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INHIBITION OF CYCLOOXYGENASE-2 USING BIOACTIVE COMPOUND DERIVED FROM *MORINGA OLEIFERA* THROUGH MOLECULAR DOCKING APPROACH

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ABSTRAK

Respon tubuh terhadap iritasi, radiasi, infeksi, dan cedera menyebabkan peradangan. Penelitian ini menggunakan pendekatan molecular docking untuk mengeksplorasi potensi senyawa bioaktif dari *Moringa oleifera* terhadap reseptor COX-2. Penelitian diawali dengan *redocking* untuk validasi metode. Sepuluh senyawa bioaktif diperoleh dari https://pubchem.ncbi.nlm.nih.gov dan reseptor COX-2 dari www.rcsb.com. Senyawa dari *Moringa oleifera* dianalisis menggunakan Lipinski's *Rule of File* dan properti ADMET. Energi pengikatan dan interaksi residu asam amino dihitung menggunakan Autodock Tools 1.5.6 dengan memanfaatkan Lamarckian Genetic Algorithm (LGA). Hasil perhitungan menunjukkan delapan senyawa bioaktif yang memenuhi Lipinski's *Rule of Five*. Benzil glukosinolat memiliki energi pengikatan yang lebih rendah daripada ligan asalnya, yaitu -8,06 kcal mol^{-1} dengan residu asam amino TYR385, SER530, TYR355, HIS90, ARG513, LEU352, dan GLN192. Studi ini menunjukkan bahwa benzil glukosinolat, senyawa yang diisolasi dari *Moringa oleifera*, menunjukkan potensi yang menjanjikan sebagai penghambat enzim COX-2. Analisis komputasional menunjukkan bahwa molekul yang terjadi secara alami ini kemungkinan memiliki sifat antiinflamasi yang signifikan dengan menargetkan jalur siklooksigenase-2 secara selektif. Penelitian ini memberikan dasar yang kuat untuk riset masa depan di bidang penemuan dan perancangan obat, khususnya dalam pencarian agen antiinflamasi alami. Metodologi dan hasil yang disajikan di sini dapat menjadi referensi berharga bagi para peneliti yang mengeksplorasi fitokimia lain, sebagai petunjuk potensial untuk mengembangkan terapi antiinflamasi yang lebih aman dan lebih efektif.

Kata kunci: antiinflamasi, COX-2, docking molekuler, *Moringa oleifera*

ABSTRACT

The body's response to irritation, radiation, infection, and injury causes inflammation. This research used a molecular docking approach to explore the potential of bioactive compounds from *Moringa oleifera* against the COX-2 receptor. It was initiated with redocking for method validation. Ten bioactive compounds were obtained from https://pubchem.ncbi.nlm.nih.gov and COX-2 receptors from www.rcsb.com, respectively. The compounds from *Moringa oleifera* were analyzed using Lipinski's Rule of File and ADMET properties. The binding energy and amino acid residue interaction were calculated using Autodock Tools 1.5.6 utilizing the Lamarckian Genetic Algorithm (LGA). The calculation results showed eight bioactive compounds that fulfill Lipinski's Rule of Five. Benzyl glucosinolate has a lower binding energy than the native ligand, namely -8.06 kJ mol-1with amino acid residues TYR385, SER530, TYR355, HIS90, ARG513, LEU352, and GLN192. This study demonstrates that benzyl glucosinolate, a compound isolated from Moringa oleifera, exhibits promising potential as a COX-2 enzyme inhibitor. The computational analysis suggests that this naturally occurring molecule may possess significant anti-inflammatory properties by selectively targeting the cyclooxygenase-2 pathway. Furthermore, this research provides a solid foundation for future investigations in the field of drug discovery and design, particularly in the pursuit of natural anti-inflammatory agents. The methodology and results presented here can serve as a valuable reference for researchers exploring other phytochemicals as potential leads for developing safer and more effective anti-inflammatory therapeutics.

