Jurnal Ilmiah Kesehatan
(Journal of Health Science)
Fakultas Keperawatan dan Kebidanan | Universitas Nahdlatul Ulama Surabaya
ISSN 1978-6743
E-ISSN 2477-3948

An Activity of Flavonoid Derived Compounds from *Medinilla Speciosa* Extract as Anti-hyperpigmentation against Tyrosinease Proteins with In Silico Methods

Elshinta Riantica[1], *Awik Puji Dyah Nurhayati*[2], *Noor Nailis Sa'adah*[3]

[1,2,3] *Biologi Department, Faculty of Sains and Data Analitics, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia*

ARTICLE INFORMATION

Received: November 29, 2023
Revised: February 16, 2023
Available online: May 2024

KEYWORDS

Docking, Flavonoid, Parijoto, Tirosinase

CORRESPONDENCE

E-mail: elriantica@gmail.com

A B S T R A C T

Parijoto (Medinilla Speciosa), a typical plant from Indonesia, contains flavonoid compounds as antioxidants. It can depigment skin by inhibiting tyrosinase activity during melanogenesis or melanin formation. Melanogenesis occurs through UV light exposure; it stimulates ROS production and triggers the formation of free radicals and melanogenesis. If not prevented, it will have negative impacts on health. Further research is needed regarding the existence of flavonoid compound derivatives found in parity fruit. This research aims to initially identify potential flavonoid derivative compounds as tyrosinase inhibitors using in silico methods. The results can be used as an initial reference for making products based on natural ingredients with minimal side effects. This research uses a bioinformatics approach with the molecular docking method of ligands towards proteins. The stages in this research include downloading and preparing receptors and ligands, docking with Autodock Vina, visualization of results with Biovia Discovery Studio, data analysis, and discussion. The study is carried out by looking at the affinity energy values and complex conformation between the receptor-ligand. The results show flavonoid derivative compounds have the potential to act as tyrosinase inhibitors, as proven by compounds interactions with the active site of tyrosinase to produce the amino acid residues phenylalanine, proline, asparagine, arginine, and histamine. Produce respective affinity energy values, namely -8.9, -7.7, -7.6, -7.5, -7.5, and 7.4 kcal/mol for chalcone, catechin, flavonol, flavanol, flavone, and flavonone compounds. Meanwhile, the comparison compound used is kojic acid, with an affinity energy of -5.5 kcal/mol.

INTRODUCTION

Hyperpigmentation is a condition of inequality pigmentation characterized by dark spots around the skin area (Allgisna *et al.*, 2021). Hyperpigmentation affects the physical appearance of the skin by providing visible signs of aging, including wrinkling, irregular pigmentation, sagging, and elastosis (Nautiyal & Wairkar, 2021). Indonesia is a tropical country exposed to the sun's ultraviolet rays throughout the year, so people are very susceptible to hyperpigmentation (Ahmad & Damayanti, 2018). Hyperpigmentation is triggered by free radical (ROS) formation, which stimulates melanogenesis or melanin pigment formation. If this is not treated, it will hurt health due to the accumulation of free radicals in the body (Hastiningsih *et al.*, 2019). Melanogenesis is catalyzed by the enzyme tyrosinase (Kusumawati *et al.*, 2018). When the number of melanocytes produced is uncontrolled, it will cause an abnormal amount of melanin, triggering hyperpigmentation. One preventive measure of hyperpigmentation is inhibiting the process of melanin synthesis through inhibiting tyrosinase, or the enzyme that controls pigmentation, and its activity provides valuable information about the melanogenic potential of melanocytes (Allgisna *et al.*, 2021).

