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In-silico study of antioxidant-anticancer activity of phenolic and flavonoid compounds of Mangifera species using molecular docking PLANTs

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Abstract

Background: The development of science and technology has created a study of anticancer drug discovery through molecular docking. This method can be applied to screening natural compounds that have antioxidant properties as anticancer candidates.
Objective: The aim of the *in-silico* study is to find out the potential antioxidant-anticancer activities using molecular docking of phenolic and flavonoid compounds contained in the Mangifera species.
Methods: Mangiferin, homomangiferin, isomangiferin, quercitrin, kaempferol 3-O-glucoside, catechin, epicatechin, daidzein, genistein, α-tocopherol, gallic acid as the test compounds and Vitamin C, doxorubicin, and hydroxyurea as comparison were prepared with MarvinSketch. The targeted protein data bank (PDB) codes used are 1V4S, 1XAN, 2BEL, 4K7O, 5M2F, 6COX, and 2W3L which were prepared with YASARA. The prepared compounds and proteins docked with each other using PLANTs software.
Result: The *in-silico* results showed that only vitamin C can exceed the native ligand docking against the 1V4S receptor. α-tocopherol has a better binding affinity compared to vitamin C on 1XAN, 2BEL, and 5M2F but could not reach the native ligand score. All of the test compounds have the potential antioxidant activity against the 4K7O protein receptor, but α-tocopherol is the only one that has the ability to inhibit the 6COX protein receptor. α-tocopherol also has better anticancer activity against breast cancer initiator (2W3L) compared to other test compounds, doxorubicin, hydroxyurea, and native ligands.
Conclusion: The conclusion is that α-tocopherol has the most potential as an antioxidant and anticancer candidate through *in silico* studies.
Keywords: Mangifera species, antioxidants, anticancer, phenolics, flavonoids, molecular docking

1. Introduction

The development of science and technology in this day and age has grown rapidly, especially in the field of health, so that many have given birth to various kinds of studies that, in their learning, can make it easier to understand various kinds of diseases, such as cancer, how to cure them, and create a study of anticancer drug discovery. Molecular docking is one of the studies that was used for the screening of compounds based on computer-assisted structural principles. This is a way to explore the interaction of a molecule, such as a drug candidate, with a target enzyme, which binds to one another. Molecular docking studies can be used to calculate the potential for anticancer drugs (Purnomo, 2011; Purnomo, 2013). This method can be applied to screening natural compounds that have antioxidant properties as anticancer candidates.

Cancer is the second deadliest disease in Indonesia. Ministry of Health data in 2019 stated that there are 42 breast cancer patients, 1 per 100,000 inhabitants. Cancer is caused

by a free radical that enters the body and can bind to amino acids so that new cells are formed that are suspected to be the beginning of cancer. The form of one of the free radicals is ROS (Reactive Oxygen Species), which is an oxygen derivative oxidizing compound that is highly reactive and causes DNA mutations, which can further trigger the occurrence of cancer. Based on this antioxidant, it is necessary to prevent or inhibit the occurrence of chain oxidation reactions of ROS (de Miguel & Cordero, 2012).

Plants that are widely proven to have antioxidant activity are the Mangifera species. The content of compounds owned by members of the Mangifera species is identical to each other. Mango contains polyphenols, carotenoids, vitamin C, and α-tocopherol compounds that are tested to have antioxidant activity (Kim *et al*., 2010). The content of polyphenols found in mangoes is mainly on the leaves, namely gallic acid, quercetin 3-β-D glucosides, α-tocopherol, 3-methyl gallate, propyl gallate, catechins, epicatechins, and also mangiferin, which is the main constituent on the leaves and bark. Mangiferin compound is proven to have antioxidant, immunomodulatory, anti-inflammatory, antidiabetic, and anticancer activity (Ramírez *et al*., 2016; Imran *et al*., 2017; Kulkarni & Rathod, 2018).

Previous research by Ribeiro *et al*. (2008) concluded that, in addition to mangiferin and its derivatives, mango fruit contains kaempferol 3-O-glucosides. Sulaiman & Ooi (2012) mentioned that mango fruit meat contains ellagic acid, protocatechuic acid, and m-digallic acid. Plants of bacang (*Mangifera foetida*), kuini (*Mangifera odorata*), and bambangan (*Mangifera pajang*) were identified as containing isoflavones which are daidzein and genistein (Khoo & Ismail, 2008). These compounds have been shown to play a role in producing antioxidant activity *in vitro* even though some of them have anticancer activity.

Plant-derived antioxidants, especially phenolics, have gained considerable importance due to their potential health benefits. Epidemiological studies have shown that the consumption of plant foods containing antioxidants is beneficial to health because it down-regulates many degenerative processes and can effectively lower the incidence of cancer and cardiovascular diseases (Ramadhan & Forestryana, 2021). The lack of *in silico* research on Mangifera species, especially its anticancer activity, has encouraged researchers to explore the lead compounds that can potentially become new drug candidates. This research was implemented to further study the potential antioxidant and anticancer activity of phenolic and flavonoids contained in the Mangifera species through testing *in silico* using the molecular docking method with PLANTS (Protein-Ligand Ant System) (Purnomo, 2011).

