruit exhibited favorable binding interactions with the p53 protein, as evidenced by their negative binding affinity values ranging from -2.7 kcal/mol to -6.0 kcal/mol. These results indicate that the bioactive compounds possess a notable affinity for the p53 protein. Oxalic acid demonstrated the strongest binding affinity at -6.0 kcal/mol, followed by niacin at -5.9 kcal/mol, choline at -5.2 kcal/mol, ascorbic acid at -5.0 kcal/mol, riboflavin and thiamine at -4.2 kcal/mol, pyridoxine at -3.6 kcal/mol, indole acetonitrile at -2.9 kcal/mol, and indole-3-acetic acid at -2.7 kcal/mol. All compounds displayed perfect structural complementarity to the p53 protein's binding site, as indicated by RMSD values of 0, which reflect optimal geometric alignment during the docking process. These findings suggest that *M. oleifera* fruit contains multiple bioactive compounds with potential therapeutic applications, particularly those targeting p53-mediated biological pathways.

From the molecular docking results of the nine bioactive compounds, two compounds were identified to exhibit binding affinity values equal to or lower (i.e., more favorable) than the validated native ligand (Nutlin-3) which had a binding affinity of -5.9 kcal/mol. These two compounds were **Niacin** (-5.9 kcal/mol) and **Oxalic acid** (-6.0 kcal/mol). A binding affinity equal to or more negative than that of Nutlin-3 indicates that these compounds have comparable or even stronger binding potential to the p53 target protein. Oxalic acid, with a binding affinity of -6.0 kcal/mol, demonstrated a slightly stronger interaction, suggesting the possibility of a more stable binding within the active site of the p53 protein. Meanwhile, niacin exhibited an identical binding affinity to Nutlin-3, indicating that it may also form strong and stable interactions with p53.

Considering that Nutlin-3 is a validated native ligand known for its activity against p53, both niacin and oxalic acid merit further investigation as potential candidates for the development of anti-senescence agents targeting the p53 signaling pathway. Subsequent to the docking analysis of the p53 protein with the native ligand and selected bioactive compounds, molecular interaction visualization was carried out to identify the specific amino acid residues involved in binding. The visualization results are presented in Table 7.

Based on the molecular docking results presented in Table 8, we can interpret the binding affinity and type of interaction of the compounds with the p53 protein, a central protein in cell cycle regulation, apoptosis, and cellular aging (senescence). In this context, Nutlin-3 was used as a positive control because it is widely known to inhibit the interaction of p53 with MDM2, thereby stabilizing p53 and enhancing its tumor suppressor activity. Two other compounds tested, niacin and oxalic acid, are potential bioactive candidates from *Moringa oleifera*, a plant known to possess various bioactive compounds with antioxidant, anti-inflammatory, and antisenescence potential.

The results showed that Nutlin-3 has a binding affinity of -5.64 kcal/mol and interacts through hydrogen bonds with glutamine residues (A:38), as well as several hydrophobic and pi-pi stacking interactions with residues such as phenylalanine, isoleucine, valine, and methionine. This reflects a strong and specific interaction with the active pocket of p53, which is in accordance with the literature that Nutlin-3 mimics the p53 interaction region on MDM2, blocking p53 degradation and enhancing its activity[28]. These findings are consistent with the study conducted by Joseph TL et al in 2010, which demonstrated that Nutlin-3 directly occupies the p53-binding pocket on MDM2, thereby blocking the p53–MDM2 interaction. This aligns with the classical mechanism in which Nutlin mimics the p53-binding helix. Consequently, Nutlin-3 effectively enhances p53 activity through its specific therapeutic efficacy[29].

Niacin exhibited a lower binding affinity value (–5.90 kcal/mol), indicating a stronger affinity toward p53 compared to Nutlin-3. The hydrogen bond interaction of Niacin was observed with leucine (A:33), alongside hydrophobic interactions involving phenylalanine,

Tabel 5. Validation results of molecular docking between p53 protein and Nutlin-3 based on RMSD and binding affinity values.

