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Original Article

Alpha-mangostin from *Garcinia mangostana* L.: A potential Nrf2 inhibitor for long COVID-19 explored through molecular dynamics

[Alfa-mangostina de *Garcinia mangostana* L.: Un posible inhibidor de Nrf2 para la COVID-19 prolongada explorado a través de la dinámica molecular]

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Abstract

Context: Long COVID, also referred to as persistent COVID-19, encompasses a heterogeneous group of individuals who continue to experience long-term symptoms after going through the acute phase of COVID-19. Long COVID is caused by poor expression of antioxidant enzymes and cytoprotective proteins, regulated by the reaction of antioxidant elements to deoxyribonucleic acid (DNA), resulting in oxidative stress. Nuclear Factor Erythroid 2 (Nrf2) and inflammasome production are recently known to play key roles in the development of long COVID-19. It is denoted that the natural antioxidant alpha-mangostin can activate Nrf2, suggesting its potential as long-term therapy for COVID-19.

Aims: To demonstrate the potential and stability of chemical interactions between alpha-mangostin and Nrf2.

Methods: This work involved various stages, including molecular docking, molecular dynamics simulation, drug-likeness prediction, pharmacokinetic profile prediction, and toxicity prediction. The target receptor Nrf2-Keap1 (PDB ID: 4L7B) was employed to be analyzed for its molecular interaction against alpha-mangostin.

Results: The free binding energy (ΔG) value of alpha-mangostin to Nrf2-Keap1 was -9.71 kcal/mol. The molecular dynamics simulation analysis on the OpenMM toolkit for 50 ns demonstrates that the alpha-mangostin maintained a stable interaction with the Nrf2-Keap1 complex.

Conclusions: This study results demonstrate the potential for alpha-mangostin to serve as a viable long-term medication following COVID-19.

Keywords: alpha-mangostin; COVID-19; Nrf2; molecular docking; molecular dynamics.

Resumen

Contexto: La COVID prolongada, también denominada COVID-19 persistente, engloba a un grupo heterogéneo de individuos que siguen experimentando síntomas a largo plazo tras pasar por la fase aguda de la COVID-19. La COVID larga está causada por una expresión deficiente de enzimas antioxidantes y proteínas citoprotectoras, regulada por la reacción de elementos antioxidantes con el ácido desoxirribonucleico (ADN), lo que provoca estrés oxidativo. Recientemente se sabe que el factor nuclear eritroide 2 (Nrf2) y la producción del inflamasoma desempeñan papeles clave en el desarrollo de la COVID-19 larga. Se denota que el antioxidante natural alfa-mangostin puede activar Nrf2, lo que sugiere su potencial como terapia a largo plazo para COVID-19.

Objetivos: Demostrar el potencial y la estabilidad de las interacciones químicas entre alfa-mangostin y Nrf2.

Métodos: Este trabajo implicó varias etapas, incluyendo el acoplamiento molecular, la simulación de dinámica molecular, la predicción de similitud de fármacos, la predicción del perfil farmacocinético y la predicción de toxicidad. Se utilizó el receptor diana Nrf2-Keap1 (PDB ID: 4L7B) para analizar su interacción molecular con la alfa-mangostina.

Resultados: El valor de la energía de unión libre (ΔG) de la alfa-mangostina con Nrf2-Keap1 fue de -9,71 kcal/mol. El análisis de simulación de dinámica molecular en el kit de herramientas OpenMM durante 50 ns demuestra que la alfa-mangostina mantuvo una interacción estable con el complejo Nrf2-Keap1.

Conclusiones: Los resultados de este estudio demuestran el potencial de la alfa-mangostina como medicamento viable a largo plazo tras la COVID-19.

Palabras Clave: alfa-mangostina; COVID-19; Nrf2; acoplamiento molecular; dinámica molecular.

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Abbreviations: ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; BBB: Blood-brain barrier; COVID-19: Coronavirus disease of 2019; CYP450: Cytochrome P450; EEL: Electrostatic energy; GI: Gastrointestinal; GPU: Graphics processing units; iLOGP: Implicit log P [n-octanol/water partition coefficient (log Po/w)]; Keap1: Kelch-like ECH-associated protein; MD: Molecular dynamic; MLOGP: Moriguchi octanol-water partition coefficient; MM-GBSA: Molecular mechanics-generalized born surface area; MMP: Matrix metalloproteinase; NF-κB: Nuclear factor *kappa* B; Nrf2: Nuclear factor erythroid 2–related factor 2; PDB: Protein Data Bank; Pgp: P-glycoprotein; ProTox: Prediction of toxicity; Rg: Radius of gyration; RMSD: Root mean squared deviation; RMSF: Root mean squared fluctuation; ROS: Reactive oxygen species; TPA: 12-O-Tetradecanoylphorbol-13-acetate; TPSA: Topological polar surface area; VdW: Van der Waals interaction; WHO: World Health Organization; WLOGP: Water partition coefficient; XLOGP: Lipophilicity scores.