2. Method

2.1 Tools

The tools used in this study are ASUS X450C Windows 7 Ultimate 64-bit laptop hardware, an 1.8GHZ Intel Core i3 processor, 6GB of RAM, 500GB of HDD, Intel HD 4000 graphics and software PLANTS v.1.1 for docking, VirtualBox v.5.1.10.12026, Marvin Sketch v.5.2.5, YASARA v.10.1.8 for preparation of ligand; ref_ligand; and protein, Discovery Studio 2021 Visualizer for visualization of ligand-receptor interactions.

2.2 *Material*

The materials used are the 2D structure of mangiferin, homomangiferin, isomangiferin, quercitrin (quercetin 3-β-D glucosides), kaempferol 3-O-glucoside, catechin, epicatechin, daidzein, genistein, α-tocopherol, and gallic acid. The 2D structure of vitamin C, hydroxyurea, and doxorubicin as comparator compounds. Target proteins downloaded from https//www.rscb.org/ with pdb (protein data bank) codes are 1V4S (glucokinase isoform 2), 2BEL (corticosteroid 11-beta-dehydrogenase isozyme 1), 2C9V (superoxide dismutase), 1QQW (catalase), 1XAN (human gluthathion reductase), 5M2F (aldo-keto reductase family 1 member B10), 4K7O (peroxiredoxin-5 mithocondrial), 3PPO (HER-2 or human epidermal growth factor receptor 2), 6COX (cyclooxygenase-2), 2W3L (phenyl tetrahydroisoquinoline amide complex) along with each native ligand.

2.3 Method and data analysis

2.3.1 Preparation of protein

The complex structure of proteins (.pdb) is obtained from the Protein Data Bank (PDB) downloaded from the http://www.rscb.org/ website. Protein preparation with the YASARA program. The preparation result obtained mol2 protein file and ref_ligand mol2 (Purnomo, 2011; Rachmania *et al*., 2018).

2.3.2 Preparation of native ligands, comparative compounds, and test compounds

Preparation of native ligands, comparative compounds, and test compounds using Marvin Sketch version 5.2.5. Ligan in ligand_2D.mrv format was selected into 10 conformers, then saved as ligand.mol2. The procedure is performed for native ligands, comparative compounds, and test compounds (Purnomo, 2011; Martati *et al*., 2019).

2.3.3 Protein validation and RMSD value assignment

PLANTs application is running via VirtualBox. Native ligands are prepared, then optimized with the target protein structure using the PLANTs application to obtain docking scores. Selected the lowest score is then saved in the form of a mol2 file. The calculation of

RMSD (Root Mean Square Distance) from the optimization results using the YASARA program according to the results of experiments or structure proteins (Purnomo, 2011; Martati *et al*., 2019).

2.3.4 Docking of compounds

PLANTs application is run via VirtualBox. Native ligand files, test compounds, and comparative compounds obtained from the preparation procedure are then docked using the PLANTs program against the predicted proteins, by writing a specific shell script on VirtualBox until a docking score output obtained (ChemPLP) (Purnomo, 2011; Martati *et al*., 2019).

2.3.5 Analysis and visualization of molecular docking

Docking results are viewed through the output in notepad format. The determination of docking results is done by selecting the conformation that has the lowest ChemPLP, or free energy score. The docking results were visualized using YASARA to see the hydrogen bond distance of <3.5 Å (Rachmania *et al*., 2018).

3. Result and discussion

Docking simulation is used in this study using the PLANTS method, which has the advantages of free application and easy operation. The match between the ligand molecule and the active site, or the specificity of the mooring status of the protein, is likened to a keyhole. The match of an active site or urgent mooring site induces the conversion of ligand conformation, known by the release of a certain amount of energy called Gibbs docking energy (ΔGbind). Docking score is calculated among others with ChemPLP value based on Gibbs free energy, where the smaller (the negative) the value of the test compound against the comparative compound, then it can be said that the compound has a good binding affinity to receptors (Martati *et al*., 2019; Tegar & Purnomo, 2013). Protocol molecular docking is accepted when the RMSD heavy atoms of the docking result of compounds against the target protein are less than 2.0 Å (Purnomo, 2013). PLANTS docking simulation is incompatible with receptors of 2C9V (superoxide dismutase), 1QQW (catalase), and 3PPO (HER-2, or human epidermal growth factor receptor 2) because of the resulting error condition when protein preparation on the YASARA program. The determination of the RMSD of the native ligand is done by loading the ref_ligand.mol2 file and the docked ligand-protein complex file in the YASARA application so that the RMSD will be calculated. Based on Table 1, the best score of the 10 conformations from the docking results selected one of each protein with RMSD <2.0 Å for testing the antioxidant-anticancer activity further. All

RMSD results meet the criteria of less than 2 Å, so it can be said that the docking protocol is declared valid.