Keywords: anti-inflammation, COX-2, molecular *docking*, *Moringa oleifera*

INTRODUCTION

Inflammation occurs when the body responds to irritation, radiation, cell destruction, toxic substances, infection, and wounds. It is a non-specific immune response (Nunes *et al.*, 2020). Inflammation symptoms also contribute widely to chronic diseases in the human body, namely cancer and tumors (Utami *et al.*, 2020). Inflammatory symptoms must be prevented for termination of development into chronic and acute.

Chronic inflammation has a long duration and can occur for years. Meanwhile, acute inflammation can occur spontaneously within minutes to hours, characterized by symptoms such as neutrophils and increased blood flow to the inflammatory area due to vasodilation (Marchi *et al.*, 2023). Excessive activity of anti-inflammatory mediators can cause immunopathological impacts on the body. Inflammatory responses in the body are mediated by various pro-inflammatory enzymes, with cyclooxygenases (COXs) and lipoxygenases (LOXs) playing crucial roles in cellular and tissue metabolism. Of particular interest is cyclooxygenase-2 (COX-2), an inducible enzyme that is upregulated in response to tissue injury or inflammatory stimuli. COX-2 catalyzes the synthesis of prostaglandins, which are key mediators of inflammation. When activated, COX-2 contributes to the classic signs of inflammation: pain, heat, redness, and swelling (Marek-Jozefowicz *et al.*, 2023). Consequently, selective inhibition of COX-2 has emerged as a promising therapeutic strategy for managing inflammatory conditions (Zakiyah *et al.*, 2022).

Reducing inflammatory reactions in the human body is carried out by inhibiting COX2, an enzyme that plays a role in the inflammatory mechanism. The COX2 enzyme is an inducible form of cyclooxygenase responsible for inflammatory, physiologic stimuli, growth factors, and prostaglandin production. Prostaglandin mediates pain during the inflammation process (Chen *et al.*, 2018). Generally, non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation. However, prolonged consumption of these treatments can create various health problems in the human body, including kidney infections, bleeding, and stomach irritation (Sohail *et al.*, 2023). Therefore, the search for potential drugs from natural compounds continues to be carried out. Drug candidates from natural ingredients can be an alternative in treating inflammation, such as flavonoids in the ethanol extract of *Moringa oliefera* leaves (Ehigiator *et al*., 2023).

Moringa oleifera is an Indonesian medicinal plant that has many benefits. They live in the high and lowlands of Indonesia. All components of the Moringa leaf have been utilized as food and medicine (Emelda *et.al*., 2023), so the Moringa leaf itself can be applied as an antitumor, antioxidant, and anti-inflammatory. In vivo, research conducted by Wulan, *et al*. (2021) showed that ethanol extract at 8.33mg/100gwas an effective dose that can be used as an anti-inflammatory. Moringa leaf and seed extracts ranging from 1.1–100 µg mL^{-1} had anti-inflammatory properties, inhibiting the production of nitric oxide (NO) (Xu *et al*., 2019). *Moringa oliefera* has been studied as an anticancer medication both in vitro and in silico (Mumtaz *et al.*, 2021). Recent in silico research conducted by Hamdy (2024) has shed light on the potential anti-inflammatory properties of compounds found in Moringa oleifera. This computational study focused on the interaction between routine phytochemicals isolated from *Moringa oleifera* and the interleukin-1 receptor antagonist (IL-1RA) receptor. The findings revealed a notable binding affinity, with a calculated binding energy of -8.96 kcal mol^{-1}.,.

In silico research, an approach that utilizes computing technology and information available in databases is utilized. The in silico method has advantages such as having a short and economical research time, so this method is very efficient in identifying drug candidates to complement the field of in vivo and in vitro research (Hartanti *et al.*, 2022) . This research aimed to determine potential drug candidates from the active compound of *Moringa oliefera* leaves, which acts as an anti-inflammatory agent for inhibiting the COX2 enzyme using the molecular docking method.