Protein Target	Native Ligand	PDB Code	Binding Affinity (kcal/mol)	RMSD
P53	Nutlin-3	DB17039	-5,9	0

Table 6. Docking scores of *M. oleifera* bioactive compounds against the p53 protein. Bold are the strongest bond affinity.

Protein	Senyawa Bioaktif	Binding Affinity (kcal/mol)	RMSD
P53	Ascorbic Acid	-5,0	0
	Choline	-5,2	0
	Indole-3-Acetic-Acid	-2,7	0
	Indole-3-Acetonenitrile	-2,9	0
	Niacin	-5,9	0
	Oxalic Acid	-6,0	0
	Riboflavin	-4,2	0
	Thiamine	-4,2	0
	Pyridoxine	-3,6	0

Table 7. Visualization results of p53 protein with native ligand and bioactive compounds of *M. oleifera*

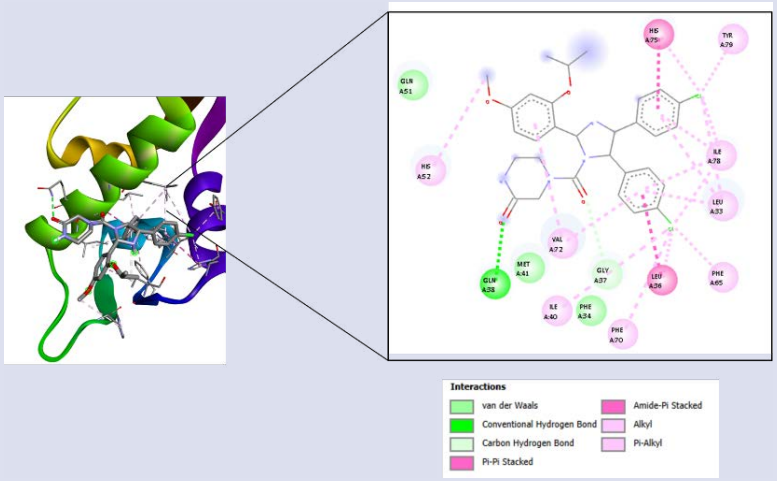
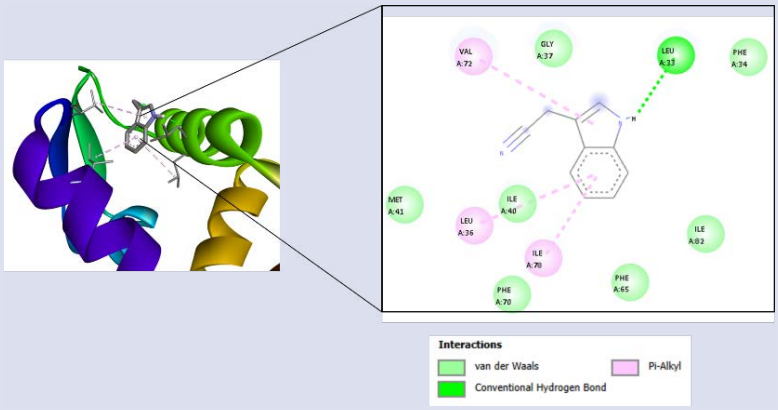
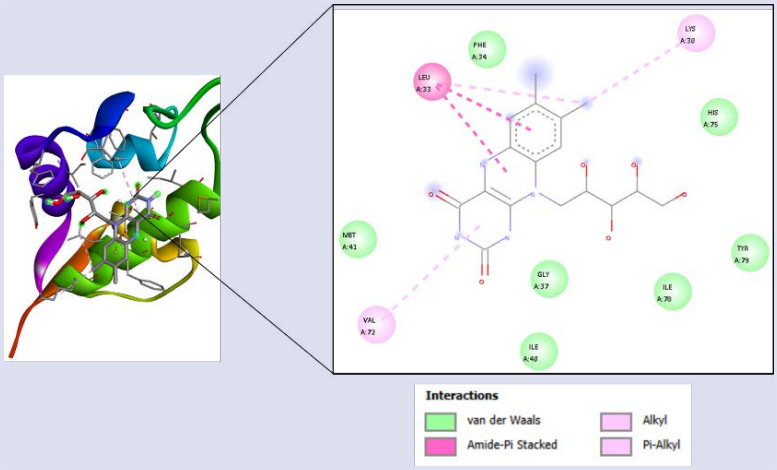
Protein	Ligand	Visualization
p53	Nutlin-3 (native ligand)	
	Niacin	
	Oxalic acid	