Table 1. Determination of RMSD value of native ligand

Reseptor code	Conformer	Docking score	RMSD (Å)
1V4S	1	-73.8522	1.8684
1XAN	2	-147.413	1.4765
2BEL	3	-73.5834	1.2412
4K7O	3	-38.7969	1.2516
5M2F	1	-189.877	1.2703
6COX	1	-91.8011	1.6437
2W3L	2	-59.8591	1.4257

Docking results show that each receptor has one test compound with the lowest ChemPLP value (docking score), which was a compound that was more selective against target receptors than the other compounds (Table 2). All of the test compounds have a lower docking score than vitamin C and native ligands against 4K7O receptors, with the lowest score being α-tocopherol. A receptor of 4K7O contains the enzyme peroxiredoxin-5 mitochondrial, so it shows that all of the test compounds have antioxidant activity by activating the enzyme peroxiredoxin-5 mitochondrial with reduced hydrogen peroxide. Gallic acid is a compound with the lowest docking score of -68.0188 on receptor 1V4S; the native ligand has a value of -73.8522, but vitamin C has a smaller value, which is -84.5925. Receptor 1V4S contains glucokinase isoform 2, so it can be concluded that vitamin C has been proven as a potential antioxidant compound compared to other compounds by inhibiting the enzyme glucokinase that can facilitate the oxidative phosphorylation process in carbohydrate metabolism that produces reactive oxygen speciation such as superoxide and hydrogen peroxide that cause the formation of free radicals (Martati *et al*., 2019).

Table 2. The docking score of native ligands, test compounds, and comparative compound against antioxidant receptors

Compound	Docking Score against antioxidant receptors					
	1V4S	1XAN	2BEL	5M2F	6COX	4K7O
Native ligand	-73.8522	-148.19	-161.203	-189.877	-91.8011	-38. 7969
Gallic acid	-68.0188	-68.9021	-72.3554	-78.039	-62.8694	-58.106
Daidzein	-20.4995	-75.0065	-73.355	-103.992	-74.7195	-57.4268
Epicatechin	-39.8876	-76.819	-79.0837	-102.053	-70.496	-56.0451
Genistein	-25.1592	-77.8097	-76.7999	-103.922	-80.6402	-61.7899
Homomangiferin	54.088	-97.7078	-98.2113	-118.623	-52.9464	-67.9367
Isomangiferin	19.0787	-99.2696	-94.3552	-124.456	-55.3055	-63.426
Kaempferol	113.974	-91.1038	-97.6784	-97.6392	-76.7229	-66.7814
Catechin	-46.1641	-88.647	-87.0881	-110.602	-87.7596	-66.8457
Quercitrin	108.179	-95.4329	-96.5797	-94.632	-79.9368	-77.5224
Mangiferin	34.2173	-90.674	-93.1494	-110.902	-57.8965	-65.5338
α-tocopherol	121.866	-114.675	-113.528	-149.596	-98.6633	-78.5999
Vitamin C	-84.5925	-73.9033	-72.6454	-83.8254	-66.1691	-60.9388

Based on the docking result against 1XAN, 2BEL, 5M2F, and 6COX receptors, α-tocopherol has the lowest docking score against those receptors than the other test

compounds (Table 2). Each antioxidant compound has different ways to stimulate receptors in inhibiting oxidation reactions to reduce free radicals. The human glutathione reductase enzyme (1XAN receptor) can be activated by antioxidant compounds by oxidizing glutathione reduced form (GSH) into oxidized form (GSSG). Antioxidant compounds can activate corticosteroids 11-beta-dehydrogenase isozyme 1 (2BEL receptor) by inhibiting the formation of cortisone into cortisol, which can cause obesity and oxidative stress. The enzyme Aldo-keto reductase family 1 member 10 (5M2F receptor) can be catalyzed by antioxidants through the reduction of various intracellular cytotoxic carbonyl compounds, including oxidative stress processes (Martati *et al*., 2019). The results of the docking score show α-tocopherol unplaced with 1XAN, 2BEL, and 5M2F receptors as antioxidants, even though it has the lowest score of vitamin C but could not reach the native ligand score.

The docking result of α-tocopherol against the 6COX receptor shows the lowest score among native ligands and vitamin C. It proved that α-tocopherol enables the deactivation of cyclooxygenase-2 (6COX receptor), which plays a role in the formation of prostaglandins in the body. Besides triggering the inflammatory response, COX-2 (cyclooxygenase-2) activity has also been suggested to decrease cell reducing power by depletion of GSH (needed to reduce prostaglandin G_2 (PGG_2) to prostaglandin H_2 (PGH_2)) as well as to increase Fe^{2+}-toxicity in neuronal cells via generation of O_2 (Laube *et al*., 2016). In addition, it has been revealed that COX-2 is overexpressed in numerous human cancers such as gastric and breast cancer (Razzaghi-Asl *et al*., 2018).

