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Article

Identification of Active Compounds of Leaf Extract and Seed Oil of *Moringa oleifera* in TrkB Receptor as Neuroprotective by Molecular Docking

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Abstract. The functional ability of the brain will decline progressively during aging which usually involves changes in plasticity. BDNF is one of the neurotrophins that regulates plasticity via TrkB receptors. So the potential of *Moringa oleifera* leaf and seed oil extracts was identified as neuroprotective on the interaction of TrkB receptors with molecular docking. The active compounds of *Moringa oleifera* leaf extract and seed oil were obtained from literature studies. Drug-likeness and ADMETox analysis were carried out using the SwissAdme and the AdmetSAR webserver. The molecular docking was carried out using the Pyrx Vina application and visualization is done using the Discovery Studio Biovia application. The docking results showed the best compounds namely luteolin, stigmasterol, and moringin as a marker compound and showed the interaction of hydrogen and hydrophobic bonds at the active site of the prediction results. So it can be concluded that compounds in the leaves and seed oil of *Moringa oleifera*, namely luteolin, stigmasterol, and moringin are predicted as ingredients that can activate TrkB receptors in the aging process.

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1. Introduction

Neurodegenerative diseases are diseases that cause a lot of morbidity and mortality in the elderly [1-2]. The functional ability of the brain will decline progressively during aging. As we age, cognitive performance generally declines resulting in decreased learning, memory, attention, decision-making speed, sensory perceptions such as sight, hearing, touch, smell, and taste, and motor coordination [3-4]. In addition, genetic, environmental, and lifestyle factors also have an impact on the aging process that occurs within individual cells and tissues [5-6].

Brain aging can manifest as memory and cognitive decline, which usually involves changes in the structural plasticity of the dendritic spines. In old animals and humans, there is a decrease in the number and maturity of dendritic spines associated with changes in synaptic transmission that may reflect impaired neural plasticity, leading to the development of neurodegenerative diseases that severely disrupt basic brain function [7-8]. Excessive oxidative stress and inflammation associated with neurodegenerative diseases [9-10].

To date, there is no effective treatment for neurodegenerative diseases. Some medications are used to relieve symptoms although they usually cause many side effects. Therefore, research and development of drugs from various plants that have neuroprotective effects as supplements to improve brain function are widely carried out. *Moringa oleifera* (MO)), which is a member of the Moringaceae family, has been widely used as daily food and as medicine in various countries and has spread almost all over the world including Indonesia [11-12]. MO is known to have anti-inflammatory, and anti-apoptotic activity, in addition to having good nutritional value [13-14]. For MO, there are 2 dosage forms commonly used as traditional medicine, namely MO leaf extract and MO seed oil. The content of secondary metabolites contained in MO extracts including triterpenoids, polyphenols, saponins, tannins, and flavonoids is thought to be neuroprotective.

Neurotrophins are important mediators of the structure and function of neuroplasticity that can protect neurons against various brain damage [15-16]. BDNF is one of the neurotrophins that has many biological aspects that are regulated by neural activity. The synergistic interaction between neuronal activity and synaptic plasticity by BDNF makes it an ideal and essential regulator of the cellular processes underlying cognition and behavior. Neurotrophin signaling regulates cell survival, proliferation, and growth of axons and dendrites via TrkB receptors [17-18]. Research on the activity of Moringa leaf extract and seed oil as neuroprotective with potential TrkB protein targets has not been conducted. So the potential of *Moringa oleifera* leaf and seed oil extracts was identified as neuroprotective on the interaction of TrkB receptors with molecular docking.

Technological developments have resulted in drug development starting with the stages of bioinformatics methods (in silico) which are simply computer-based methods and use several software programs that are easily available on the public web [19]. In these in silico studies, it is generally preferred at an early stage to predict and can provide provisional estimates from studies relating to ligand/compound activity. This in silico method does not require expensive costs and does not require a very long time [20].

2. Experimental Section

2.1. Protein Preparation and Validation

The 3D structure of the TrkB receptor was selected and downloaded from the Protein Data Bank webserver (https://www.rcsb.org/) [21-22]. A well-resolution structure was selected and Ramachandran analysis was performed using the SAVES v6.0 web server (https://saves.mbi.ucla.edu/) using the PROCHECK tool to determine protein quality. Active site search of 3D protein structure was conducted through the CASTp webserver (http://sts.bioe.uic.edu/castp/index.html) using the default parameters of the webserver including Area and Volume to be used in the docking process between the target protein and the test ligand. The active site conformation chosen is the one that has the highest Area and Volume values

2.2. Ligand Preparation

A total of 18 active compounds of *Moringa oleifera* leaf extract and seed oil were obtained from literature studies and their 3D structure was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in pdb format. All test ligands were analyzed for drug-likeness and ADMETox using SwissADME (http://www.swissadme.ch/) and AdmetSAR web servers (http://lmmd.ecust.edu.cn/admetsar1/home/).