MATERIALS AND METHOD

Lipinski's Rule of Five and ADMET Properties

It is essential to study Lipinski's Rule of Five before proceeding with molecular docking calculations as an initial selection. This step aimed to determine which compounds from the medicinal plant *Moringa oliefera* comply or do not comply with Lipinski's rules. It concerns the physicochemical properties of drugs that resemble drugs (druglikeness). Lipinski's rules are drug

molecular mass ≤500 Da, octanol/water partition coefficient (MlogP) ≤5, Hydrogen Bond Acceptor (HBA) ≤10, and Hydrogen Bond Donor (HBD) ≤5. Lipinski's data was obtained using a website-based application: http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp (Jayaram, 2017). Apart from that, ADMET predictions were also carried out to determine the pharmacokinetic properties of drug candidates derived from the medicinal plant Moringa oliefera. Lipinski and ADMET tests are carried out by inputting compounds into a software website-based application: https://preadmet.webservice.bmdrc.org/ (Pitaloka *et al.*, 2023).

Molecular Docking Calculation

Molecular docking was calculated using computer hardware processor intel ® Celeron N4020 CPU, installed RAM 8,00 GB, system type 64-bit operating system x64-based processor, windows specifications edition Windows 11 home single language. Computational chemistry software was also used to prepare and dock ligands and receptors. Molecular docking calculation is conducted to predict the binding energy of a ligand-COX2 complex. To validate a method in this study, we carried out a redocking step between mefenamic acid and COX2 receptor.

The bioactive compound derived from *Moringa oliefera* was retrieved from the database (http://www.knapsackfamily.com/), namely astragalin, amino (methoxy sulfinyl) pentasulfide, benzyl glucosinolate, d-allose, isorhamnetin, gallic acid, gentisic acid, glucoconringin, syringic acid, and marumoside A (Deng *et al*., 2023). Moreover, these ligands were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The COX2 receptor was obtained from the PDB database (https://www.rcsb.org/) (PDB ID: 5IKR) (Wiyono *et al*., 2023).

Ligands and receptors were generated using the Chimera application using Dock prep tools (Haugland, 2021). After that, ten ligands from *Moringa oliefera* leaves were docked against the COX-2 receptor in a grid box 40 x 40 x 40 Å spacing 0.375 Å. The Lamarckian genetic algorithm of 100 runs was applied to molecular docking calculations. The lowest energy complex will be visualized using Biovia Discovery Studio to determine the type of bond formed (Neelaveni *et al*., 2024).

RESULTS AND DISCUSSION

Lipinski's Rule of Five and ADMET Properties

Lipinski's rule analysis aims to determine adherence of the compound to the rules, such as molecular weight of less than 500 DA, log P of less than 5, and HBA and HBD of less than 10 and 5, respectively. The results of Lipinski's analysis can be seen in Table 1. Drug candidates with a molecular mass of more than 500 DA will have difficulty distributing on cell membranes, so the drug fails to diffuse (Karami *et al.*, 2022). Table 1 shows that all ligands from *Moringa oleifera* have a molecular mass below 500 DA, concluding that all compounds fulfill the first rule.

Furthermore, the MlogP value of a therapeutic candidate chemical must not exceed five as it affects the drug's hydrophobicity or lipophilicity in the distribution processes.

MlogP > 5 ligands have higher hydrophobic features and are preserved in the lipid bilayer for longer. Thus, the medicine will harm the human body (Sahu *et al*., 2022). All active chemicals found in *Moringa oleifera* have a MlogP value of less than 5.

The HBA value for a drug-candidate molecule cannot exceed 10. Violation of this rule decreases permeability when the drug passes through the membrane. Moreover, the drug does not dissolve easily in lipids. In this investigation, two pharmacological molecules, astragalin, and glucoconringin, did not match Lipinski's criteria.

If the HBD value exceeds 5, the medicinal molecule may slowly adsorb (Truong, George and Holien, 2021). The amount of HBD is closely related to oral bioavailability and pharmacokinetic properties. HBD is vital in donating hydrogen atoms and forming hydrogen bonds to form complex compounds (Karami *et al.*, 2022). Of the ten active *Moringa oleifera* compounds, two have an HBD value above 5: astragalin and glucoconringin.