Table 8. Types of bonds between p53 and native ligands and bioactive compounds of *M. oleifera*

Compounds	Binding Affinity (kcal/mol)	Interactions with amino acid residues	
		Hydrogen bond interaction	Other bonding interactions
Nutlin-3	-5.64	Gln (A:38)	Gln (A:51), His (A:52), His (A:75), Tyr (A:79), Ile (A:78), Ile (A:40), Leu (A:33), Leu (A:36), Phe (A:65), Phe (A:70), Phe (A:34), Gly (A:37), Val (A:72), Met (A:41),
Niacin	-5.90	Leu (A:33)	Val (A:72), Gly (A:37), Phe (A:34), Phe (A:65),Phe (A:70), Ile (A:82), Ile (A:78), Ile (A:40), Leu (A:36), Met (A:41)
Oxalic acid	-6.0	-	Phe (A:34), Lys (A:30), His (A:75), Tyr (A:79), Ile (A:78), Gly (A:37), Ile (A:40), Val (A:72), Met (A:41), Leu (A:33)

histidine, and tyrosine residues, which contribute to the stabilization of the protein–ligand complex. This suggests the potential of niacin to modulate p53 activity indirectly, especially considering that niacin (vitamin B3) is known to enhance NAD+ metabolism—a crucial cofactor in sirtuin activity. In particular, SIRT1 plays a critical role in p53 deacetylation and the regulation of cellular senescence [30]. A study conducted by Andriyani et al. (2023) demonstrated that bioinformatics analysis revealed the active compounds in *M. oleifera* leaf extract directly affect the p53 protein within the aging pathway. Among these compounds, those derived from *M. oleifera* exhibited the strongest binding affinity to p53 compared to the native ligand. *M. oleifera* has the potential to suppress intracellular reactive oxygen species (ROS) levels and modulate p53 activity, thereby playing a role in preventing cellular aging[31]

In contrast, oxalic acid demonstrated the most favorable binding affinity (–6.0 kcal/mol), yet notably did not exhibit detectable hydrogen bonding interactions. Nonetheless, it engaged with several amino acid residues including phenylalanine, histidine, tyrosine, and methionine. This may suggest that although oxalic acid possesses strong affinity, the orientation or geometry of its interaction may lack the specificity or stability seen in Nutlin-3 or niacin. Nevertheless, its high binding affinity still implies potential bioactivity, warranting further *in vitro* or *in vivo* validation. Although oxalic acid exhibited a relatively high predicted binding affinity to p53 in the docking simulations, its potential as a therapeutic candidate is limited by several factors. The docking analysis revealed minimal or absent hydrogen-bonding interactions with critical residues within the p53 binding pocket, suggesting that the observed affinity may primarily arise from non-specific interactions, which could reduce the stability of the complex under physiological conditions. Moreover, oxalic acid is a metabolic precursor of oxalate, and excessive oxalate accumulation in the body is associated with adverse clinical outcomes, including nephrolithiasis and renal impairment[32]. These metabolic concerns, combined with the lack of strong hydrogen-bond stabilization, indicate that oxalic acid is unlikely to be a suitable lead compound despite its favorable docking score, and further in vitro or in vivo validation would be required before considering its pharmacological application.

These findings support the potential of *Moringa oleifera* compounds as candidates for anti-aging or anti-senescence therapies via modulation of the p53 regulatory pathway. Recent studies also corroborate this potential. A study by Rath et al. (2021) demonstrated that *M. oleifera* extract can induce apoptosis and inhibit cell proliferation through upregulation of p53 expression[33]. Moreover, *M. oleifera* is known to contain compounds such as quercetin, niazimicin, and phenolic acids, which can stimulate antioxidant pathways and reduce oxidative stress—key triggers of cellular senescence[34]. A similar study conducted by Abdul et al. (2019) reported that a combination of *Moringa oleifera* and *Centella asiatica* extracts was able to reduce ROS levels, 8-OHdG, and p53 expression following oxidative stress, indicating a reduction in cellular stress and senescence through the downregulation of p53[35].

Accordingly, the present study suggests that niacin and oxalic acid from *M. oleifera* possess the ability to interact with p53 and potentially modulate its activity in the context of cellular aging. Niacin, with its specific hydrogen bonding interactions and high binding affinity, appears particularly promising for further investigation, both through advanced *in silico* approaches (e.g., molecular dynamics simulations) and experimental biological assays. p53 is a central regulator of the cell cycle and apoptosis. In the context of aging, its activation can trigger senescence or apoptosis in abnormal cells, thus reducing cancer risk, though potentially accelerating tissue aging if left unchecked[36]. Previous research has shown that selective modulation of p53, such as that achieved by Nutlin-3, can promote tissue regeneration without inducing excessive oxidative stress[37]. The administration of *Moringa oleifera* extract can reduce the number of senescent cells, and bioinformatics studies have shown that the p53 protein is the most directly affected by its bioactive compounds in the senescence pathway[31]. Natural compounds such as niacin (vitamin B3) and oxalic acid—both found in *M. oleifera*—therefore present promising potential as anti- premature senescence modulators of the p53 protein and should be further investigated using methods such as **in vitro** and **in vivo** studies.

This study has several limitations inherent to in silico molecular docking approaches. First, the docking simulations were conducted in the absence of an explicit solvent model, which may limit the accuracy of binding energy estimations and fail to capture solvation effects that occur under physiological conditions. Second, the analysis was performed using a rigid protein structure, without accounting for the conformational flexibility of p53, which can influence ligand binding dynamics and affinity. Third, the docking results provide only a static snapshot of the ligand–protein interaction, without considering the temporal stability and dynamic behavior of the complex. Therefore, molecular dynamics (MD) simulations and solvent-inclusive models are recommended in future studies to validate the docking predictions and provide a more comprehensive understanding of the interaction mechanisms between *Moringa oleifera* bioactive compounds and p53.

CONCLUSION

The analysis revealed that although all compounds passed Lipinski's Rule of Five, only a few—namely thiamine and pyridoxine—fully met all drug-likeness filters. Bioavailability scores do not rely solely on compliance with these filters, but are also influenced by molecular features such as size and polarity. Compounds such as niacin and oxalic acid stood out due to their high predicted bioavailability, despite not fulfilling all classical pharmacokinetic criteria.

All bioactive compounds derived from *Moringa oleifera* fruit were classified under toxicity class 6, indicating that they are "relatively non-toxic." This further supports their potential use in drug or supplement development, particularly as most are naturally occurring vitamins that have already been proven safe for human consumption. The high LD_{50} values (>2000 mg/kg) suggest a very low risk of acute toxicity.

Out of the nine bioactive compounds evaluated, two—niacin (–5.9 kcal/mol) and oxalic acid (–6.0 kcal/mol)—were selected based on their binding affinity values, which were equal to or better than that of the native ligand Nutlin-3 (–5.9 kcal/mol). These findings indicate that both compounds may serve as alternative inhibitors of the p53 protein and are worthy of further investigation through *in vitro* and *in vivo* studies, particularly in the context of developing p53-based therapeutic strategies.

Niacin and oxalic acid exhibited stronger binding affinities than Nutlin-3, highlighting their potential as effective p53 modulators. Niacin formed stable hydrogen bonds and interacted with key residues also targeted by Nutlin-3. Oxalic acid demonstrated the strongest binding affinity but lacked hydrogen bonding, which may reduce the stability of its interaction. The anti-senescence potential of *M. oleifera* bioactive compounds is likely attributed to their capacity to modulate p53 in a mild and selective manner, thereby supporting cell regeneration and protecting DNA from damage.

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