2.3. Screening and Analysis of Interactions using Molecular Docking

Target protein preparation was performed using Autodock Tools- 1.5.7 software. All water molecules, ligands, or other impurity molecules are removed. Furthermore, the addition of polar hydrogen atoms to the crystal structure of the target protein is carried out [23-24]. The prepared protein file is saved in .pdb format. The docking process is carried out using the Vina wizard tool in the Pyrex software (https://pyrx.sourceforge.io/) [25]. For the preparation of macromolecules (target protein), all the .pdb files were converted to .pdbqt format " by clicking on the right button of the protein name and then clicking on "make macro-molecule" For ligand preparation, all ligands were uploaded one by one and minimized the total energy of the ligands and all ligands were converted into .pdbqt format.

The grid box was set based on the prediction active site for the docking process with sizes X 55.0875 Å, Y 33.6045 Å, Z 234759 Å and the coordinate dimensions of the X, Y, Z axes respectively are 42.9959 Å, 41.7106 Å, 36.9023 Å. The grid box will select the search space in the protein. Next, running is done by clicking "forward". Visualization of docking results using the BIOVIA application (https://discover.3ds.com/discovery-studio).

3. Results and Discussion

3.1. Protein Preparation and Validation

The protein used in the study was the structure of Tropomyosin receptor kinase B (TrkB) Homo sapiens with PDB ID 4ASZ obtained from the Protein Data Bank webserver (https://www.rcsb.org/) [26-27] and Ramachandran analysis using the Procheck webserver (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) to determine the quality of the protein to be used at the time of docking (Fig. 1)

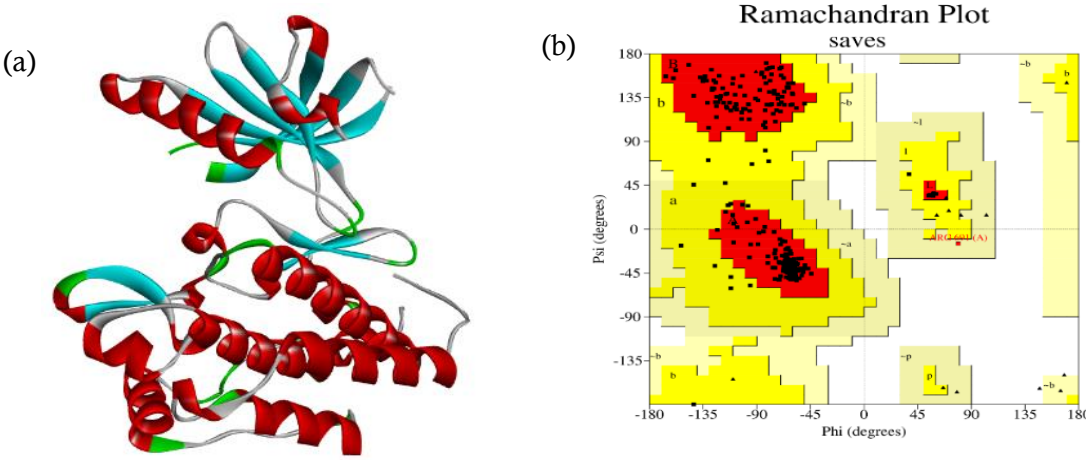


Figure 1. (a) 3D macromolecule of TrkB, (b) Validation of TrkB structure

Table 1. Results of Ramachandran plot analysis of TrkB protein

Parameter	No of Residues	%-tage
Most favoured regions [A,B,L]	235	92.9%
Additional allowed regions [a, b, l, p]	17	6.7%
Generously allowed regions [~a,~b,~l,~p]	1	0.4%
Disallowed regions	0	0.0%
Non-glycine and non-proline residues	253	100%
End-residues (excl. Gly and Pro)	5	
Glycine residues	19	
Proline residues	12	
Total number of residues	289	

Based on the PDB database, the selected TrkB protein has a resolution of 1.70 Å. One of the requirements for a good protein structure is a structure that has a resolution of ≤2.5 Å [28]. The Ramachandran plot is a distribution plot between the φ (phi) and psi angles of ψ that can reflect the area of available conformational space allowed for proteins [29]. The results of Ramachandran's analysis of the amino acids that make up the TrkB protein have an angle of φ (phi) and an angle of ψ (psi) in the allowed area with a value of 92.9% and the prohibited area with a value of 0%. Ramachandran plot has an area marked with letters A, B, L (red color) as an allowed region which is the coordinate area of the secondary structure of the protein as the maximum tolerance limit area of steric strains, if the protein is in a white area, then the amino acid is in the outliner that is in the disallowed region. A good quality model ideally has more than 90% in the most favoured regions [30]. So based on these results, the selected protein structure is good quality.