Table 1 lists two *Moringa oleifera* chemicals contradict Lipinski's rules: astragalin and glucoconringin. Meanwhile, eight medicinal compounds found in Moringa leaves can be indicated as potential anti-inflammatory drugs. The remaining ligands in Table 1 were docked to

COX2 using molecular docking computation. Meanwhile, the ADMET test results may be found in Table 2.

ADMET testing on a new drug candidate compound aims to see the ability of a drug compound to be adsorbed and distributed in the human body. The HIA value shows the ability of a candidate drug compound to be absorbed in the human intestine. The standard HIA value for good absorption in the human body is 20-100 (Cáceres *et al*., 2020). In this study, eight active compounds in *Moringa oliefera* had HIA values in the excellent range. So, these eight compounds can be adequately absorbed in the human intestine.

Table 1. Lipinski's rule of five bioactive compounds from *Moringa oliefera*

No	Compounds	Lipinski's Rule of Five					
		PUB ID	Molecular Mass ≤500 (DA)	MlogP ≤ 5	Hydrogen Bond Acceptor (HBA) ≤10	Hydrogen Bond Donor (HBD) ≤5	Violation
1	Astragalin	5282102	448	-0.436	11	7	Violate
2	Benzylglucosinolate	9548605	409	-0.02	10	5	Unviolate
3	D-Allose	439507	180	-0.22	6	5	Unviolate
4	Gallic acid	370	170	0.51	5	4	Unviolate
5	Gentisic acid	3469	154	0.79	4	3	Unviolate
6	Syringic acid	10742	198	1.10	5	2	Unviolate
7	Marumoside A	101794623	297	-1.07	7	5	Unviolate
8	Glucoconringiin	656537	391	-1.10	11	6	Violate
9	Isorhamnetin	5281654	316	2.31	7	4	Unviolate
10	Amino (methoxy sulfinyl) pentasulfide	85756567	255	3.27	3	2	Unviolate

Table 2. ADMET properties of bioactive compound from *Moringa oleifera*

No.	Compounds	%HIA	BBB	Caco-2 (nm/sec)	%PPB	Ames Tes mutagenicity
1	Benzylglucosinolate	96.55	2.10	31.51	97.85	Mutagen
2	D-Allose	22.35	0.05	2.56	7.31	Mutagen
3	Gallic acid	53.69	0.34	13.84	65.38	Mutagen
4	Gentisic acid	74.75	0.44	18.30	69.61	Mutagen
5	Syringic acid	74.75	0.44	18.30	69.61	Mutagen
6	Marumoside A	60.54	0.03	1.25	25.73	Mutagen
7	Isorhamnetin	78.34	0.05	4.93	83.54	Mutagen
8	Amino (methoxy sulfinyl) pentasulfide	92.69	0.09	53.51	00	Mutagen

Caco-2 cell value modeling aims to predict the ability of drug candidates to be absorbed into active compounds orally. Caco-2 parameter must have a value >0.9nm/sec, indicating high permeability in penetrating cell membranes (Venkatraman, 2021). In this study, all drug candidates for *Moringa oliefera* active compounds had Caco-2 values >0.9 nm/sec, which indicated high permeability.

PPB is the distribution rate of a drug compound that binds proteins so that the body will distribute drug-bound blood in all body parts. Meanwhile, PPB shows that the drug candidate can be distributed well in the human body, marked with a PPB value that is smaller than 90. If the PPB value exceeds 90, the medication candidate is bound to plasma. Consequently, the medicine requires an extended period to be distributed (Cáceres *et al*., 2020). In this study, eight active

compounds in *Moringa oliefera* had PPB values below 90.

The BBB distribution parameter is related to the central nervous system (CSN). It regulates the movement of ions, molecules, and cells between the brain and blood. When the BBB value is greater than > -1, it is thought to be easier to distribute in the brain (Wei *et al.*, 2022). The therapeutic candidate for the active ingredient in moringa leaves has a BBB value greater than -1, indicating that the eight components can be circulated effectively.