Table 3. The docking score of native ligands, test compounds, and comparative compounds against anticancer receptor of 2W3L

Compound	Docking score	Compound	Docking score
Native ligand	-59.8591	Gallic acid	-61.1358
Mangiferin	-71.2039	Daidzein	-57.1798
Homomangiferin	-71.4521	Epicatechin	-60.4968
Isomangiferin	-70.4024	Genistein	-62.9551
α-tocopherol	-82.2205	Kaempferol 3-O-glucoside	-69.5438
Doxorubicin	-74.4892	Catechin	-64.6806
Hydroxyurea	-50.5939	Quercitrin	-71.5258

The result of docking *in silico* against the 2W3L receptor shows all of the test compounds have a lower score compared to the native ligand (-59.8591) except daidzein (-57.1798). A compound with the lowest score, α-tocopherol (-82.2205), can show more selectivity results than doxorubicin (-74.4892) and hydroxyurea (-50.5939). Receptor 2W3L is a phenyl tetrahydroisoquinoline amide complex with the ability to release MCF-7 (Michigan Cancer Foundation-7) breast cancer cells from pleural effusion breast adenocarcinoma. The result proved mangiferin, homomangiferin, isomangiferin, quercitrin, kaempferol 3-O-glucoside, catechin, epicatechin, genistein, α-tocopherol, and gallic acid

have *in silico* anticancer activity by inhibiting the activity of MCF-7 breast cancer cells through stimulate, which causes the process of cell apoptosis (Lelita *et al*., 2017).

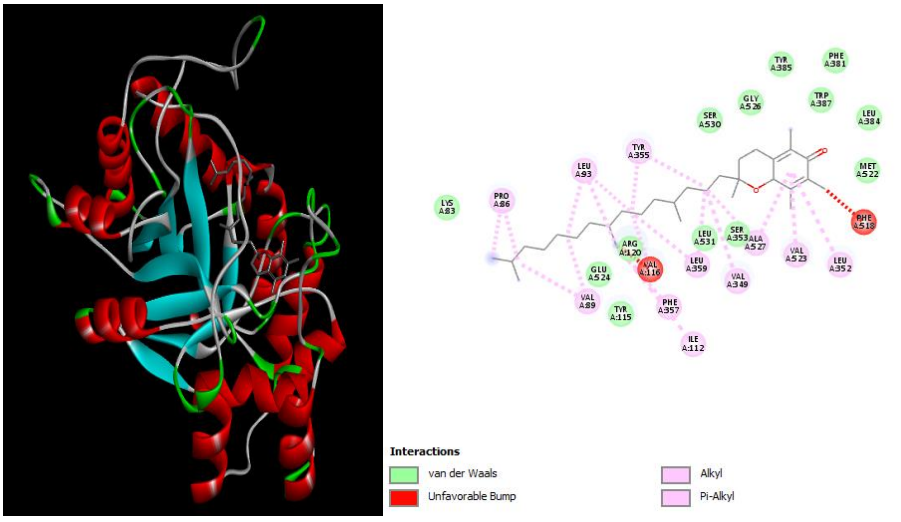
Quercitrin (quercetin 3-β-D glucosides) and genistein are flavonoids that have been shown to inhibit the cancer cells from making heat shock proteins in breast cancer, leukemia, and colon cancer cell lines. Kumar & Pandey (2013) have extensively reviewed the anticancer effects of genistein on in vitro and in vivo models. The determination of the effects of isoflavones genistein, daidzein, and biochanin A on mammary carcinogenesis has been studied. Genistein was found to suppress the development of chemically-induced mammary cancer without reproductive or endocrinological toxicities. Neonatal administration of genistein exhibited a protective effect against the subsequent development of induced mammary cancer in rats. Quercetin is known to produce antineoplastic activity and exert growth-inhibitory effects on several malignant tumor cell lines in vitro, including human breast cancer cells. Tumor cell growth inhibition by quercetin may be due to its interaction with nuclear type II estrogen binding sites (EBS). It has been experimentally proven that increased signal transduction in human breast cancer cells is markedly reduced by quercetin acting as an antiproliferative agent.

The predictivity of the QSAR models was tested with α-tocopherol and its analogs for antiproliferative activity on the MCF-7 breast cancer cell line. Based on the estimated ADMET properties, it was concluded that tocopherol and several analogs represent good drug candidates for the treatment of breast cancer (Gagic *et al*., 2016). Research by Diao *et al*. (2016) shows α-tocopherol significantly accelerates breast cancer growth in vivo by reducing ROS production and p53 expression. ROS levels and p53 expression were decreased in tumor tissues. Water-solvable α-tocopherol Trolox significantly promoted MCF-7 cell proliferation in vitro while reducing intracellular ROS levels and p53 expression. p53 knockdown by p53-siRNA transfection in MCF-7 cells significantly reduced p53 expression and increased MCF7 cell proliferation. The antiproliferative activity of mangiferin was also tested in vitro against the MCF-7 breast cancer cell line. The higher concentrations of mangiferin with doxorubicin for 96 hours have the ability to resensitize MCF-7 breast cancer cells through reducing cell viability and inhibiting P-glycoprotein (P-gp) expression (Louisa *et al*., 2014).

Receptor interaction with ligands after the docking process is visualized using Discovery Studio 2020 software. A dotted line is a bond or interaction that occurs between ligands and receptors. Observation of amino acid interactions aims to identify interactions that occur between ligands and receptors. Interactions that occur are usually hydrogen bonds, electrostatic interactions, van der Walls interactions, hydrogen interactions, and

hydrophobic interactions. The more amino acid residue bond, the better their activity (Rollando, 2017). The most active compound compared to other test compounds was α-tocopherol in docking simulation, which reacted with 6COX and 4K7O protein receptors (total binding energy of -98.6633 and -78.5999) because it has similar amino acid residue bonding to native ligands (Figures 1 and 2). The α-Tocopherol compound has the same amino acid residues with (cyclooxygenase-2) 6COX native ligand of 53.85% than vitamin C (7,69% amino acid similarity). This assumes that these compounds can play a role in producing antioxidant activity through the pathway of inhibition of the cyclooxygenase-2 (COX-2) enzyme.

Visualization of docking results using Discovery Studio 2021. The result of the visualization showed ligand interactions with amino acid residues in protein macromolecules and can determine the type of bond that occurs between the ligand and the protein in 2D and 3D forms. Visualization in 3D makes it possible to see the ability of linked compounds to bind to the active sites of proteins. The results of the 2D visualization show the types of bonds that interact between protein and ligand. The type of bond that underlies the parameters of the docking test is hydrogen bonding, which is the greatest bond strength interaction with an energy of 1-7 kcal/mol. The other chemical bonds can occur as a result of flexible ligands interacting with receptors. The interactions can be in the form of electrostatic (5 kcal/mol) and van der Walls bonds (0.5-1 kcal/mol), which can increase the affinity of the ligand for the receptor, thereby increasing conformational stability (Syahputra *et al*., 2014). The 2D α-tocopherol visualization results show the bond interactions between the structure and amino acids of the 6COX and 4K7O proteins, which only consist of van der Waals bonds and alkyl groups. Although it does not contain hydrogen bonds as is the case in native ligands, it consists of many bonds, that accumulates, resulting in a greater bond affinity.



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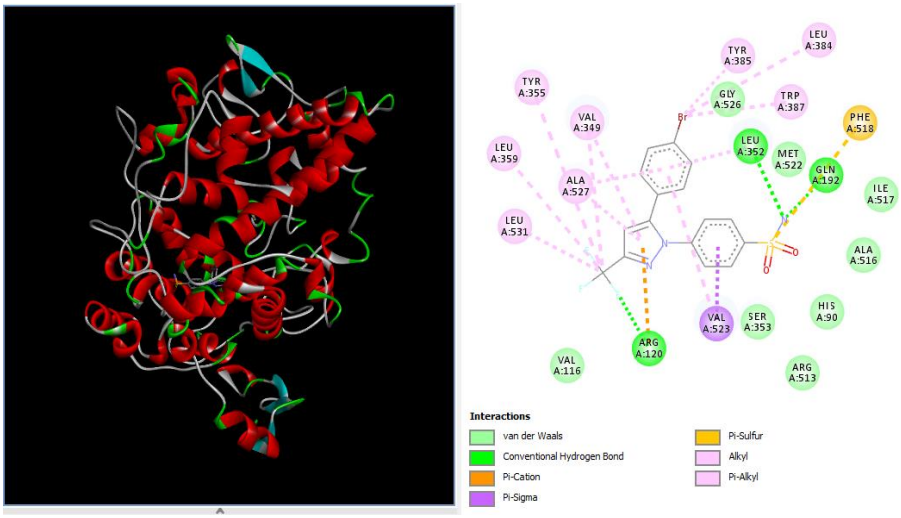

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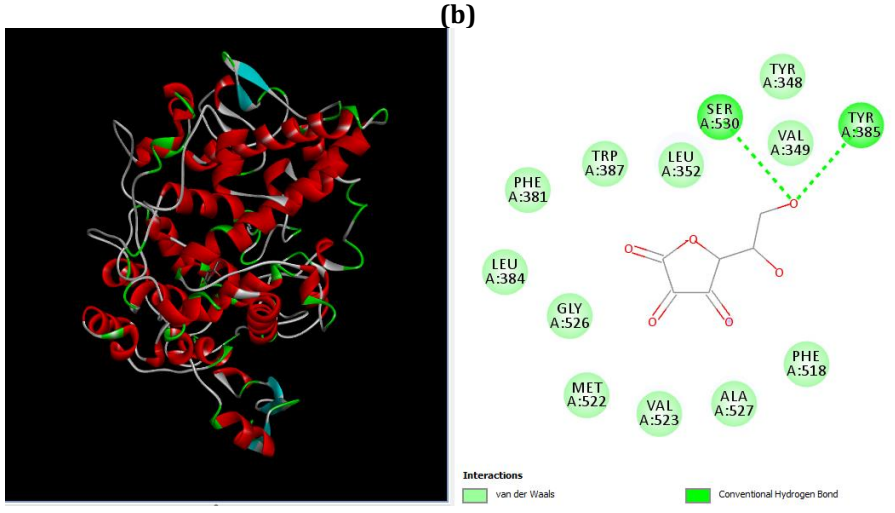

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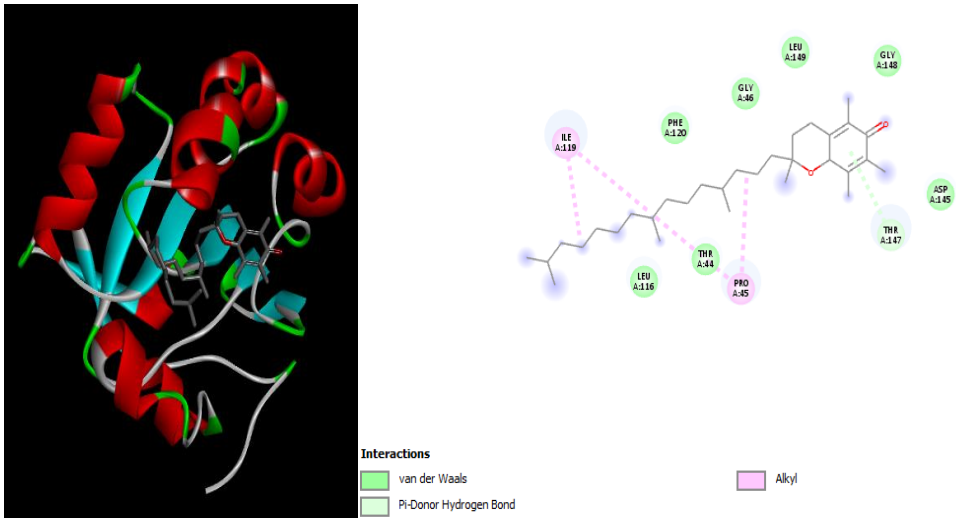
Figure 1: Visualization of interaction between (a) α-tocopherol, (b) native ligand, and (c) vitamin C against 6COX receptor using Discovery Studio 2020 Software.

The structure of α-tocopherol contains a saturated phytyl C16 side chain and a hydroxyl group at position 6, which makes it the most biologically active isoform of the vitamin E family. The side chain located at position 2 of the 6-chromonal ring helps with the incorporation of vitamin E in the membrane so that the 6-position of hydroxyl groups is in optimal locations to scavenge free radicals and slow down lipid peroxidation inside the membrane (Alqahtani *et al*., 2015). The substituent groups of α-tocopherol contributed to the bonding form with amino acid residues with antioxidant receptor, as shown in Table 4.

Table 4. The result of amino acid residue bonding between α-tocopherol, native ligand, and vitamin C against on 6COX receptor

Compound	Amino acid residue bonding
α-tocopherol	ALA527, ILE112, LEU93, LEU352, LEU359, PHE518, PRO86, TYR355, VAL89, VAL349, VAL523
Native ligand	ALA527, ARG120, GLN192, LEU352, LEU359, LEU384, LEU531, PHE518, TYR355, TYR385, TRP387, VAL349, VAL523
Vitamin C	SER530, TYR385

Based on Figure 2, the native ligand of 4K7O has one amino acid residue bonding, which is LYS63, compared with α-tocopherol and vitamin C, which have more form bonding with amino acid residues. It is also possible to state that tocopherols are antioxidant compounds that may contribute to the antioxidative properties of Mangifera species (Mirfat *et al*., 2016). All test compounds have the ability to bond with more than one amino acid residue, and this result is correlated with the value of the docking score of each compound that has a lower score than the native ligand (Table 5). The result shows phenolic and flavonoid compounds in Mangifera species proved to have antioxidant properties through *in silico* studies and exhibit antioxidative action through a variety of mechanisms.



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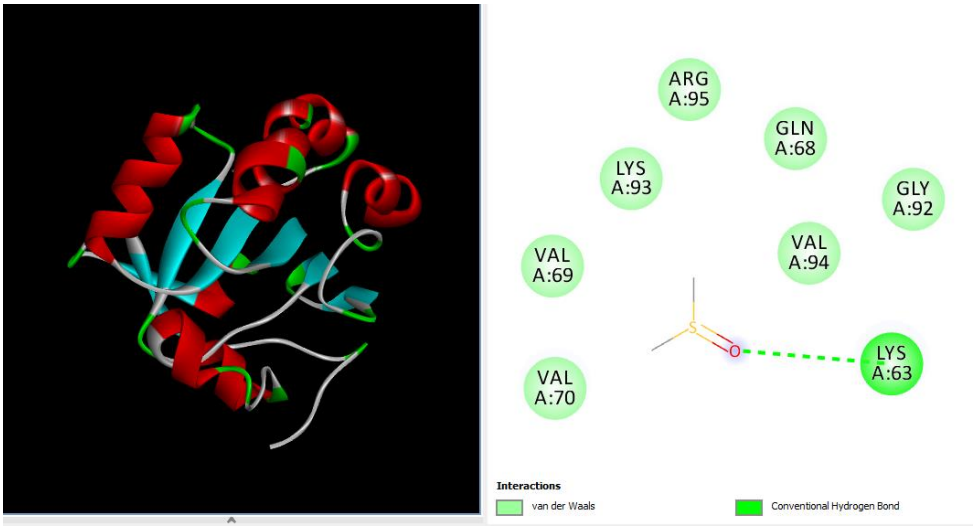

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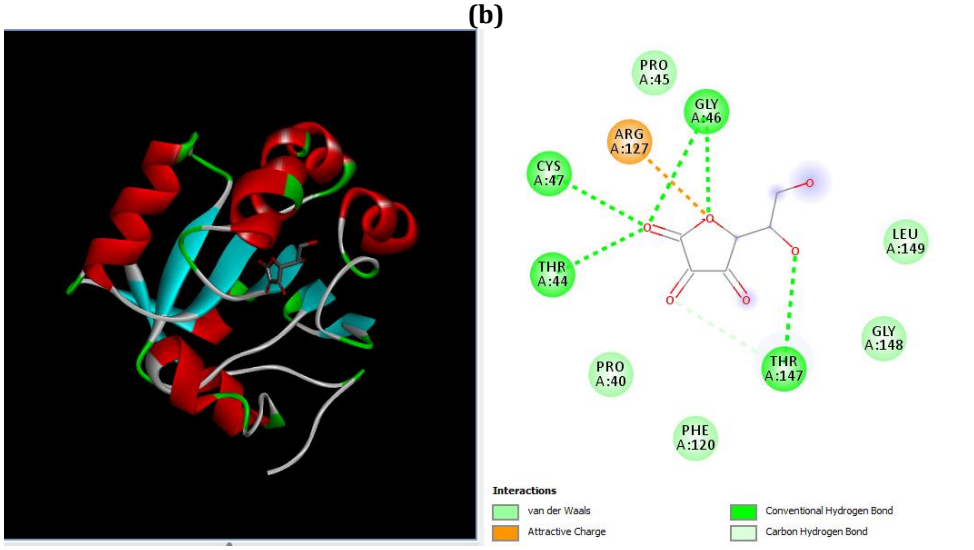

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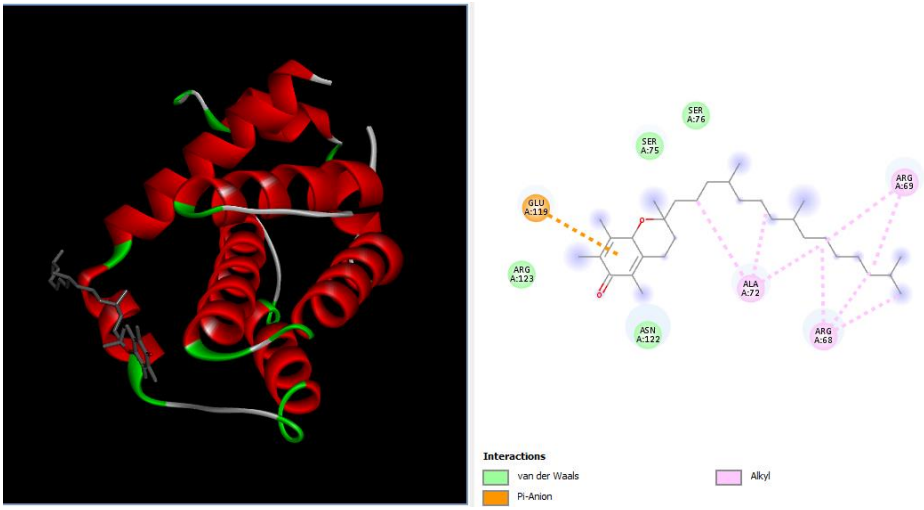
Figure 2. Visualization of interaction between (a) α-tocopherol, (b) native ligand, and (c) vitamin C against 4K7O receptor using Discovery Studio 2020 Software.

Table 5. The result of amino acid residue bonding between test compounds, native ligand, and vitamin C against on 4K7O receptor

Compound	Amino acid residue bonding
Gallic acid	ARG127, CYS47, GLY46, THR44, THR147
Daidzein	ARG127, CYS47, GLY46, THR44
Epicatechin	ARG127, GLY46, PRO45, THR147
Genistein	CYS47, ILE119, PHE120, PRO45, THR44
Homomangiferin	ASP145, GLY148, THR147
Isomangiferin	ARG127, ILE119, PRO45, THR147
Kaempferol 3-O-glucoside	LEU116, PHE120, PRO45, THR147
Catechin	ARG127, CYS47, GLY46, PRO45, THR44, THR147
Quercitrin	ILE119, THR147
Mangiferin	ARG127, CYS47, GLY46, ILE119, PRO45, THR44, THR147
α-tocopherol	ILE119, PRO45, THR147
Native ligand	LYS63
Vitamin C	ARG127, CYS47, GLY46, THR44, THR46, THR147

Phenolics in Mangifera species have also received considerable attention because they significantly contribute to many physiological functions, including antioxidant, anti-carcinogenic, anti-inflammatory, antimutagenic, and anti-tumor activities (Ismail *et al*., 2019). This *in silico* study also shows the ability of phenolic and flavonoid compounds to inhibit the activity of the MCF-7 breast cancer cell line through their capability to compete with native ligand in the placement of the active site on the 2W3L receptor by forming amino acid residue bonding. The most potent compound in this *in silico* study is α-tocopherol which has the lowest docking score, especially when compared with doxorubicin as a comparative compound and native ligand. This α-tocopherol activity is shown by the similarity of amino acid residue bonding with native ligand (ALA72) and doxorubicin (GLU119); even α-tocopherol has more amino acid residue bonding than native ligand, as shown in Figure 3 and Table 6.



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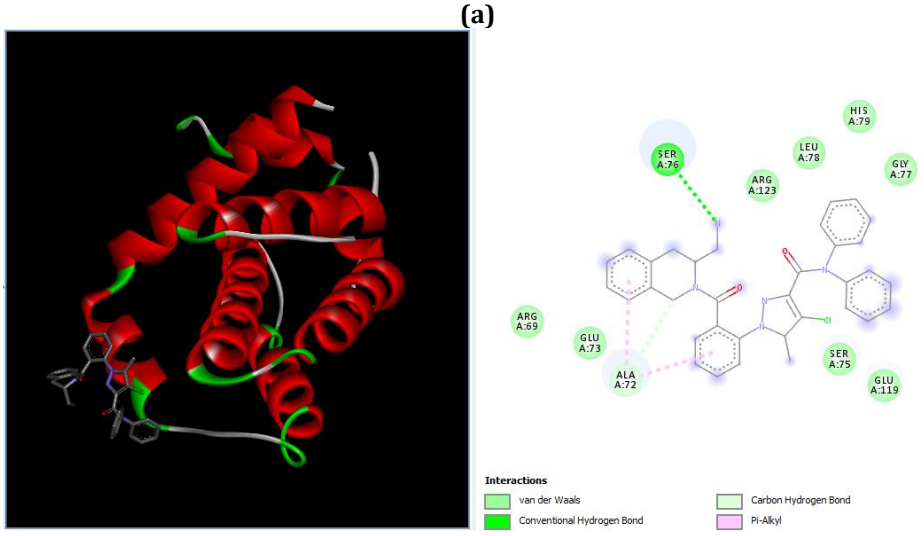

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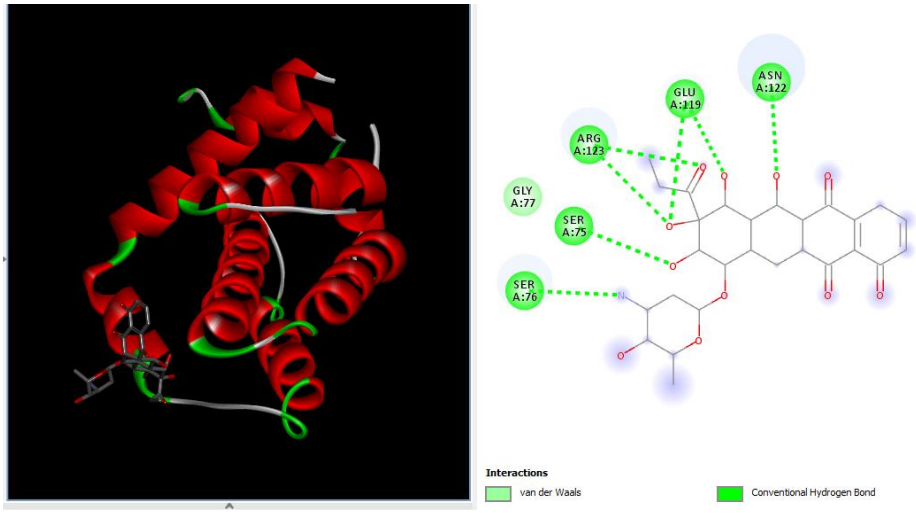

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Figure 3. Visualization of interaction between (a) α-tocopherol, (b) native ligand, and (c) doxorubicin against 2W3L receptor using Discovery Studio 2020 Software.

Table 6. The result of amino acid residue bonding between test compounds, native ligand, and doxorubicin against on 2W3L receptor

Compound	Amino acid residue bonding
Gallic acid	ARG123, GLU119, HIS79, LEU78
Epicatechin	ARG68, SER75
Genistein	ARG123, SER76
Homomangiferin	ARG123, GLU119, HIS79, SER75
Isomangiferin	ARG123, GLU119, GLY77, HIS79
Kaempferol 3-O-glucoside	ALA72, ARG68, ARG69, PHE71, SER64, SER75
Catechin	ALA72, SER75
Quercitrin	ALA72, ARG26, ARG68, PHE71, SER75
Mangiferin	ARG123, ASN122, GLU119, HIS79
α-tocopherol	ALA72, ARG68, ARG69, GLU119
Native ligand	ALA72, SER76
Doxorubicin	ARG123, ASN122, GLU119, SER75, SER76

Accordingly, the *in-silico* study shows α-tocopherol contributes to having the most antioxidative properties in Mangifera species. The development of the activity of α-tocopherol can be selected for further synthesis as drug candidates for the treatment of breast cancer and oxidative-mediated diseases. This study also shows α-tocopherol can be a leading compound to develop new anti-inflammatory and anticancer drugs.

4. Conclusion

Phenolic and flavonoid compounds contained in the Mangifera species have potential antioxidant activity *in silico* using the PLANTS method against the 4K7O protein receptor. Vitamin E, or α-tocopherol, is the only one that has the ability to inhibit the 6COX protein receptor. All test compounds have anticancer activity except daidzein against the 2W3L protein receptor. Based on research conducted, α-tocopherol has the most potential

as an antioxidant and anticancer candidate compared to other tested compounds through *in silico* studies using the PLANTS method.

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