Prediction of protein receptor binding sites that may be occupied by target protein native ligands is performed via the CASTp webserver (http://sts.bioe.uic.edu/castp/index.html) using the webserver's default parameters to determine the binding pocket to be used for docking between the target protein and the test ligand.

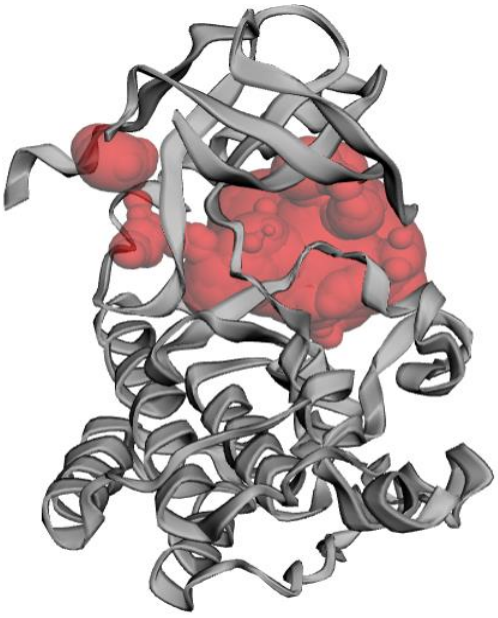


Figure 2. Active site prediction (red pocket)

Active site prediction was carried out due to the absence of a complex native ligand with the TrkB protein. The analysis results from the CASTp web server showed that the TrkB protein has 41 binding pocket conformations characterized by reaching a residual probe radius of 1.4 Å indicated by the highest Area and Volume values on the default web server. The Area and Volume values are cavity values describing the depth and breadth of the cavity at the binding site location.

The conformation chosen in this prediction result has an Area value of 837.229 Å2 and a Volume of 1055.715 Å3. The consensus binding site that belongs to the selected conformation is Asp543, Phe545, Gln547, Glu562, Phe565, Gly566, Lys567, Val568, Lys588, Leu590, Ala597, Asp600, Phe601, Arg603, Glu604, Leu607, Leu608, Thr609, Leu611, Gln612, His613, Ile616, Val617, Lys618, Phe619, Tyr620, Gly621, Met631, Phe633, Leu683, Phe688, Val689, His690, Arg691, Asp692, Asn697, Ile708, Gly709, Asp710, Phe711, Gly712, Met713, Ser714, Arg715, Asp716, Tyr722, Thr729, Met730, Leu731, Pro732, dan Phe746

3.2. Analysis of Drug-likeness and ADMETox

The physicochemical properties of drugs will regulate the mechanism of absorption, distribution, metabolism, and excretion (ADME) of drug compounds in the body. The ideal drug molecule is a drug molecule that conforms to the physicochemical properties contained in Lipinski's rule (Rule of Five (RO5)). The RO5 guidelines would predict the drug-likeness of a chemical compound with a specific biological activity designed for the oral administration route [31]. Therefore, drug-likeness analysis is important to determine the chances of the compound becoming a candidate drug molecule for the oral administration route. According to RO5, a drug-like compound must have a molecular weight (WM) of no more than 500 g/mol, a log P value of no more than 5, Hydrogen bond donors (HBD) of no more than 5, and a hydrogen bond acceptor (HBA) of no more than 10 [32].

Lipinski's rule can determine the physicochemical properties of compounds to determine the hydrophobicity of a compound to pass through the cell membrane by passive diffusion. The log P value expresses the coefficient of solubility in fat/water which has a range of -0.4 – 5. Molecular weights greater than 500 g/mol cannot diffuse through cell membranes. The greater the log P value, the more hydrophobic the molecule.

Molecules that have too hydrophobic properties tend to have a high level of toxicity because they will be retained in the lipid bilayer and distributed more widely in the body so that the bond specificity of compounds and desired targets is reduced causing off-target binding [31-32]. Log P values that are too negative are also not recommended because molecules cannot pass through the lipid bilayer membrane. The number of hydrogen bond donors and acceptors describes the energy level required in the absorption process. The higher the number of hydrogen bonds, the higher the energy required for the absorption process to occur [32].