https://doi.org/10.33086/jhs.v17.i02.5107

Inhibition of tyrosinase can be done by utilizing bioactive compounds in plants (Fatmawaty *et al.*, 2010). Indonesia is mega biodiversity; it has many kinds of plants, including herbs, which have the potential to be used as medicine. One plant that has not been widely explored pharmacologically is parity (*Medinilla speciosa*). *M. speciosa* is a plant from the Melastomataceae family that grows wild in rainforests on mountain slopes at an altitude of 800-2,300 m above sea level. In Indonesia, *M. speciosa* is often found in Mount Muria, Kudus, and Central Java (Milanda et al., 2021), but nowadays, it has begun to be cultivated as an ornamental plant with medicinal purpose (Maria et al., 2014; Kunarto & Sani, 2020). *M. speciosa* is usually used as a fertilizer and treats various diseases, such as mouth ulcers and diarrhea. Besides that, it is approved by studies as well as antibacterial, anti-inflammatory, antioxidant, anticancer, anti-hyperlipidemic, anti-obesity, and immunostimulant activities (Hanum *et al.*, 2017; Sa'adah *et al.*, 2017, 2018, 2019).

Further study on *M. speciosa* natural products leads to identifying active compounds that have the potential for skin depigmentation with fewer negative effects. Based on several previous studies, natural compounds in plants have potential activity as inhibitors of tyrosinase enzymes, such as phenolic and flavonoid compounds (Fatmawaty *et al.*, 2010; Kurniasari *et al.*, 2018; Allgisna *et al.*, 2021). Based on a study by Febrilian and Pujiastuti (2017), flavonoids have antioxidant properties that can protect against damage to pancreatic β cells by free radicals. Flavonoids are a large group of polyphenolic compounds found in various types of medicinal plants and are known to have the ability to depigment skin by directly inhibiting tyrosinase activity in the melanogenesis process. The bonds between flavonoids and copper (Cu), and their antioxidant effects are reported to play a role in inhibiting the action of tyrosinase enzyme (Allgisna *et al.*, 2021). According to El-Nashar *et al.* (2021), flavonoids are known as tyrosinase inhibitors because they have inhibitory activity (IC50 = 0.12-266.67 μM) based on tests from various herbal plants. Flavonoids are divided into several subgroups based on carbon substitution in the central aromatic group (C). These subgroups are flavones, flavonols, flavanones, catechins, and chalcones (Panche *et al.*, 2016). Until now, no study has discussed the existence of this flavonoid subgroup in *M. speciosa* fruit, so the authors desire to test and early identify the potential of flavonoid derivative compounds as tyrosinase inhibitors in silico using the molecular docking method. Molecular docking is a computational method that aims to imitate the interaction of a ligand molecule with its target protein in an in-vitro test (Motiejunas & Wade, 2006). So, this method can be used to predict the most likely activity, position, orientation, and conformation between ligands with proteins (Quiroga *et al.*, 2016). The resulting score from the docking process explains whether a compound is potent or not as a drug candidate. The smaller docking result means the protein-ligand complex is more stable, so the compound is considered more potential (Purnomo, 2011). Suppose the docking results in this study prove that

https://doi.org/10.33086/jhs.v17.i02.5107

derivative compounds or flavonoid subgroups can inhibit tyrosinase. In that case, identification and testing for the presence of these compounds in *M. speciosa* fruit can be carried out, followed by in vivo tests on animal models to see the biological activity of melanogenesis inhibition. The prospect of further study results could lead to the production of drugs or skin care products that have anti-hyperpigmentation capabilities with minimal side effects.

METHOD

This study using the molecular docking method, begins with downloading the protein receptor and ligand or test compound. The receptor, tyrosinase protein, was obtained by downloading the file (PDB format) via the Protein Data Bank (PDB) website (http://www.rscb.org/pdb/) with specific code 5I38. The ligands or compounds, such as 3D structures of kojic acid, flavones, flavonols, flavanones, flavanols, catechins, and chalcones (PubChem CIDs 3840, 10680, 11349, 265703, 253959, 9064, and 17341 respectively), downloaded via PubChem website (http://pubchem.ncbi.nlm.nih.gov/). The structure of kojic acid is used as a reference or control in this study. Next, the molecular docking process can begin. It starts with separating the protein from its accompanying components using Biovia Discovery Studio 2020 software. It is known that the protein structure downloaded from the Protein Data Bank is a complex structure with several components. All components in the protein structure must be removed to start the molecular docking, leaving only one protein molecule which will later be used as a receptor during the docking treatment. Next, the molecule file used to be saved in the '.pdb (Protein Data Bank)' storage format. The protein that has been stored is ready to be used for the docking process. The next stage is receptor preparation using PyRx software to prepare the protein receptor used in molecular docking in the appropriate format, namely '.pdbqt.'

Next, the ligands or test compounds must be prepared. The energy value of ligands must be reduced and then stored in the same format as the receptor, namely '.pdbqt.' The next stage is docking with PyRx which has been integrated with AutoDock Vina. Before the docking process begins, first ensure that the receptor and ligand are compatible by looking at the information in the software window that says, *'7 ligand(s) selected and C:\Users\hp\Document\reseptor\reseptor.pdbqt selected'*. The docking process is directed at the receptor's active site, which can bind to the ligand by adjusting the location and dimensions of the grid box. The amino acids as active sites in this study include Phe197, Pro201, Asn205, His208, and Arg209. After the grid box settings on the active side are complete, Autodock Vina can be run. The software will automatically carry out the docking process and then wait for the process to complete until the binding affinity and RMSD values appear in the Controls box, which is displayed in tabular form. The docking results will be automatically saved on the device. Next, the docking results

https://doi.org/10.33086/jhs.v17.i02.5107

were visualized using Biovia Discovery Studio 2020 to determine the interaction of ligands on protein receptors in a 2-dimensional diagram illustration. The diagram will show the various amino acid residues and the types of bonds that occur between protein and ligand. The visualization results are saved in image format on the device.

This study uses a bioinformatics approach and molecular docking analysis for ligands and proteins, so the analysis in this research is descriptive. A molecular docking analysis was carried out to see the conformation of the receptor-ligand complex because of docking with Autodock Vina. The result shows an affinity energy value (kcal/mol). A good level of stability between the ligand and receptor is indicated by the more negative the affinity energy value, the stronger the bonds formed will be. The analysis results will be related to the activity of the compounds and amino acids that play a role in the interaction of the ligand with tyrosinase protein.

RESULT

Based on the study, docking results showed nine of the best conformations for each compound. The best docking score was shown by the chalcone compound with a binding affinity value of -8.9 kcal/mol, followed by the catechin, flavonol, flavanol, flavone, and flavanone compounds, which have values -7.7; -7.6; - 7.5; -7.5; and -7.4 kcal/mol, respectively. The docking score values are in Tables 1 and 2.

Table 1 Results of Compound Docking Values

Conformation	Docking Energy / Binding Affinity (kcal/mol)						
	Kojic acid	Flavone	Flavonol	Flavanol	Flavanone	Catechin	Chalcone
	E=76.93	E=187.70	E=318.24	E=209.12	E=214.21	E=204.84	E=730.70
5I38_001_conf_1	-5.5	-7.5	-7.6	-7.5	-7.4	-7.7	-8.9
5I38_002_conf_1	-5.4	-7.4	-7.5	-7.2	-7.2	-7.6	-8.1
5I38_003_conf_1	-5.4	-7.3	-7.3	-7.2	-6.8	-7.5	-8.1
5I38_004_conf_1	-5.3	-7.2	-7.0	-7.0	-6.8	-7.4	-7.8
5I38_005_conf_1	-5.3	-7.2	-7.0	-6.9	-6.7	-7.3	-7.7
5I38_006_conf_1	-5.1	-7.1	-6.7	-6.7	-6.6	-7.2	-7.6
5I38_007_conf_1	-4.8	-6.7	-6.6	-6.6	-6.5	-7.2	-7.3
5I38_008_conf_1	-4.8	-6.6	-6.3	-6.6	-6.4	-6.7	-6.9
5I38_009_conf_1	-4.7	-6.6	-6.1	-6.5	-6.1	-6.6	-6.9

Based on the data obtained after molecular docking as in Table 1, the best docking score value is used as a representation of the form of interaction between the ligand and receptor, that has been previously docked. The following are the results of the best docking scores from all tested ligands (Table 2).

https://doi.org/10.33086/jhs.v17.i02.5107

Table 2 Results of Docking Value Selection

Ligands	Best docking conformation scores against tyrosinase (kcal/mol)
Kojic acid	-5.5
Flavone	-7.5
Flavonol	-7.6
Flavanol	-7.5
Flavanone	-7.4
Catechin	-7.7
Chalcone	-8.9

The visualization results show protein macromolecules' interaction between ligands (compounds) and amino acids. The amino acid residues that interact with the ligand will determine the type of bond that occurs between the ligand and the protein. This is a table of visualization results of amino acids that bind to ligands and target receptors.

Table 3 Visualization of Amino Acid Ligands in Tyrosinase Receptors

Ligands and Reseptor Target	Asam Amino Visualization	*Binding Site*	
Kojic acid and tyrosinase	His42, His60, **Asn205, His208**, Val217, Val218		
Flavone and tyrosinase	**Phe197, His208**, Val218, Ala221		
Flavonol and tyrosinase	**His208, Arg209**, Val218, Ala221	Phe197,	Pro201,
Flavanol and tyrosinase	**Pro201, Asn205, His208**, Val218, Ala221	Asn205,	His208,
Flavanone and tyrosinase	**His208, Arg209**, Val218, Ala221	Arg209	
Catechin and tyrosinase	His60, **Phe197, Asn205, His208**, Val218		
Chalcon and tyrosinase	His42, Met61, **Pro201, His208, Arg209**, Val218		

Based on the results of amino acid visualization using the Biovia Discovery Studio software, it is seen that the docking and visualization process of flavones, flavonols, flavanols, flavanones, catechins, and chalcones is proven to be able to bind through the binding site on the target protein receptor. The visualization results of flavone, flavonol, flavanol, flavanone, catechin, chalcone, and kojic acid compounds are shown in Figures 1-7.

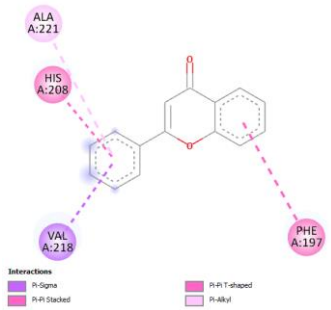

Figure 1 3D visualization results of molecular docking of flavone compounds and tyrosinase protein

https://doi.org/10.33086/jhs.v17.i02.5107

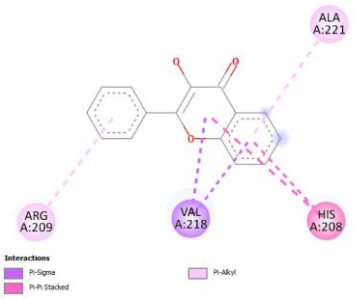

Figure 2 3D visualization results of molecular docking of flavonol compounds and tyrosinase protein

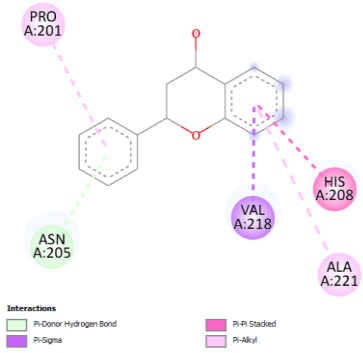

Figure 3 3D visualization results of molecular docking of flavanol compounds and tyrosinase protein

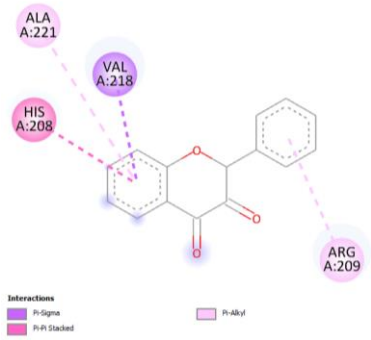

Figure 4 3D visualization results of molecular docking of flavanone compounds and tyrosinase protein

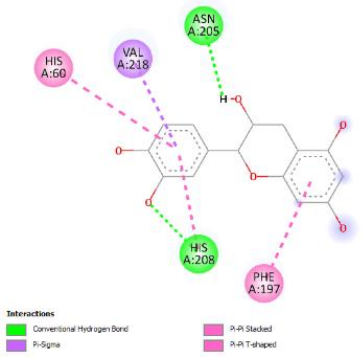

Figure 5 3D visualization results of molecular docking of catechin compounds and tyrosinase protein

https://doi.org/10.33086/jhs.v17.i02.5107

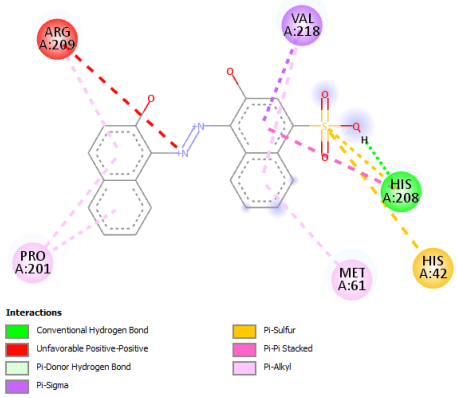

Figure 6 3D visualization results of molecular docking of chalcone compounds and tyrosinase protein

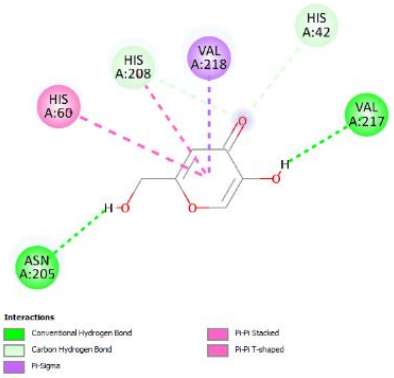

Figure 7 3D visualization results of molecular docking of kojic acid compounds and tyrosinase protein

Based on molecular docking of flavone, flavonols, flavanols, flavanones, catechins, chalcone, and kojic acid compounds against the tyrosinase protein, the results showed that flavone compounds can bind to the amino acids phenylalanine (Phe197) and histamine (208) via π-π bonds in the binding site area. Flavonol compounds can inhibit tyrosinase in the amino acid histamine (His208) via a π-π bond and arginine (Arg209) via a pi-Alkyl bond in the binding site area. Flavanol compounds can inhibit tyrosinase in the amino acid proline (Pro201) via pi-Alkyl bonds, asparagine (Asn205) via hydrogen bonds, and histamine (His208) via π-π bonds in the binding site area. Flavanone compounds can inhibit tyrosinase in the amino acid histamine (His208) via a π-π bond and arginine (Arg209) via a pi-Alkyl bond in the binding site area. Catechin compounds can inhibit tyrosinase in the amino acid histamine phenylalanine (Phe197) via π-π bonds, asparagine (Asn205), and histamine (His208) via conventional hydrogen bonds in the binding site area. The chalcone compound can inhibit tyrosinase on the amino acid proline (Pro201) via a pi-Alkyl bond, histamine (His208) via a conventional hydrogen bond, and arginine (Arg209) via an unfavorable positive-positive bond in the binding site area. Meanwhile, the control compound in the form of kojic acid can inhibit tyrosinase in the amino acid asparagine (Asn205) through conventional hydrogen bonds, and histamine (His208) through hydrogen bonds in the binding site area.

https://doi.org/10.33086/jhs.v17.i02.5107

DISCUSSION

Based on the steps in the method, it is known that the process of downloading receptors and ligands used in this study only requires hardware, such as a computer that is connected to the internet network. All files can be freely accessed on the website or at the link listed in the method. Next, the receptor preparation process is carried out by removing water molecules, ions, and ligands so that these molecules will not interfere with the interaction between target molecules and proteins in AutoDock (Ravi & Krishnan, 2016). Meanwhile, ligand preparation is used to adjust the ligand format and receptor so it can be easier to interact with each other. Before docking begins, the protein and ligand formats are changed to '.pdbqt', so that the docking process runs well in accordance with the terms of PyRx software.

Referring to the study, the docking results obtained nine conformations for each compound where the best docking score is shown in the first conformation by the chalcone compound with a binding affinity value of -8.9 kcal/mol, followed by the first conformation also from the catechin compound, flavonol. , flavonols, flavones, and flavanones which have values of -7.7, -7.6, -7.5, -7.5, and 7.4 kcal/mol, respectively (Table 1-2). The docking score is a parameter of the binding affinity strength of the ligands and receptors. The lower docking score reflects the more stable ligand-protein interaction (minus). The comparison of these scores explains whether a compound is potent or not to inhibit the performance of the specific protein being targeted. The smaller docking result means the compound is more potent in inhibiting protein activity (Purnomo, 2011). The docking score from the results of this study showed that flavonoid derivative compounds such as chalcone, catechin, flavonol, flavanol, flavones, and flavonone have the potential to inhibit tyrosinase activity, which can cause hyperpigmentation. Of the six test ligands used, chalcone showed the greatest affinity value, which means that chalcone was predicted to be the most effective compound in inhibiting tyrosinase compared to other compounds.

The mechanism of melanin production through the melanogenesis pathway begins with the oxidation of L-tyrosine or L-DOPA as a starting material for dopaquinone by tyrosinase, and the second step results in the formation of quinone, which functions as a substrate for the next step, which produces melanin. Tyrosinase is a central glycoprotein enzyme in the membrane region of typical endosomal compartments, called melanosomes. Tyrosinase catalyzes its substrates in a rate-limiting mechanism of action of the melanogenesis reaction in two steps. First, tyrosinase catalyzes the addition of a hydroxyl group from its substrate L-tyrosine to the intermediate 3,4-dihydroxyphenylalanine (DOPA). Second, the oxidation of DOPA is used to produce the final product, DOPA-quinone. Tyrosinase and tyrosinase-related protein (TRP), catechol oxidase, and hemocyanin belong to the type-III copper protein family. The catalysis of the conversion of L-tyrosine to L-DOPA depends only on copper ions. Type-III copper oxidase has a paired copper binding site of two copper ions Cu(A) and Cu(B). Each copper ion is coordinated to bind to

https://doi.org/10.33086/jhs.v17.i02.5107

a His residue in the catalytic site of the tyrosinase enzyme. If an inhibitor inhibits tyrosinase, melanin production will also be inhibited. Human tyrosinase inhibitors are beneficial in the pharmaceutical and cosmetic fields. Historically, a representative whitening agent is L-ascorbic acid (vitamin C) because it has been shown to inhibit melanin synthesis by reducing dopaquinone to L-DOPA by L-ascorbic acid. However, L-ascorbic acid is unstable in formulations used in cosmetics. To overcome this problem, several other compounds have also been developed and proven to be representative of tyrosinase inhibitors, including kojic acid, which was used as a control in this study. However, it is cytotoxic to normal cells in specific doses and cannot penetrate dermal skin tissue. Therefore, many herbal-based products have been developed to obtain the same benefits with a lower risk of side effects and high bioavailability (Kim *et al.*, 2023).

Flavonoid derivative products contained in the fruit of *M. speciosa* could be one answer to the development of processed natural products as inhibitors of tyrosinase which triggers hyperpigmentation. Looking at the docking results that have been carried out in this study, it can be assumed that the presence of flavonoid derivative compounds can imitate tyrosine substrates to result in competitive inhibition of melanin formation. Competitive inhibitors recognize and occupy the active site enzyme of a free enzyme in solution to prevent binding of its substrate to the enzyme's active site. Phenolic compounds, as parent of flavonoids, are inhibitors that have been proven to exhibit tyrosinase inhibitory activity because they have one or several aromatic rings with a 9C-OH group or several -OH groups in their backbone structure. They are conjugated to saccharides or organic acids (Kim *et al.*, 2023). This is also possible for the chalcone, catechin, flavonols, flavanols, flavones, and flavonones compounds because they are supported by previous research that proves other flavonoid derivative compounds such as quercetin, kaemferol, apigenin, quercitrin, etc., by molecular docking with value around -6.0 to -7.2 kcal/mol (Priani & Fakih, 2021; Jakimiuk *et al.*, 2022). However, to confirm the results of this test, in-vitro and in-vivo tests are needed on animal models to see the biological activity, possible toxicity, and other side effects if the ingredient is used as a skincare product.

The results of the visualization showed interactions between ligands (compounds) and amino acids in protein macromolecules. The amino acid residues interacting with the ligand will determine the bond between ligands and proteins. Based on the visualization results, showed that the docking and visualization process of chalcone, catechin, flavonol, flavanol, flavone, and flavanone compounds had been proven to bind through the binding site on the target receptor. The amino acids involved in the tyrosinase binding site have a role in binding the functional group of the compound that has been docked. The amino acids that bind to the active site have their characteristics, and these characteristics can determine the type of interaction that occurs with the compound. The active site or binding site in

https://doi.org/10.33086/jhs.v17.i02.5107

tyrosinase consists of four types of amino acids with different structures. The four types include Phenylalanine, Proline, Asparagine, Arginine, and Histamine (Deri *et al.*, 2016). A binding site is a protein binding area for ligands that will influence the protein's conformation and function. This area also shows amino acid residues that form interactions between receptors and ligands (Arwansyah *et al.*, 2014). In general, amino acids consist of an amino group, a carboxyl group, a hydrogen group, and a side chain (R group) (Simamora, 2015). According to Deri *et al.* (2016), the possibility of compound movement, which in this case uses kojic acid, in the active site can be shown in two positions: the peripheral and active sites. In the peripheral site, the compound is stabilized by interactions with Phe197, Pro201, Asn205, and Arg209, whereas in the active site, the compound is stabilized by His208 coordinating Cu(B), like tyrosinase substrates. The hydroxyl group of the compound is oriented towards Cu(A) with a distance of 3.3 Å, while the distance of the carbonyl group to Cu(A) is 5.5Å. This is in accordance with the theory of melanogenesis where type III copper will bind to His residues to stimulate the conversion of L-tyrosine to L-DOPA (Kim *et al.*, 2023).

All molecules that go through the docking process will have interactions with each other, and these intermolecular interactions will determine the biological properties of the molecules in the cell. In general, these molecular interactions are noncovalent interactions, such as hydrogen bonds, ionic bonds, van der Waals interactions, and hydrophobic interactions (Yuwono, 2005). The interactions formed as a result of docking are usually van der Waals and hydrogen bonds, but there are also other hydrophobic interactions such as pi-pi, pi-sigma, and pi-alkyl bonds, each of which has different strengths. Based on testing of chalcone, catechin, flavonol, flavanol, flavone, and flavanone ligands in tyrosinase, it is known that there was an interaction between the ligand and the protein, which produced the same amino acid residue as the positive control kojic acid in the active site area of tyrosinase. