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Molecular Docking and Toxicity Test of Apigenin Derivative Compounds as an Anti-Aging Agent

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ABSTRACT

The intrinsic factors that caused aging are enzymes such as hyaluronidase and elastase by oxidative stress mechanism. Antioxidants are the bioactive compounds which are highly important to fight against oxidative stress that can cause aging. Modified compounds of apigenin have reported can act as an antioxidant. The aim of this study was to determine candidate of apigenin derivative compounds that were potential to inhibit hyaluronidase and elastase enzyme by using molecular docking method with human target protein with pdb.id : 2JIE and 5JMY. Molecular docking was done using windows operating system with several softwares, i.e: PLANTS, YASARA and MarVinSketch. Visualization of bonding modes between the ligand and amino acid residues was done with PyMol. Of the 50 apigenin derivative compounds tested, obtained 9 compounds with lower docking scores than apigenin in inhibiting hyaluronidase and 5 compounds in inhibiting elastase enzyme. 3'6-diamineapigenin had the lowest docking score (-62.39) in inhibiting hyaluronidase enzyme (2JIE) and 3'amineapigenin had the lowest docking score (-91.31) in inhibiting elastase enzyme (5JMY). The binding interactions of the actively docked conformations of the ligand and the target protein have been identified and showed the most amino acid residues that considered affect hyaluronidase and elastase inhibition process such as VAL_710, GLU_762 and VAL_763. Based on these results, there are some antioxidants of the apigenin derivative compounds that recommended as an anti-aging agent.

Keywords: apigenin, antioxidant, anti-aging, molecular docking

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1. Introduction

Aging is a process of gradual disappearance of the network's ability to repair or replace itself and maintain its structure, as well as its normal function. Skin can experience aging, one of which is marked by the emergence of wrinkles (wrinkle). It does not occur solely because of the increasing age of the skin itself, but can also occur due to external factors. Aging can also be caused by various factors, i.e factors that come from within the body itself (intrinsic factor) or factors that come from outside body (extrinsic factor). The intrinsic factors include the activity of certain enzymes. Increased activity of certain enzymes involved in the aging process of the skin includes elastase, hyaluronidase, and collagenase (Thring et al., 2009).

Elastase is a proteolytic enzyme involved in the degradation of the extracellular matrix (ECM) that includes elastin. Elastin provides much of the elastic recoil properties of skin, arteries, lungs, and ligaments. Loss of elastin is a major part of what causes visible signs of aging (wrinkles, sagging) in the skin (Thring et al., 2009).

Hyaluronic acid or hyaluronan is one of the important components of the tissue matrix substance and has a role in the development, growth, and repair of damaged tissue (Pogrel et al., 1996). Meanwhile, elastin plays a role in the maintenance of skin elasticity, but elastase can degrade it (Thring et al., 2009). Degradation of the Extracellular Matrix (ECM) has been directly linked to skin aging and is correlated with an increase in activity of certain enzymes involved in skin aging (Longo et al., 2003; Makrantonaki et al., 2010). Inhibition of these enzymes is crucial in anti-aging prevention (Ndlovu et al., 2013).

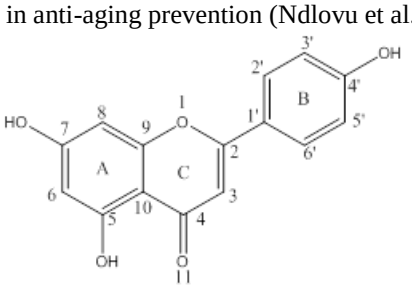

Fig. 1. Based structure of apigenin

Apigenin is the main organic compound of flavonoids contained in celery belonging to the flavon group (Fig.1). The compound that has three aromatic rings with the -OH- grouped branches. Apigenin can be synthesized and developed into a potent antioxidant compound (Seyoum et al., 2006; Ray, 2012). Several studies related to the development of apigenin structure have been done to

increase the ability of antioxidant activity (Perwira et al., 2015; Mumpuni et al., 2017). Mumpuni et al. (2017) has modified the structure of the apigenin and predicted its ability as antioxidant using the QSAR method (Semiempirical Austin Model 1). Of the 90 modified compounds of apigenin, obtained 50 compounds that have better activity as antioxidant than apigenin.

Antioxidants are the bioactive compounds which are highly important to fight oxidative stress that can produce free radicals which are affected for various diseases such as heart attack, cancer, cataract, atherosclerosis, neurodegenerative disease, decreased kidney function, diabetes, and premature aging (Uttara et al., 2009).

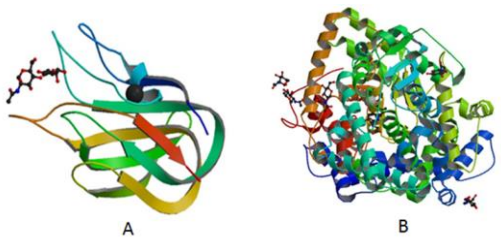

Fig.2. Crystalline Structure of Receptor (A) 2JIE; (B) 5JMY (http://rcsb.org)

In this study carried out analysis of apigenin derivative compounds that have been reported by Mumpuni et al. (2017) as an antioxidant that plays a role in inhibition of hyaluronidase and elastase enzyme using molecular docking method with enzyme of hyaluronidase and elastase that present in protein data bank (PDB) data base with id 2JIE and 5JMY (Fig. 2). Visualization and elucidation of the bonding mode between a ligand and target protein to determine amino acids residues that play a role in ligand-protein affinity in the inhibition process. In this study will also be screened about the toxicity of apigenin derivative compounds using a protox webserver.

2. Materials and Methods

2.1. Tools.

The hardware used in this research is a laptop with AMD A6 processor, 4 GB RAM, Windows 10, 64-bit operating system. Software used includes: PLANTS, Marvin Skecth, PyMol, and YASARA.

2.2. Materials

Fifteen (50) 2D structures of apigenin derivative compounds of research results by Mumpuni et al. (2017), crystalline form of protein with PDB ID : 5JMY and 2JIE.

2.3. Research Procedure

2.3.1. Preparation of Proteins and Ligands

Preparation of proteins and ligands was done using YASARA software. Proteins with 5JMY.pdb.id and 2JIE.pdb.id that have been downloaded from database of Protein Data Bank loaded on YASARA worksheets, proteins, and native ligands are separated and saved with file names protein.mol2 and ref_ligand.mol2. Native ligands and new ligands (apigenin derivative compounds) are continuously prepared to form conformations using Marvin Skecth and saved with file name ligand.mol2.

2.3.2. Docking protocol validation

The docking protocol validation is performed by calculating the Root Mean Square Deviation (RMSD) values between the native ligand of pdb.id with the conformation of the docked ligand. Docking protocol is considered good and can be used for the further docking process if it has a value of 2 - 2.5 Å, closer to 0 considered the alignment is better. This validation result used to configure the plantsconfig.file.

2.3.2. Molecular docking

The docking process has done with standard procedure of molecular docking using PLANTS (Korb et al., 2009; Purnomo, 2010). Validated protocol (plantsconfig.file), ref_ligand.mol2, protein.mol2, and ligand.mol2 prepared as input data. Docking process has done by typing commands on cmd. The PLANTS application will read the validated protocol and start the docking process. Docking process obtained docking scores as output data that showed the energy of the ligand in binding to the target protein. The more negative of docking scores the affinity of the ligand binding to the protein is stronger.

2.3.2. Toxicity Screening

Toxicity screening has done using ProTox webserver, test compounds were prepared with SMILES file type. Then input on ProTox webserver then run the calculating program of toxicity. The toxicity level performed in LD_{50}, mg/kg unit.

3. Results and Discussion

The RMSD value obtained from the docking protocol validation process was 1.1 Å for 5JMY enzyme and 1.69 Å for the 2JIE enzyme, this value is eligible for the protocol to be used for further docking process. The alignment between the reference ligand and the conformation of the docked ligand is very good (Fig. 3). The docking process of apigenin derivative compounds was performed on 50 apigenin derivative compounds that were tested in silico by QSAR method that having better antioxidant activity than apigenin by Mumpuni et al. (2017) (Table 1). Of the 50 compounds tested, obtained 9 compounds having a lower docking score than apigenin in inhibiting hyaluronidase enzyme i.e 3,6-diethoxy apigenin, 3'-amine apigenin, 3,6-dimethoxy apigenin, 3,6-dipropoxy apigenin, 3'-ethoxy apigenin, 3'-fluoro apigenin, 3'6 diamine apigenin, 6-amine apigenin,

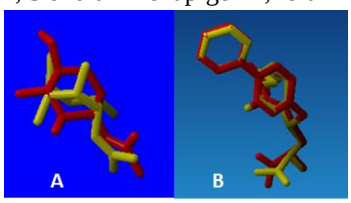

Fig. 3. Alignment of ref_ligand (red) of (A) 2JIE and (B) 5JMY receptor with docked ligand conformation (yellow)

Table 1. Docking score of apigenin derivative compounds

No	apigenin derivative compounds (test ligand)	Receptor		LD_{50} (mg/Kg)
		Hyaluronidase (2JIE)	Elastase (5JMY)	
1.	3 3'6 trimethoxy apigenin	-49.64	-57.80	5000
2.	3 6 diethoxy apigenin	-57.40	-76.31	5000
3.	3' amine apigenin	-57.58	-91.31	3919
4.	3 amine apigenin	-49.21	-72.05	3919
5.	3 ethoxy apigenin	-48.08	-77.21	5000
6.	3 ethyl apigenin	-51.23	-77.71	3919
7.	3- isopropyl apigenin	-51.15	-72.96	3919
8.	3 isopropoxy apigenin	-49.08	-73.14	3919
9.	3- methyl apigenin	-52.48	-73.7	3919
10.	3- methoxy apigenin	-52.47	-79.98	3919
11.	3- propyl apigenin	-51.27	-82.29	3919
12.	3 propoxy apigenin	-46.30	-75.31	5000
13.	3,3',6-triethyl apigenin	-47.74	-75.51	159
14.	3,3',6-triethoxy apigenin	-44.73	-59.77	5000
15.	3,3',6-triisopropyl apigenin	-51.25	-73.18	159
16.	3,3',6-triisopropoxy apigenin	-42.65	-72.59	5000
17.	3,3',6-trimethyl apigenin	-50.66	-75.81	159
18.	3,3',6-tripropyl apigenin	-48.08	-70.90	159
19.	3,3',6-tripropoxy apigenin	-47.95	-62.57	5000
20.	3,3'-diethyl apigenin	-48.85	-80.59	159
21.	3,3'-diisopropyl apigenin	-51.15	-73.94	159
22.	3,3'-dimethyl apigenin	-50.79	-86.94	3919
23.	3,3'-dipropyl apigenin	-52.78	-87.74	159
24.	3,3'-dipropoxy apigenin	-50.38	-71.62	5000
25.	3,6-diisopropyl apigenin	-50.37	-69.35	159
26.	3,6-dimethyl apigenin	-50.02	-77.24	159
27.	3,6-dimethoxy apigenin	-58.83	-82.98	5000
28.	3,6-dipropyl apigenin	-49.60	-75.58	159
29.	3,6-dipropoxy apigenin	-58.83	-82.97	5000
30.	3'- ethoxy apigenin	-58.85	-82.97	4000
31.	3'- fluoro apigenin	-57.44	-86.97	3919
32.	3'- chloro apigenin	-55.88	-81.80	1070
33.	3',6-diethoxy apigenin	-53.82	-83.14	3919
34.	3',6-dimethoxy apigenin	-48.85	-83.29	3919
35.	3',6-dipropoxy apigenin	-53.83	-83.28	5000
36.	3'6 diamine apigenin	-62.39	-85.21	1070
37.	6-amineapigenin	-57.14	-83.65	1070
38.	6-ethyl-apigenin	-55.81	-80.17	3919
39.	6-ethoxy apigenin	-53.88	-80.73	4000
40.	6-propoxy-apigenin	-49.39	-75.75	4000
41.	6-methyl-apigenin	-56.71	-78.86	3919
42.	6-methoxy apigenin	-50.83	-75.72	4000
43.	33' diamine apigenin	-50.76	-76.02	3919
44.	33' diethoxy apigenin	-51.53	-74.58	5000
45.	33' diisopropoxy apigenin	-51.05	-74.49	159
46.	33' dimethoxy apigenin	-51.19	-74.49	5000
47.	33'6 triamine apigenin	-53.63	-74.39	3919
48.	36 diamine apigenin	-48.16	-67.84	3919
49.	36 diethyl apigenin	-51.56	-72.07	159
50.	36 diisopropoxy apigenin	-50.34	-68.79	159
51.	Apigenin	-56.15	-84.80	2500

6-methyl-apigenin and 5 compounds that having a lower docking score than apigenin in inhibiting elastase enzyme i.e 3'-amine apigenin, 3,3'-dimethyl apigenin, 3,3'-dipropyl apigenin, 3'- fluoro apigenin, 3'6-diamine apigenin. The 3'6-diamine apigenin had the lowest docking score in inhibiting hyaluronidase enzyme (2JIE) and the 3'amineapigenin had the lowest docking score in inhibiting elastase enzyme (5JMY). The chemical structures of these compounds are shown in Fig. 4. Through molecular docking can be known the potential of compounds to be a drug candidates based on the affinity of binding to target proteins (Kastritis et al., 2012). Binding affinity is an important

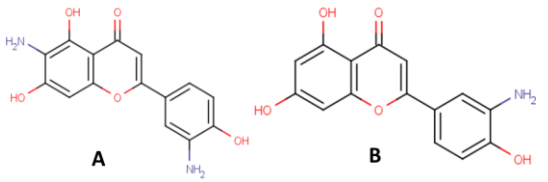

Fig.4. Structure of (A) 3'6-diamine apigenin (B) 3'amine apigenin

aspect to be considered in molecular and macro molecular interactions (Seo et al., 2014). This binding affinity was illustrated by the value of the docking score. Lower binding affinities indicate that a compound requires less energy to engage in binding or interaction. In other words, the lower affinity value of binding increases the potential for binding to the target protein (Baker et al., 2007; Tassa et al., 2010). Score docking of 3'6 diamine apigenin and 3'amine apigenin were -62.39 and -91.31 (Table 1). These were the lowest score than the others derived compounds of apigenin. Its considered that 3'6 diamine apigenin and 3'amine apigenin have the best affinity in the binding site of anti-aging receptor 2JIE and 5JMY respectively. The negative value of binding energy change (ΔG) reveals that the binding process is spontaneous, it can fit well in the binding pocket receptor forming most stable drug receptor energetically (Kumar et al., 2014). Larger the negative value of binding energy, greater the chemical be accepted as a drug (Balavignesh et al., 2013).

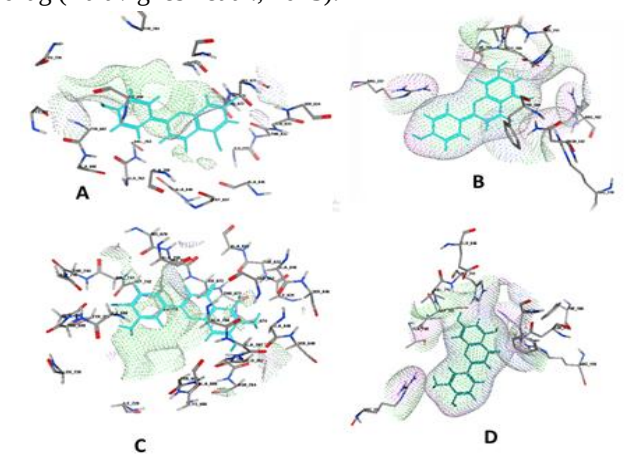

Fig.5. Hydrophobic areas (green) and hydrogen bond mapping (purple) in binding pocket of ligand-receptor (A) apigenin – 2JIE (B) apigenin – 5JMY (C) 3'6-diamine apigenin – 2JIE (D) 3' amine apigenin – 5JMY

The better affinity was due to the replacement of substituent affecting the properties of the ligand, based on the receptor properties mapping showed in Fig. 5. It was seen that the binding pocket of the 5JMY receptor was more hydrophobic than the binding pocket of the 2JIE receptor (green). The substitution of hydrogen had an effect on the lipophilicity of ligand, replacing H atom with an amine group at position 3' have made the log P value of 3'amine apigenin (2.002) not much different from apigenin (2.1). A large log P value makes 3'amine apigenin had a good interaction in binding pocket of the 5JMY receptor. Whereas replacement of hydrogen with amine groups at positions 3' and 6 on apigenin actually decreases the log P value of 3'6-diamine apigenin (1.5). It makes the hydrophobicity of 3'6-diamine apigenin was lower than apigenin. 3'6-diamine apigenin had a better affinity in the binding pocket of the 2JIE receptor. The log P value could be used to show the ability of a molecule to penetrate a biological membrane that was like a fat layer (Hansch et al., 1972).

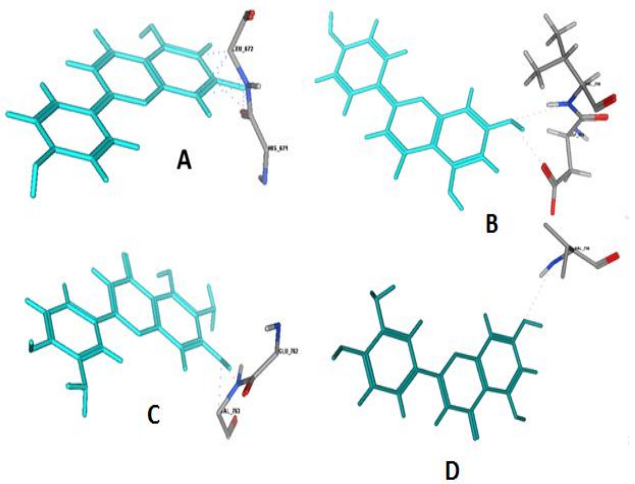

Fig. 6. Hydrogen bond (red) and Van Der Walls interaction (blue) of ligand and active amino acid residues of the receptor (A) apigenin – 2JIE (B) apigenin – 5JMY (C) 3'6-diamine apigenin – 2JIE (D) 3' amine apigenin – 5JMY

The binding interactions of the most active docked conformation of the ligands of apigenin derivative compound and the target proteins have been identified using PyMol. Checked one by one all amino acids within 4 Å of the active site of the target protein, the important binding interactions were identified. The interaction of ligands with the binding pocket receptor is shown in Fig. 3. Apigenin and 3' amine apigenin occupied the same binding pocket of the 5JMY receptor with some active amino acid residues i.e THR_708, ASP_709, HIS_711, ARG_222, VAL_710, ARG_102. ARG_110, ASN_542, PHE_544. Hydrogen bonds formed between apigenin and active amino acid residue ASP_709 and VAL_710 with distances 1.89 and 2.1 Å respectively, whereas in 3'amine apigenin formed a hydrogen bond with active amino acid residue VAL_710 with distance 3.23 Å (Fig. 6). Apigenin and 3'6-diamine a pigenin occupied the same binding pocket of 2JIE receptor with some active amino acid residues i.e LUE_672, THR_673, HIS_671, SER_634, ALA_633, THR_632, ILE_631, ALA_645, TRP_657, ALA_648, ALA_760, GLU_762, VAL_763, ALA_688, TYR_687, LYS_727, LEU_720, TYR_701, ILE_698, VAL_743, and LEU_741. Apigenin formed Van Der Walls interactions with active amino acid residue HIS_671 and LEU_672 with distance 2.17 Å and 1.62 Å, whereas 3'6-diamine apigenin forming a hydrogen bond with active amino acid residue GLU_762 with distance 0.74 Å and formed a Van Der Walls interaction with active amino acid residue VAL_763 with

distance 1.97 Å. In addition to hydrogen bonding, the activity of ligand inhibiting on receptor was also influenced by electronic bonding, hydrophobic and van der walls interaction (Khalid et al., 2013).

Toxicity of compounds is very important on determining a candidate of a drug. So, in this research has done tested the toxicity of apigenin derivative compounds (Table 1). The most apigenin derivative compounds have very low toxicity, included the both of active compounds i.e 3'6-diamine apigenin and 3'amine apigenin.

4. Conclusion

The most active conformation of apigenin derivative compound as antioxidant acting as anti-aging agent is the 3'6-diamine apigenin that active to inhibit hyaluronidase enzyme (2JIE) and the 3'amine apigenin that active to inhibit elastase enzyme (5JMY).

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