Table 2. Results of drug-likeness analysis

Ligands	Pubchem CID	Canonical Smile	MW (g/mol)	HBA	HBD	LogP
Alpha-Tocopherol	14985	CC1=C(C2=C(CCC(O2)(C)CCCC(C)CCCC(C)CCCC(C)C)C(=C1O)C)C	430.71	2	1	5.92
Apigenin	5280443	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	270.24	5	3	1.89
Beta-Sitosterol	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	414.71	1	1	4.79
Caffeic Acid	689043	C1=CC(=C(C=C1C=CC(=O)O)O)O	180.16	4	3	0.97
Campesterol	173183	CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C	400.68	1	1	4.92
Erucic Acid	5281116	CCCCCCCCC=CCCCCCCCCCCCC(=O)O	338.57	2	1	5.22
Ferulic Acid	445858	COC1=C(C=CC(=C1)C=CC(=O)O)O	194.18	4	2	1.62
Gallic Acid	370	C1=C(C=C(C(=C1O)O)O)C(=O)O	170.12	5	4	0.21

Kaempferol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	286.24	6	4	1.70
Moringin	14865502	CC1C(C(C(C(O1)OC2=CC=C(C=C2)CN=C=S)O)O)O	311.35	6	3	2.00
Myricetin	5281672	C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	318.24	8	6	1.08
Naringenin	932	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O	272.25	5	3	1.75
Oleic Acid	445639	CCCCCCCCC=CCCCCCCCC(=O)O	282.46	2	1	4.27
Quercetin	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	302.24	7	5	1.63
Vanillic Acid	8468	COC1=C(C=CC(=C1)C(=O)O)O	168.15	4	2	1.40
Luteolin	5280445	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O	286.24	6	4	1.86
*Rutin	5280805	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O	610.52	16	10	1.58
Stigmasterol	5280794	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	412.69	1	1	5.01

*** Excluded compound**

Table 3. Results of ADMETox Analysis

Ligands	GI Abs	Inhibitor CYP					Toxicities		
		1A2	2C19	2C9	2D6	3A4	AMES	Carcinogenesis	AOT
Alpha-Tocopherol	Low	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	III
Apigenin	High	Yes	No	No	Yes	Yes	Non-AMES Toxic	Non-Carcinogens	III
Beta-Sitosterol	Low	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	I
Caffeic Acid	High	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	IV
Campesterol	Low	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	I
Erucic Acid	Low	Yes	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	IV
Ferulic Acid	High	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	IV
Gallic Acid	High	No	No	No	No	Yes	Non-AMES Toxic	Non-Carcinogens	III
Kaempferol	High	Yes	No	No	Yes	Yes	Non-AMES Toxic	Non-Carcinogens	II
Moringin	High	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	III
Myricetin	Low	Yes	No	No	No	Yes	Non-AMES Toxic	Non-Carcinogens	II
Naringenin	High	Yes	No	No	No	Yes	Non-AMES Toxic	Non-Carcinogens	II
Oleic Acid	High	Yes	No	Yes	No	No	Non-AMES Toxic	Non-Carcinogens	IV
Quercetin	High	Yes	No	No	Yes	Yes	Non-AMES Toxic	Non-Carcinogens	II
Vanillic Acid	High	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	III
Luteolin	High	Yes	No	No	Yes	Yes	Non-AMES Toxic	Non-Carcinogens	II
Rutin	Low	No	No	No	No	No	Non-AMES Toxic	Non-Carcinogens	III
Stigmasterol	Low	No	No	Yes	No	No	Non-AMES Toxic	Non-Carcinogens	I

A compound will have low permeability or poor absorption when it does not meet any of Lipinski's rules [25]. From the results of the drug-likeness analysis in Table 3, all compounds meet Lipinski's rule except Rutin. Rutin has a molecular weight of 610.52 g/mol (≥500) and a number of HBA 16 (≥10). The predicted pharmacokinetic profile results obtained as can be seen in Table 4 show

that compounds that have a high absorption rate in the gastrointestinal (GI) are Apigenin, Caffeic acid, Ferulic acid, Gallic Acid, Kaempferol, Moringin, Naringenin, Oleic acid, Quercetin, Vanillic acid, and Luteolin. While other compounds have low absorption rates in GI. The results of the prediction of metabolic rate, there are only 7 compounds that are not inhibitors of metabolizing enzymes, both CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, namely alpha-tocopherol, beta-sitosterol, caffeic acid, campesterol, ferulic acid, moringin, vanillic acid, and rutin. So that these compounds are predicted not to cause interactions with drugs or other compounds that are substrates of the metabolizing enzyme if given simultaneously.