INTRODUCTION

The global health crisis coronavirus disease of 2019 (COVID-19) pandemic is instigated by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus, posing a significant challenge on a global scale. Coronaviruses are enveloped RNA viruses with a single-stranded positive genome classified within the *Coronaviridae* family (Pérez-González et al., 2022). The World Health Organization (WHO) announced that there were 754,018,841 confirmed cases of COVID-19 from 216 countries and 6,817,478 deaths. While 60% of those infected recover within 28 days without hospitalization, most patients continue to experience various symptoms for an extended period of 3-12 weeks, a condition known as long COVID syndrome (WHO, 2022). One of the contributing factors to long COVID-19 is the disruption of the balance between of cytoprotective proteins and antioxidant enzymes, which can be triggered as the response of antioxidant elements to deoxyribonucleic acid (DNA) and induce oxidative stress. Recent reports elucidated that long COVID-19 can also be affected by the nuclear factor erythroid 2–related factor 2 (Nrf2) modulation, as the activation or inhibition of Nrf2 implicated in the oxidative stress and virus replication (Deramaudt et al., 2013). The transcription factor Nrf2 has been acknowledged to initiate the transcription of numerous reactive oxygen species (ROS)-scavenging enzymes (Sun et al., 2021). In a healthy cell, Nrf2 binds to the cytoplasmic repressor protein Keap-1 and is degraded by the proteasome (Verma et al., 2022). Several investigations have been instigated into the crosstalk between Nrf2 activity and potential compounds that are able to induce Nrf2 translocation and promote transcription of antioxidant-related genes.

Antioxidants are vital for the body because they can stop, slow down, or postpone the oxidation process (Warraich et al., 2020). Endogenous antioxidants serve as the body's inherent defense mechanism against oxidative damage (Mirończuk-Chodakowska et al., 2018). Exogenous antioxidants or those derived from sources other than the body, such as food and medications, are required when the free radicals and reactive species in the body surpass its inherent capacity (Ercegovac et al., 2022; Sharifi-Rad et al., 2020). Phytochemicals (flavonoids, flavones, polyphenols, and others) demonstrate antioxidant potential through numerous mechanisms, from directly scavenging ROS to increasing the expression of cellular antioxidant enzymes and preventing against oxidative stress-mediated cell damage (Hannan et al., 2020).

Studies have demonstrated that phytochemicals may interact with Nrf2 signaling to alleviate pathological conditions such as neurotoxicity and liver damage (Thiruvengadam et al., 2021). Alpha-mangostin (Fig. 1A), abundantly found in mangosteen, is known for its antioxidant activity mediated through Nrf2 mediation and inhibition of the nuclear factor *kappa* B (NF-κB) pathway. Furthermore, alpha-mangostin acts as a ROS inducer, which causes apoptosis in cancer cells, and as a ROS inhibitor which can prevent extensive cell damage, thereby endangering other tissues (Muchtaridi et al., 2024). The protective mechanism of cell damage against oxidative stress by alpha-mangostin involves the accumulation and nuclear translocation of Nrf2 along with upregulation of heme oxygenase-1 expression (Fang et al., 2016). The molecular docking studies indicate that alpha-mangostin demonstrated good interactions with Kelch-like ECH-associated protein (Keap1)-Nrf2 and may serve as an inhibitor, presenting potential benefits for long COVID-19 (Muchtaridi et al., 2022).

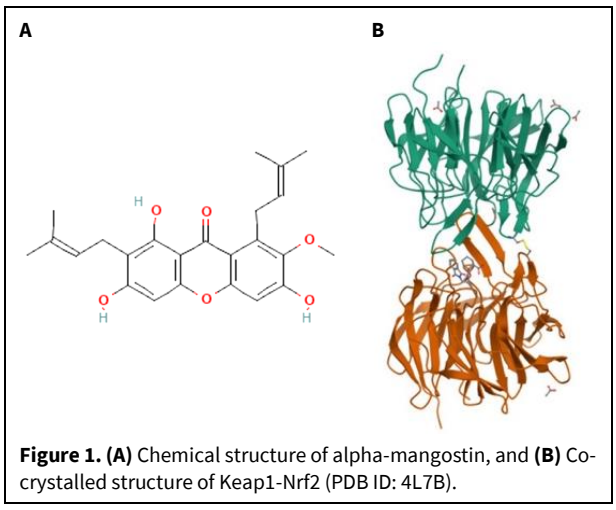


Figure 1. (A) Chemical structure of alpha-mangostin, and **(B)** Co-crystalled structure of Keap1-Nrf2 (PDB ID: 4L7B).

Previous studies have shown that alpha-mangostin can help protect the liver from autoimmune hepatitis by influencing the SIRT1/Nrf2/NF-κB signaling pathway (Shehata et al., 2022). Other research has explored how xanthone metabolites affect

the interaction between Keap1 and Nrf2 using molecular models and lab tests (Alzain et al., 2023). While earlier work has highlighted alpha-mangostin's impact on the Keap1-Nrf2 pathway, our study stands out by using molecular dynamics simulations to look at how alpha-mangostin interacts with Nrf2 over time. Unlike past studies that mainly focused on static binding assessments, we wanted to understand how the interaction changes and behaves under real-world conditions (Teguchi and Yamamoto, 2021). Our approach provides new insights into how alpha-mangostin and Nrf2 interact, including their stability, conformational changes, and key molecular interactions. This deeper understanding not only improves our knowledge of the interaction but also lays the groundwork for developing new treatments for conditions like long COVID-19. Plus, by demonstrating the value of molecular dynamics simulations, our study offers new possibilities for advancing pharmaceutical research.

MATERIAL AND METHODS

Prediction using Lipinski's Rule of Five (drug-likeness)

Lipinski's Rule of Five (drug-likeness) prediction was performed online at (http://scfbio-iitd.res.in) (Jayaram and Prajapat, 2023). The test substance was prepared by minimizing energy and stored in (mol2) format. To perform the prediction, the test compound (mol2) file was opened using Notepad, and all contents were copied, pasted on the website (http://scfbio-iitd.res.in), and submitted. The results of Lipinski's Rule of Five Prediction were then displayed based on the parameters used.

Prediction of pharmacokinetics and pharmacodynamics

In this study, the physicochemical and pharmacokinetic properties of alpha-mangostin and dimethyl fumarate were examined using Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analysis. SWISSADME, an accessible online tool (http://www.swissadme.ch/), was employed to assess the drug-like qualities of these compounds (Daina et al., 2017). The ligand structures, sourced from PubChem, were entered into SWISSADME to determine their ADMET profiles. The evaluation followed Lipinski's Rule of Five, which considers criteria such as molecular weight under 500 g/mol, LOGP <5, a topological polar surface area (TPSA) of less than 140 Å², and fewer than ten hydrogen bond acceptors and five hydrogen bond donors. Compounds demonstrating advantageous properties regarding drug-likeness, blood-brain barrier (BBB) permeability, bioavailability, gastrointestinal (GI) absorption, and synthetic accessibility were chosen for further study.

Prediction of toxicity profiles

ProTox-3.0 - Prediction of TOXicity of chemicals is an online platform used to evaluate the toxicity of selected compounds (accessible at https://tox.charite.de/protox3/) (Drwal et al., 2014). Assessing the toxicity of natural compounds is a vital step in the drug design and development process. This study considered various factors to determine toxicity, such as toxicity classification, lethal dose 50, hepatotoxicity, immunotoxicity, mutagenicity, carcinogenicity, and cytotoxicity. Based on the predicted toxicity levels, the compounds were categorized into different classes: Class I and II (fatal), Class III (potentially toxic), Class IV/V (potentially harmful), and Class VI (non-harmful).

Preparation of ligand structure (alpha-mangostin) and nuclear factor erythroid 2 (Keap1:Nrf2) macromolecule

The ligand structure was described and optimized prior to use in the molecular docking process. The two-dimensional structure of alpha-mangostin was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov). MarvinSketch was used for preparation, involving optimization stages with a minimized energy process. This process aims to stabilize and facilitate the bond arrangement that occurs during the molecular docking process, adding hydrogen atoms, and converting the structure from two dimensions to three dimensions and the protonation stage by conditioning the test compound at pH 7.4, which is adjusted to the body's pH (Cheraghi et al., 2023; Duay et al., 2023).

The three-dimensional structure of Nrf2 macromolecule was obtained from the Protein Data Bank (http://www.rcsb.org/pdb) (PDB ID: 4L7B) (Fig. 1B) with a resolution of 2.41 Å. The X-ray crystal structure of Kelch-like ECH-associated protein (Keap1) co-crystallized with (1S,2R)-2-[(1S)-1-[(1,3-dioxo-2,3-dihydro-1H-isoindol-2-yl)methyl]-1,2,3,4-tetrahydroisoquinolin-2-carbonyl]cyclohexane-1-carboxylic acid (compound (S,R,S)-1 a) was obtained from the Protein Data Bank (PDB) (PDB ID: 4L7B) (Jnoff et al., 2014). Subsequently, Kollman charges and polar hydrogen atoms were added to the pure receptor, adapting it to the docking environment to perform accurate calculations. Meanwhile, Gastaiger charges and hydrogen atoms were added to the ligand, and the torque was adjusted to optimize its suitability for the docking environment (Jnoff et al., 2014).

Molecular docking study

Molecular docking studies were carried out using MGLTools 1.5.6 software equipped with AutoDock 4.2 to observe and identify affinities and interactions that occur between Nrf2 macromolecule with alpha-mangostin molecule (Rizvi et al., 2013). The distance between the surface of the macromolecule and the compound molecule being tested was limited by a boundary maximum radius of 0.375 Å. All simulations were carried out using a grid box size 50 × 50 × 50. Lamarckian genetic algorithm with 100 conformation searches was used in this simulation (Jnoff et al., 2014). The population size was set as 150, the maximum number of evaluations was set as 2,500,000, and 100 runs were performed. The data from the molecular docking study was then analyzed using the Discovery Studio 2021 Client programs (Muchtaridi et al., 2023a).

Molecular dynamics simulation

The best test ligands from docking were subjected to molecular dynamic (MD) simulation. This simulation was run on an online graphics processing units (GPU) using OpenMM software and Google Colab, which was linked to Google Drive. The simulation begins by switching the runtime for the MD simulation to GPU (Eastman et al., 2017). The topology was then created; while creating the topology, the force field General Amber Force Field 2 was employed, and the ligand was ff19SB (Jia et al., 2013).

Following the creation of the topology, solvation and neutralization were performed. During solvation, the ligand-receptor complex was dissolved in the TIP3P model solvent, and in the neutralization stage, Na+ was added at a concentration of 0.15 to neutralize the system. The equilibration step was then used to stabilize the system so that the state was consistent before entering the production stage. The final production stage is conducted for 50 ns at a temperature (T) of 310 K or 36.8°C. The characteristics of the encapsulated α-mangostin, root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and molecular mechanic-generalized born surface area (MM-GBSA) were analyzed in this simulation.

In silico data analysis

In this study, *in silico* data analysis was conducted through several key steps. The docking method was validated using MGLTools 1.5.6 and AutoDock 4.2 to explore the interactions between alpha-mangostin and the Nrf2 macromolecule. Lipinski's Rule of Five was applied through the online tool at http://scfbio-iitd.res.in to evaluate drug-likeness based on molecular weight, LOGP, TPSA, and hydrogen bonding properties. The ADMET profiles of alpha-mangostin and dimethyl fumarate were assessed using SWISSADME, providing insights into their absorption, distribution, and other pharmacokinetic characteristics. Toxicity predictions were performed with ProTox-3.0, categorizing the compounds from fatal to non-harmful based on their predicted toxicity levels. The ligand structure was optimized and compared with native ligands to assess chemical interactions, while molecular dynamics simulations provided free energy calculations and evaluated the stability of the ligand-receptor complex (Fakih al., 2024). The data were processed and displayed in tables and figures for a clear and comprehensive analysis.

RESULTS

Prediction Lipinski's Rule of Five (drug-likeness)

Drug similarity prediction, or Lipinski's Rule of Five, is related to the process of drug absorption and distribution and is based on various physicochemical qualities of the drug when consumed orally. Research and development of oral drugs must follow Lipinski's Rule of Five, which ensures that the drug meets the good criteria. Lipinski's Rule of Five parameters include molecular weight <500 g/mol, partition coefficient (Log P) <5, hydrogen bond donor <5, and hydrogen bond acceptor <10. Meeting these criteria is believed to enhance the drug's chances of being effectively absorbed and distributed in the body. Alpha-mangostin meets four out of the five principles (Table 1). However, its low solubility in water is by the large log P value close to 5 which is the maximum limit of LogP based on the Lipinski criteria. Despite this, the overall compliance with the majority of Lipinski's criteria indicates that alpha-mangostin could be considered a viable candidate for oral drug development (Lipinski, 2004).

Prediction of pharmacokinetics and pharmacodynamics

The pharmacokinetic and pharmacodynamic analysis showed that both alpha-mangostin and dimethyl fumarate meet the required criteria. In terms of plasma membrane permeability, a TPSA score below 140 Å² is considered significant, and all the selected compounds in this study had TPSA scores below this threshold. Each compound also had molar refractivity scores within the acceptable range of 40 to 130. Lipophilicity scores (XLOGP) for the compounds

Table 1. Drug-likeness prediction result of native ligand, comparative ligand, and alpha-mangostin based on Lipinski's Rule of Five.

Compound names	BM (g/mol)	Log P	Donor H	Acceptor H
4L7B-ligand	446	3.30	1	7
Alpha-mangostin	412	4.53	3	6
Dimethyl fumarate	312	-0.05	5	6

Table 2. Prediction result of pharmacokinetic and pharmacodynamics profiles of native ligand, comparative ligand, and alpha-mangostin based on SWISSADME.

Compound names	4L7B-ligand	Alpha-mangostin	Dimethyl fumarate
Molecular weight	446.50 g/mol	410.46 g/mol	144.13 g/mol
Topological polar surface area (TPSA)	94.99 Å²	100.13 Å²	52.60 Å²
Hydrogen bond acceptors	5	6	4
Hydrogen bond donors	1	3	0
Molar refractivity	128.80	119.99	33.05
XLOGP	3.03	6.27	0.22
iLOGP	2.71	4.14	1.93
MLOGP	2.83	2.19	0.15
WLOGP	2.21	5.09	-0.11
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
GI absorption	High	High	High
Bioavailability score	0.56	0.55	0.55
BBB permeability	No	No	No
Pgp substrate	Yes	No	No
CYP2D6 inhibitor	No	No	No
PAINS (Pan assay interference compounds)	0 alert	0 alert	0 alert
Leadlikeness	No; 1 violation: MW>350	No; 2 violations: MW>350, XLOGP3>3.5	No; 1 violation: MW<250
Skin permeation (log kp)	-6.87 cm/s	-4.35 cm/s	-7.02 cm/s

XLOGP: Lipophilicity scores; GI: Gastrointestinal; CYP: Cytochrome 450; BBB: Blood-brain barrier; Pgp: P-glycoprotein; iLOGP: Implicit log P [n-octanol/water partition coefficient (log Po/w)]; MLOGP: Moriguchi Log P [octanol-water partition coefficient (it is based on quantitative structure-logP relationships, by using topological indexes); WLOGP: Water partition coefficient (implementation of the atomistic method developed by Wildman and Crippen for Lipophilicity index).

ranged from 5.41 to -0.50, while iLOGP scores varied from 1.93 to 4.14. All the compounds exhibited high bioavailability. GI absorption was also high across the board, with none of the compounds showing limitations in GI absorption. None of the compounds were able to cross the BBB, and none showed p-glycoprotein (Pgp) substrate activity. The study also assessed the potential for inhibiting cytochrome P450 (CYP450) enzymes and their isoforms. For skin permeability, which is crucial for transdermal drug delivery, a log Kp score above 2.5 indicated lower skin permeability (Bitew et al., 2021). Table 2 summarizes these findings from the pharmacokinetic and pharmacodynamic analyses.

Prediction of toxicity profiles

The toxicity profiles of the compounds indicate that the 4L7B-ligand and alpha-mangostin are generally less toxic than dimethyl fumarate (Fig. 2, Table 3). Alpha-mangostin stands out with its lower toxicity class and reduced risks of mutagenicity and liver toxicity compared to dimethyl fumarate, which has the highest toxicity class and more significant concerns regarding carcinogenicity and cell toxicity. While the 4L7B-ligand has moderate toxicity, it shows a higher potential for affecting the immune system than alpha-mangostin. These results suggest that dimethyl fumarate poses the most significant risk of adverse effects, whereas alpha-mangostin seems relatively safer. The elevated immunotoxicity in both the 4L7B-ligand and dimethyl fumarate highlights the need for careful evaluation of their impact on immune

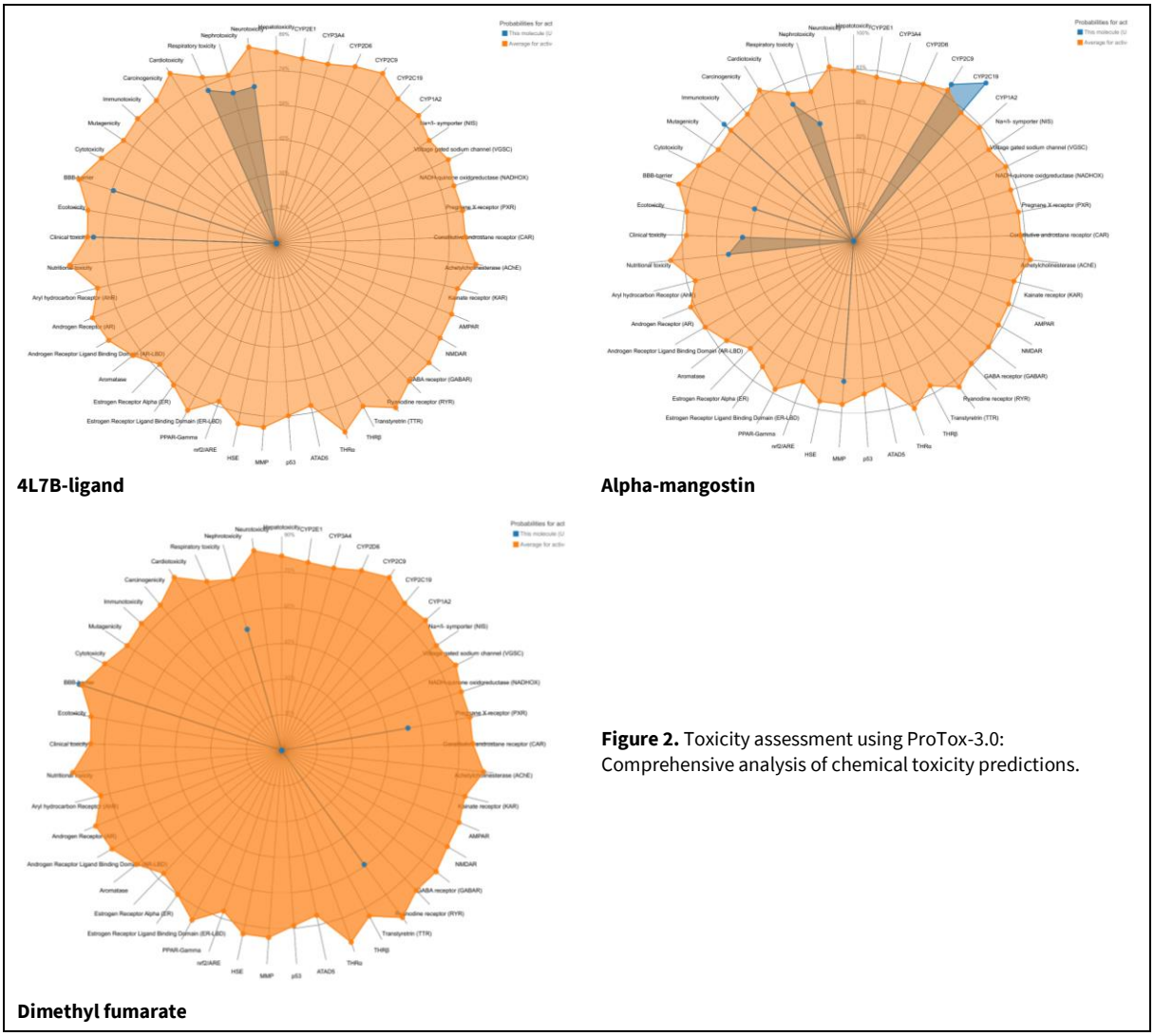


Figure 2. Toxicity assessment using ProTox-3.0: Comprehensive analysis of chemical toxicity predictions.

Table 3. *In silico* toxicity prediction for drug candidates and chemical compounds.

Compound names	4L7B-ligand	Alpha-mangostin	Dimethyl fumarate
Toxicity class	5	4	3
Hepatotoxicity	0.88	0.70	0.80
Immunotoxicity	0.99	0.84	0.99
Carcinogenicity	0.61	0.69	0.74
Mutagenicity	0.74	0.53	0.71
Cytotoxicity	0.73	0.77	0.85

health, underscoring the importance of these findings in guiding safe and effective drug development (Yuniarta et al., 2023).

Molecular docking study

The goal of molecular docking studies is to observe how the ligand interacts with the target receptor, with outcomes including ΔG value and the type of bond formed with the amino acid residue. The binding energy in the molecular docking process describes the test compound's affinity to the target protein, in this case, the Nrf2 enzyme (Fig. 3A). According to the results of molecular docking studies, α-mangostin had lower binding energy than the native ligand (1VV) (Fig. 3B), and dimethyl fumarate (Fig. 3C) as the standard ligand, with ΔG score of α-mangostin was -9.71 Kcal/mol (Fig. 3D), indicating that the affinity for Nrf2 was high due to lower

energy required for binding (Table 4). The findings revealed that alpha-mangostin forms strong hydrogen bonds with the amino acids SER602 and GLY364, as well as hydrophobic interactions with ARG415, ALA556, and TYR572, all of which have been linked to the structure of the Keap1-Nrf2 interface. These interactions highlight the potential of alpha-mangostin to activate Nrf2, underscoring its potential as a long-term treatment option for COVID-19.

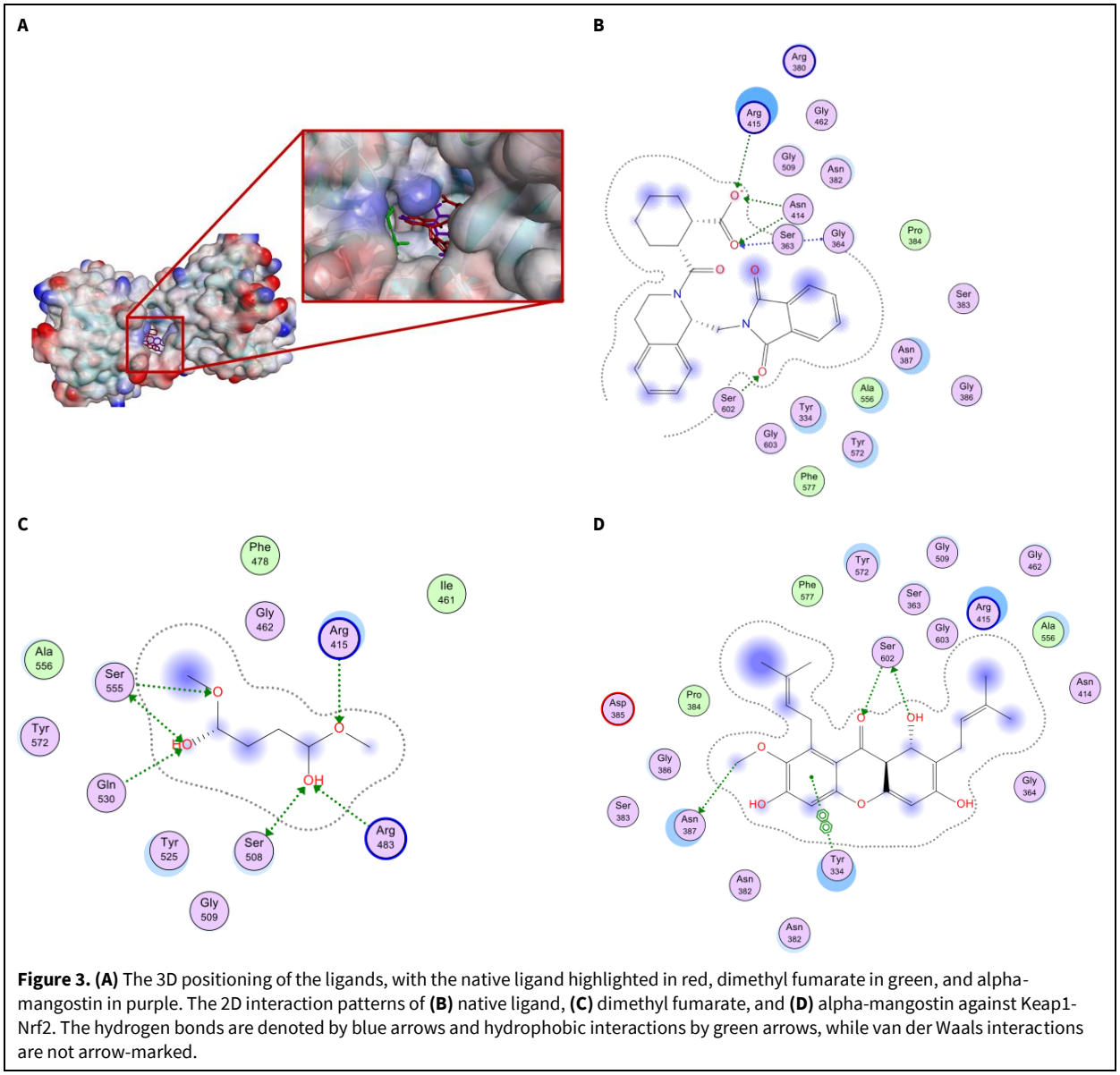


Figure 3. (A) The 3D positioning of the ligands, with the native ligand highlighted in red, dimethyl fumarate in green, and alpha-mangostin in purple. The 2D interaction patterns of **(B)** native ligand, **(C)** dimethyl fumarate, and **(D)** alpha-mangostin against Keap1-Nrf2. The hydrogen bonds are denoted by blue arrows and hydrophobic interactions by green arrows, while van der Waals interactions are not arrow-marked.

Table 4. Molecular docking result and amino acid residues of test compounds on the PDB ID: 4L7B receptor.

Compound names	ΔG (Kcal/mol)	Amino acid interaction	
		Hydrogen bonds	Hydrophobic interactions
4L7B-ligand	-9.25	ARG415, ASN414, SER602	TYR334, ALA556, TYR572
Alpha-mangostin	-9.71	GLY36, SER602	ARG415, ALA556, TYR572
Dimethyl fumarate	-4.62	ARG483, SER508, ARG415, SER555, GLN530, ILE461	ALA556, TYR572

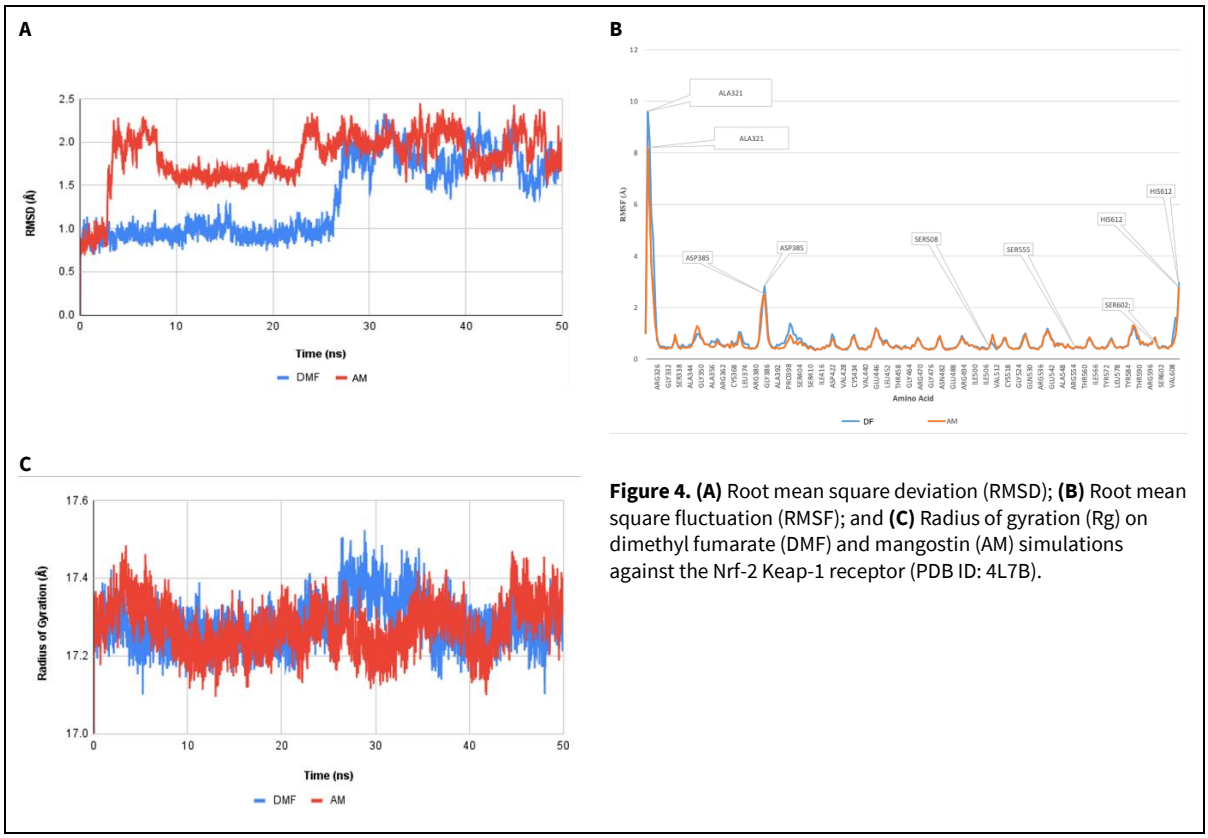


Figure 4. (A) Root mean square deviation (RMSD); **(B)** Root mean square fluctuation (RMSF); and **(C)** Radius of gyration (Rg) on dimethyl fumarate (DMF) and mangostin (AM) simulations against the Nrf-2 Keap-1 receptor (PDB ID: 4L7B).

Molecular dynamics simulation

Molecular dynamics simulations were carried out on two complexes (alpha-mangostin and dimethyl fumarate) toward Nrf-2 Keap-1 receptor using Open MM software. First, the RMSD analysis was carried out to compare changes or shifts in molecular conformation that occur during the simulation process, as the RMSD number describes the difference between the ligand-receptor conformation during the simulation process and the initial ligand-receptor conformation (Darusman et al., 2024). If the average RMSD value of the ligand binding to the receptor is <2 Å, the ligand is considered stable and meets the requirements for a good fit (Jnoff et al., 2014). A rise in the RMSD value of the test ligand complex against the Nrf-2 Keap-1, resulting from excessively large variation, suggests that the protein bonds are loosening, leading to the denaturation of the protein. The RMSD values for the two complex systems were calculated, and conformational changes can be observed in the plot of RMSD against simulation time, as it showed the correlation with conformational snapshots taken during molecular dynamics simulation (Fig. 4A). Alpha-mangostin exhibited good stability throughout the molecular dynamics' simulation, with the average RMSD values of alpha-mangostin and dimethyl fumarate were ± 1.82 Å and ± 1.36 Å, respectively.

The RMSF parameter measures the fluctuations in the amino acid residues that make up the receptor throughout the simulation process. The average RMSF value of 0.66 Å, as depicted in Fig. 4B for the alpha-mangostin ligand complex with Nrf-2 Keap-1 receptor over a 50 ns molecular dynamics simulation, indicated a relatively low level of fluctuation in the amino acid residues at the binding site. Meanwhile, dimethyl fumarate, the comparator ligand, had an average RMSF fluctuation value of 0.72 Å. With difference of 1.4 Å, it was suggested that alpha-mangostin formed stable bonds with residues in Nrf-2 Keap-1. The amino acid residues ALA321, ASP385, and HIS612 had large variations in the alpha-mangostin in comparison with the dimethyl fumarate. The analytical results indicate that the amino acid residues exhibiting large variations in the RMSF have unstable bonds and are likely not actively involved in the binding site. Furthermore, the amino acid residues SER602, SER555, and SER508 had the lowest fluctuation value in the ligand complex of the alpha-mangostin for the 4L7B receptor, with a fluctuation value of 0.42, 0.44, and 0.46 Å. Meanwhile, the amino acid residues were projected to have persistent interactions and actively participate in the binding site. In comparison, the dimethyl fumarate showed low fluctuations, with values of 0.43, 0.44, and 0.45 Å, indicating stability in the binding area. This demonstrates that the α-mangostin molecule can form a hydrogen

bond with the active site of Nrf-2 Keap-1, specifically the amino acid residue SER602.

Following those two prior analyses, an examination of the radius of Rg was performed to evaluate the stability of the complex and whether the protein is stable in a folded or unfolded state during molecular dynamics simulation. At an Rg of 50 ns, it was observed that the alpha-mangostin complex tends to be more stable than dimethyl fumarate. Apart from that, alpha-mangostin and dimethyl fumarate showed average Rg values of 17.27 nm and 17.28 nm, respectively (Fig. 4C). This finding indicated that alpha-mangostin has a lower average Rg value than dimethyl fumarate.

Molecular mechanics-generalized born surface area (MM-GBSA)

MM-GBSA was used to calculate the total binding energy between the ligand and the receptor. The total energy describes the stability of the molecular structure during the simulation. The stability of the system can be observed from the total energy analysis in Table 5. The average total energy values of alpha-mangostin and dimethyl fumarate were -13.72 Kcal/mol and -3.76 Kcal/mol, subsequently. The result could be due to Van der Waals interaction (VdW) on the alpha-mangostin that contributed to this effect. This indicated that the alpha-mangostin was more likely than the dimethyl fumarate to activate Keap1-Nrf2 and had the potential to be a long COVID-19 medication.

DISCUSSION

Over the last five years, our research has focused on the study of α-mangostin against cancer. Alpha-mangostin is the main component of the edible mangosteen fruit, which grows in tropical areas such as Indonesia (Bi et al., 2023). This compound is very active in several human cancer cells including breast, cervical, and lung cancer cells (Muchtaridi and Wijaya, 2017). Notably, most studies show that this compound is active in fighting breast cancer cells through several mechanisms (Herdiana et al., 2021).

Alpha-mangostin is effective as an antiproliferative agent in MCF-7 human breast adenocarcinoma cells by suppressing TPA-mediated matrix metalloproteinase (MMP)-2 and MMP-9 expressions (Lee et al., 2010; Nurhidayah et al., 2023). We have also proven these findings in the form of nano polymeric α-mangostin employing the same mechanism (Meylina et al., 2021; Muchtaridi et al., 2023b; Wathoni et al., 2021). Alpha-mangostin was predicted to actively inhibit cell migration of triple-negative breast cancer (Dewi et al., 2023), as proven by the latest research (Sarmoko et al., 2023). Based on a study by Sarmoko et al. (2023), alpha-mangostin altered ROS levels in MDA-MB-231 cells, indicating that alpha-mangostin regulates cell activities, including survival and migration, by modulating ROS levels (Sarmoko et al., 2023). These results were supported by previous studies that alpha-mangostin induces oxidative stress and mitochondrial dysfunction, which results in 4T1 cell death through an apoptotic mechanism (Cruz-Gregorio et al., 2023).

In the future, alpha-mangostin is predicted to increase ROS levels in cancer cells, surpassing the ROS levels observed in normal cells. The presence of excess levels of ROS is predicted to impact the metabolic capacity of tumor cells, leading to disruptions in proliferation and migration, thereby causing cancer cell death (Hayes et al., 2020).

Table 5. Values of free energy using molecular mechanic-generalized born surface area of compounds against the 4L7B macromolecule.

Energy component (Kcal/mol)	Complex system	
	Alpha-mangostin	Dimethyl fumarate
Van der Waals Interactions (VdW)	-22.56	-6.17
Electrostatic energy (EEL)	-3.14	-3.24
Electrostatic contribution to solvation-free energy (E_{GB})	14.91	6.75
Non-polar contribution to solvation-free energy (E_{SURF})	-2.92	-1.11
ΔGgas (VdW + EEL)	-25.70	-9.41
ΔGsolv (EGB + E_{SURF})	11.98	5.65
ΔGtotal (VdW+EEL+ E_{GB} + E_{SURF})	-13.72	-3.76

The involvement of alpha-mangostin in affecting ROS levels within cells is attributed to its role in activating the Nrf2 binding site on Keap1 and activating the Nrf2-ARE signaling pathway (Muchtaridi et al., 2022). In our previous study, we simulated alpha-mangostin using 50 ns OpenMM (Eastman et al., 2017) of molecular dynamics simulation against Keap1 Nrf2 (PDB ID: 4L7B) complexed with dimethyl fumarate. According to Fig. 4, alpha-mangostin interacted against amino acid residues and played a crucial role in the connection between Nrf2 and Keap1 (Jnoff et al., 2014). From the simulations, almost all amino acids interact with the native ligand dimethyl fumarate and also interact with alpha-mangostin. The compound formed hydrophobic interaction with conserved amino acid residues of ARG415, SER508, ALA556, and TYR572 that linked to the structure of the Keap1 Nrf2 interface in the binding domain of Keap1. This hydrophobic amino acid residue accommodates the interface between Nrf2 and Keap1 thus when the inhibitor binds to these amino acid residues, the bond between the Nrf2 and Keap1 is released and as a result, Nrf2 is activated to regulate the ROS. Alpha-mangostin also formed strong hydrogen bonds with the amino acids SER602 and GLY364 within the binding domain of Keap1. Similar to the native ligand in the complex crystal, the carbon-aromatic ring of alpha-mangostin forms a phi-phi stacking interaction with the aromatic ring at TYR572, contributing to the stabilization of alpha-mangostin in the Nrf2 Keap1 binding pocket. However, it is imperative to conduct *in vitro* and *in vivo* studies of alpha-mangostin to substantiate its role in enhancing ROS levels in cancer cells.

CONCLUSION

Based on the findings from molecular docking studies, the alpha-mangostin shows promising potential as an antioxidant agent targeting the Nrf2 receptor, evidenced by its lower ΔG value compared to both the original ligand and the reference ligand, which is -9.70 kcal/mol. Additionally, during the 50 ns molecular dynamics simulation, the ligand-receptor complex (4L7B) demonstrates sustained interaction upon α-mangostin binding to the receptor.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Concepts or ideas							x
Design	x						x
Definition of intellectual content							
Literature search	x					x	
Experimental studies	x	x			x	x	
Data acquisition	x	x	x	x	x		
Data analysis	x	x	x	x	x	x	
Statistical analysis		x	x				
Manuscript preparation	x						x
Manuscript editing	x					x	
Manuscript review	x	x	x	x	x	x	x

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