Next, toxicity prediction aims to see whether the compound being tested has toxic properties to the body. The toxicity test in this study was seen through mutagenicity and carcinogenicity parameters. The mutagenic potential value of a drug candidate compound using positive bacteria shows that the compound can act as a carcinogen. In this study, eight active compounds in Moringa leaves were mutagenic.

Molecular Docking Calculation

The 5IKR protein that has been prepared will undergo method validation through redocking between the natural ligand mefenamic acid and the COX2 receptor. This step is proven in the superimposed ligand before and after redocking in Figure 1. The results of 5IKR validation with native ligands show a binding energy value of -6.85 kcal mol^{-1} with an RMSD of 1.86 Å. This result shows that it can be valid if it has an RMSD value ≤ 2Å (Allouche, 2012). The validation data can be utilized to compare drug candidates from the bioactive compound of *Moringa oliefera.* The native ligand inhibits COX2 receptor performance by forming hydrogen interactions at the amino acid residues Tyr-385 and Ser-530.

Table 3 shows the results of the molecular docking calculation of bioactive compounds in Moringa oliefera. Among the eight active compounds in *Moringa oliefera*, benzyl glucosinolate has the lowest binding energy, -8.06 kcal mol^{-1}. The result shows that the benzyl glucosinolat compound has the potential to bind to the COX-2 receptor.

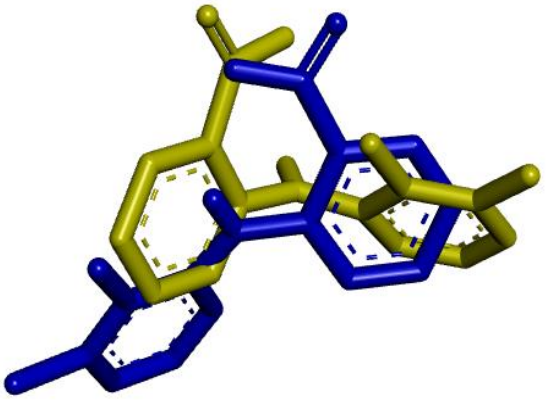

Figure 1. Superimpose of mefenamic acid+COX-2 complex (blue: before redocking docking, and yellow: after redocking)

There are six candidate ligand compounds from *Moringa oleifera* that show similar interactions with amino acid residues, namely amino (methoxy sulfinyl) pentasulfide, benzyl glucosinolate, d-allose, gallic acid, gentisic acid, and syringic acid. The similarity of the amino acids formed in the complex and the ligand indicates similar activity. Therefore, these six compounds have activities similar to those of the native ligand. Hydrogen bonds are essential in the ligands-receptors complex because hydrogen bonds stabilize the ligand and receptor.

The Inhibition Constant (Ki) value is another parameter when docking bioactive compounds discovered in *Moringa oleifera* with COX2 receptors. The Ki parameter represents the equilibrium binding affinity of the ligand-receptor complex. The Ki value is proportional to the Gibbs free energy value; the lower the Ki value, the more stable the interactions with the ligand and receptor (Sanzeni *et al.*, 2020). The benzyl glucosinolate molecule has the lowest ki value, measuring 1.24 μm. This result shows that the benzyl glucosinolate exhibits a persistent association with a higher binding affinity. The lower Ki value also indicates the small amount of medication that inhibits the receptor's activity.

Table 3. Binding energy, inhibition constant, and amino acid residue of drug candidate from *Moringa oleifera* for COX-2 inhibition

No.	Compounds	PubChem ID	Binding energy (kcal mol^{-1})	KI (µm)	Amino Acid Residue
1	Benzyl Glucosinolate	9648605	-8.06	1.24	TYR385,SER530,TYR355,HIS90,ARG513,LEU352,GLN192
2	D Allose	439507	-3.55	2.49	TYR385,MET522,SER530
3	Gallic Acid	370	-4.1	980.65	TYR385,SER530,MET522
4	Gentisic Acid	3469	-4.23	794.42	MET522,TYR385
5	Syringic Acid	10742	-5.19	157.19	TYR385,VAL523
6	Marumoside A	101794623	-6.76	11.11	ARG120,MET522,TYR355
7	Ishoramnetin	5281654	-5.18	159.9	-
8	Amino (methoxy sulfinyl) pentasulfide	85756567	-5.65	71.92	SER530,VAL523
9	Mefenamic Acid	-	-6.85	9.6	TYR385,SER530

The best conformation compared to the native ligand is the benzyl glucosinolate-COX2 complex. This ligand interacts with amino acid residues Ser-530 and Tyr-385 through hydrogen bonds. Moreover, benzyl glucosinolate has another hydrogen bond that strengthens interaction via TYR355, HIS90, ARG513, LEU352, and GLN192. Figure 2 visualizes the docking results complex of mefenamic acid+COX2 and benzyl glucosinolate+COX2.

Visualization of Figure 2A shows the 3D structure of the COX-2 receptor on 5IKR. The complex compound mefenamic acid has amino acid residues bonded via hydrogen bonds to TYR 385 and SER 530. This interaction occurs in the carbonyl group in mefenamic acid; the amino acid residue SER 530 is bound to an oxygen group (O), and TYR385 is bound to a hydroxyl group (OH), respectively. This result is similar to previous research conducted by Orlando *et al*., (2016), which found that the COX-2 receptor interacted with the amino acid residues of TYR-385.

Meanwhile, Figure 2B is an interaction of the benzyl glucosinulate+COX2 complex. The formed hydrogen bond in that complex is the TYR 385 and Ser 530 bound to the O group in sulfate ion. Based on the above result, the benzyl glucosinolate compound shows an inhibitory interaction comparable to the native ligand.

Figure 3 shows the hydrogen bond distance in the benzyl glucosinolate+ COX2 complex. The hydrogen bond distance to the amino acid residue Ser530 is 1.98 Å, and the distance to the amino acid Tyr385 is 2.27 Å. Furthermore, Figure 3 on the right depicts the polarization properties of hydrogen bonds; purple indicates donor features, while green indicates acceptor properties. The area where the amino acid residue binds to the benzyl glucosinolate compounds is colored purple, indicating acid. This allows the base part of the amino acid to interact with the benzyl glucosinolate ligand.

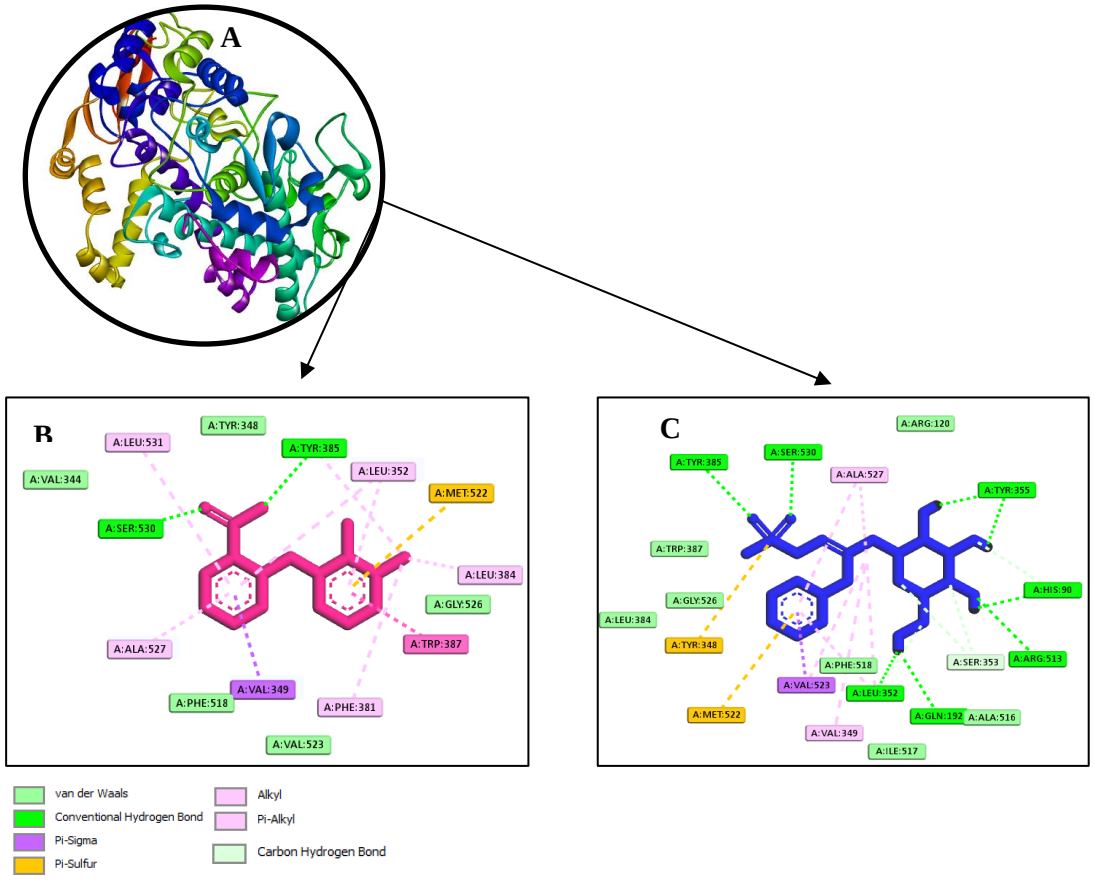

Figure 2. Visualization 3D of COX-2 receptor (A), mefenamic acid + COX-2 complex (B), and benzyl glucosinolate + COX-2 complex (C)

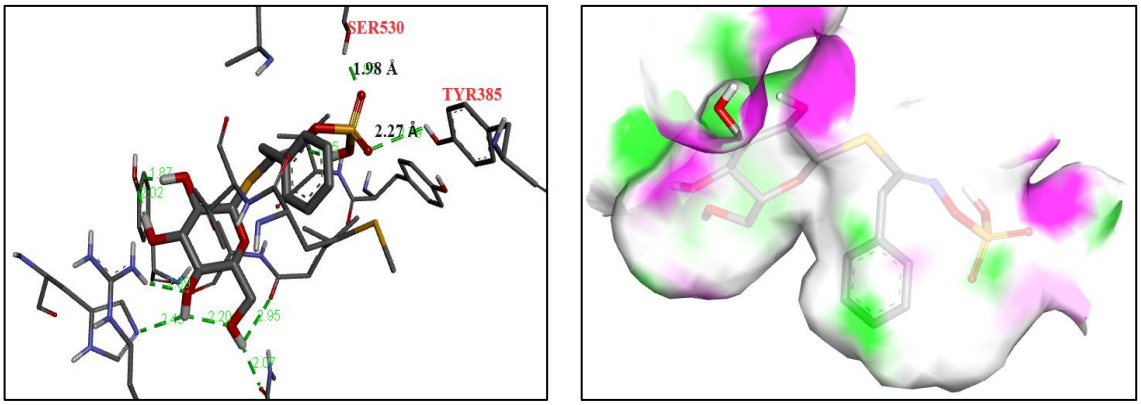

Figure 3. Hydrogen bond distance in benzyl glucosinolate + COX-2 complex (left) and representation of hydrogen bond area (purple: atom donor, green: atom acceptor)

CONCLUSION

The results of molecular docking calculations show that the benzyl glucosinolate compound contained in *Moringa oleifera* can interact with the active site of the 5IKR protein and has a low binding energy of -8.06 kcal mol^{-1} with amino acid interactions similar to the native ligand. Therefore, benzyl glucosinolate is predicted to inhibit the COX-2 receptor. The results of this research can be used as additional information in the field of in vitro and in vivo research.

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