Based on the results of toxicity analysis, all active compounds showed harmless according to the criteria of Age, Metastases, Extent, and Size (AMES) and were not carcinogenic. Based on oral acute toxicity analysis, compounds that fall into category I are beta-sitosterol, campesterol, and stigmasterol., category II are kaempferol, myricetin, naringenin, quercetin, and luteolin., category III is alpha-tocopherol, apigenin, gallic acid, moringin, vanillic acid, and rutin., category IV is caffeic acid, erucic acid, ferulic acid, and oleic acid. Classification of oral acute toxicity based on US EPA criteria that category I contains compounds with LD50 values less than or equal to 50mg/kg, and category II contains compounds with LD50 values greater than 50mg/kg but less than 500mg/kg. Category III includes compounds with LD50 values greater than 500mg/kg but less than 5000mg/kg. Category IV consists of compounds with LD50 values greater than 5000mg/kg) [33-34].

3.3 Screening and Analysis of Interactions using Molecular Docking

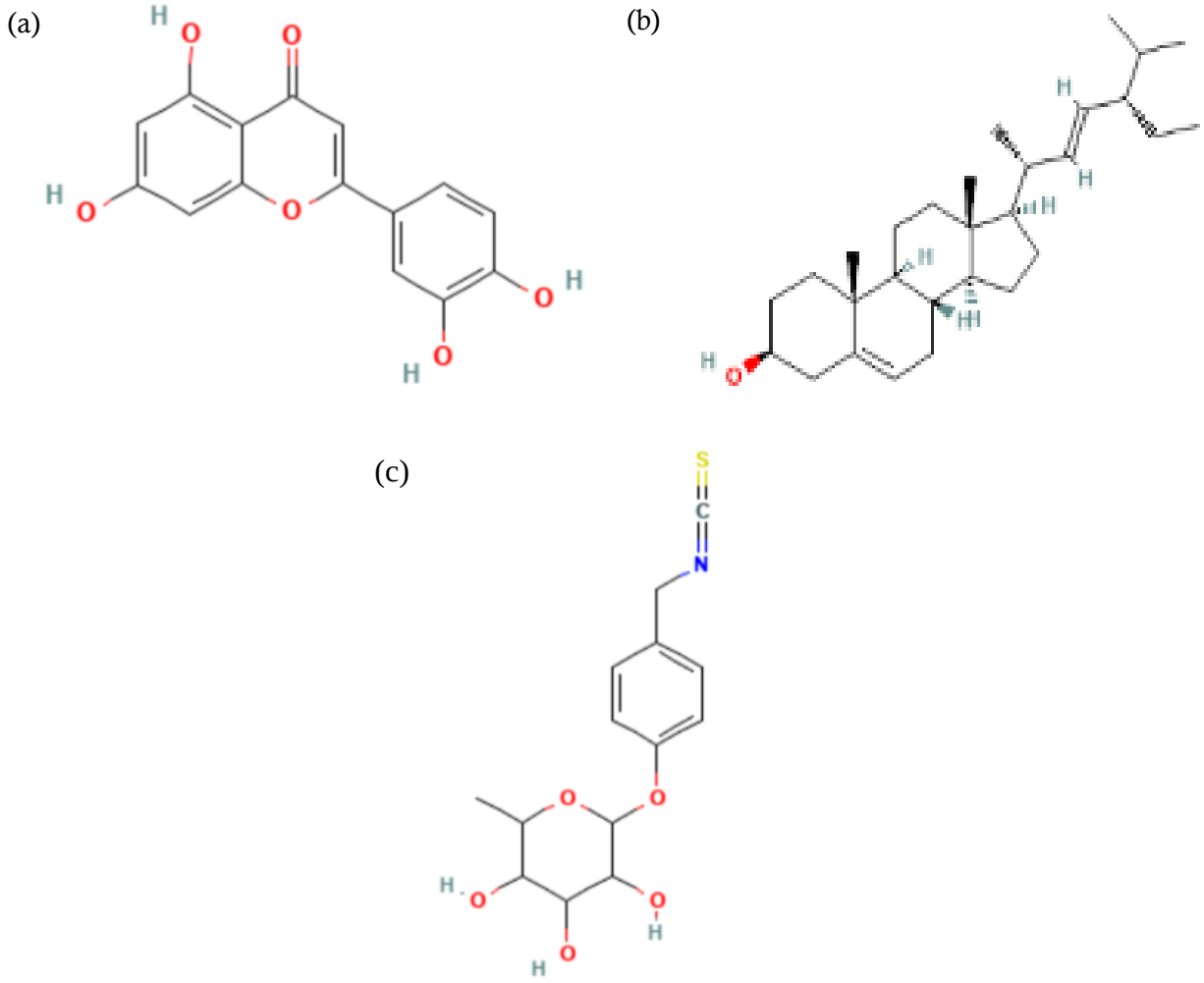
Compounds that meet the criteria for drug similarity based on Lipinski's rule and have good ADMETox analysis results are carried out docking process. The docking process is carried out using PyRx application version 0.8. The docking process is carried out between compounds and TrkB receptor (PDB ID: 4ASZ). In this study, tethering was carried out on a grid box arranged in the area of the prediction of active site protein with a size of X 55.0875 Å, Y 33.6045 Å, Z 234759 Å and the coordinate dimensions of the X, Y, Z axes respectively are 42.9959 Å, 41.7106 Å, 36.9023 Å.

Table 4. Binding Affinity of Protein Target and Active Compound of Leaf Extract and seed oil of *Moringa oleifera*

Ligand	ΔG (kcal/mol)
Alpha-Tocopherol	-7.0
Apigenin	-7.3
Beta-Sitosterol	-7.5
Caffeic Acid	-6.1
Campesterol	-7.2
Erucic Acid	-5.2
Ferulic Acid	-5.8
Gallic Acid	-5.8
Kaempferol	-7.0
***Moringin**	**5.9**
Myricetin	-7.2
Naringenin	-7.2
Oleic Acid	-5.6
Quercetin	-7.1
Vanillic Acid	-5.6
***Luteolin**	**-7.6**
***Stigmasterol**	**-8.1**

*** Selected compounds**

Two molecules that have the best affinity binding are visualized to see the interaction between ligands and receptors. The data obtained from the visualization results are in the form of bond types, bond distances formed by atoms on ligands involved in bonds between ligands and protein amino acids. The parameter used to determine the binding value of Affinity is the free energy analysis of the binding (ΔG). The stability of the interaction between the ligand and the target receptor was predicted by analysis of the free binding energy indicated by a low ΔG value [35]. Stable ligand-protein conformation if it has a low ΔG value and less stable ligand-receptor complex if the ΔG value is high. The more negative the ΔG value, the higher the ligand-protein complex affinity, the more stable interaction between the compound and its protein, and the better the activity of the compound [36]. Docking results showed that luteolin and stigmasterol had the most negative binding affinity, namely -7.6 kcal/mol and -8.1 kcal/mol. Moringin is a marker compound in *Moringa oleifera*



Gambar 3. Struktur 2D. (a) luteolin, (b) stigmasterol, (c) moringin

Table 5. Docking scores, H-bond interactions, Hydrophobic interactions, Electrostatic interactions, and the Binding affinity of the best Hit compound BIOVIA Discovery Studio

Receptor	Ligand	Binding affinity (kcal/mol)	Hydrogen bond interactions (distance, Å)	Hydrophobic interactions
TrkB	Luteolin	-7.6	Ser714 (2.18) Lys643 (2.58) Met713 (2.25)	Met713 Leu560
	Stigmasterol	-8.1	-	His690 Ile616 Val617 Leu611 Leu683 Arg603 Phe688
	Moringin	-5.9	ASP710 (1.98)	His690 Leu683 Phe688

3.4 Visualization of Docking Results

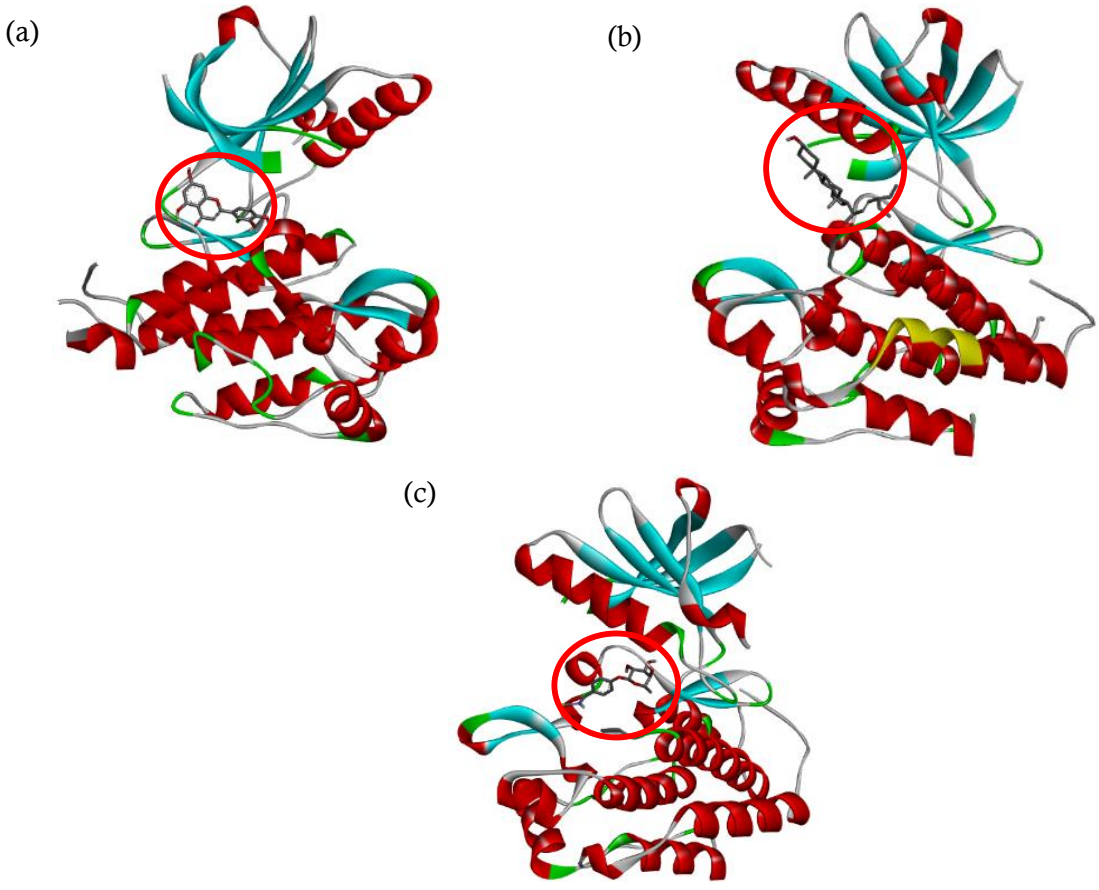


Figure 4. 3D Complex of ligands and TrkB. (a) luteolin, (b) stigmasterol, (c) moringin

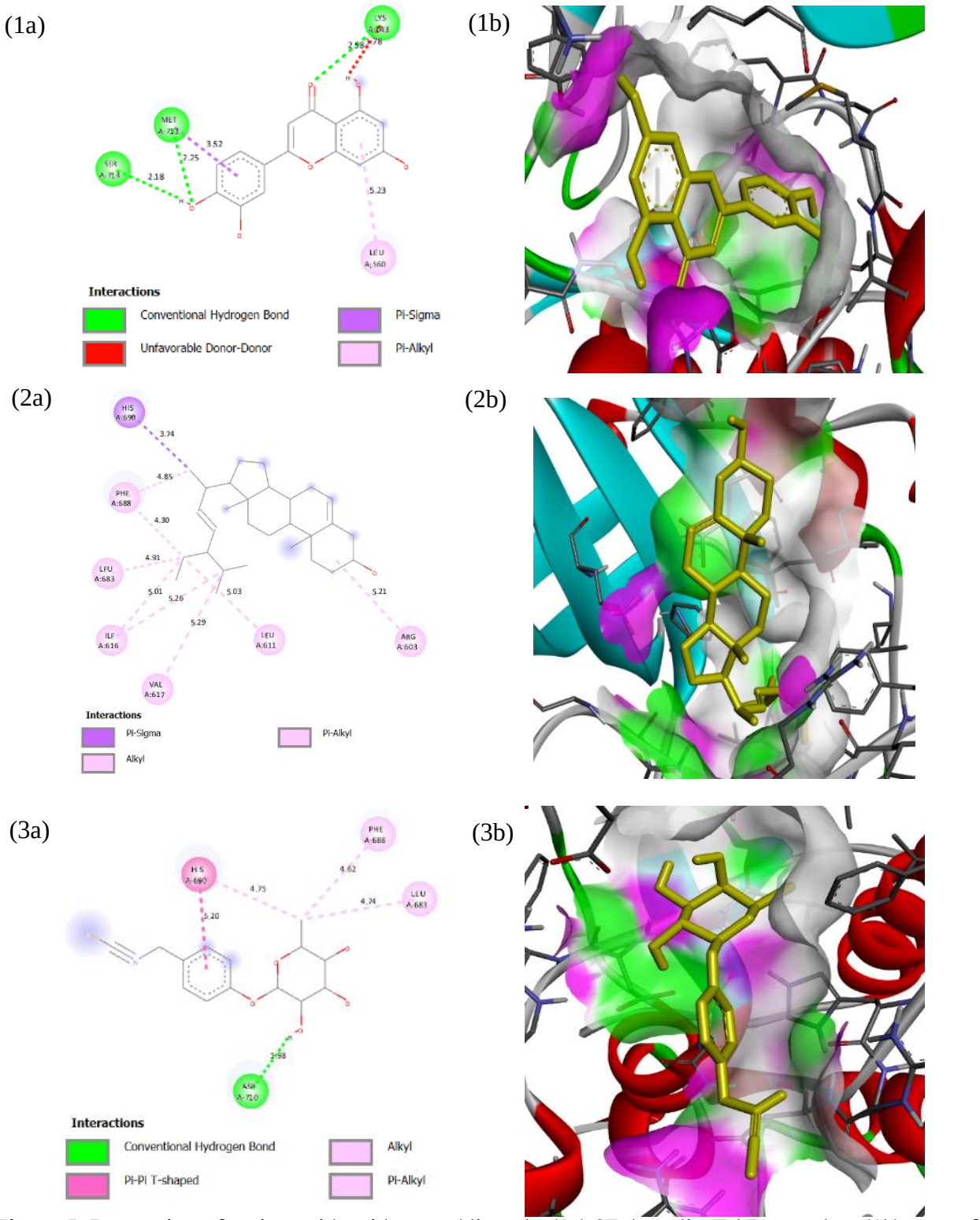


Figure 5. Interaction of amino acid residues and ligands. (1a) 2D luteolin-TrkB complex, (1b) pose of luteolin-TrkB complex, (2a) 2D stigmasterol-TrkB complex, (2b) pose of stigmasterol-TrkB complex, (3a) 2D moringin-TrkB complex, (3b) pose of moringin-TrkB complex

The docking process is carried out on the TrkB active site area (PDB ID 4ASZ) obtained based on active site predictions using the CASTp web server. The active site topology TrkB is used in molecular tethering (Fig. 2). Based on the results of amino acid residue interaction analysis, luteolin showed hydrogen bond interactions in Ser714 amino acid residues with a bond distance of 2.18 Å and Met713 with a bond distance of 2.58 Å which is part of the active site and luteolin also showed hydrophobic bond interactions in amino acid residues of Met713 which is also part of the active site (Fig 1a).

Furthermore, stigmasterol compounds do not show hydrogen bonding but show hydrophobic bonds in residues His690, Ile616, Val617, Leu611, Leu683, Arg603, and Phe688 which are part of the predicted active site (Fig. 2a). Meanwhile, moringin compounds form a hydrogen bond at the amino acid residue Asp710 with a distance of 1.98 Å and hydrophobic interactions at the amino acid residues His690, Leu683, and Phe688 which are all part of the active site (Fig 3a). The closer the distance between the ligand bonds to the amino acid residues on the receptor, the more the ligand bonds with these residues; thus, the more stable the resulting bond. Hydrogen bonding is a desirable bond in ligand-receptor interactions because it can affect the physicochemicalness of the nature of the compound and its biological activity. Hydrophobic bonds indicate the degree of solubility of the drug in the cell membrane, which is thought to be effectively bound to the receptor [36].

4. Conclusions

The results of the drug-likeness analysis, all compounds except Rutin meet the criteria based on the Lipinski rule. Based on ADMETox analysis, all compounds have a good pharmacokinetic profile and are harmless according to the criteria of Age, Metastases, Extent, and Size (AMES), are not carcinogenic, and acute oral toxicity is quite safe. The docking results showed the two best compounds, namely luteolin with ΔG -7.6 kcal/mol and stigmasterol ΔG -8.1 kcal/mol. Moringin as a marker compound with ΔG -5.9 kcal/mol. The results of the analysis of the interaction of amino acid residues from the three compounds to the TrkB protein showed an interaction through hydrogen and hydrophobic bonds at the active site of the prediction results. So it can be concluded that compounds in the leaves and seed oil of moringa oleifera with luteolin, stigmasterol and moringin marker compounds are predicted as ingredients that are able to activate TrkB receptors in the aging process (ageing).

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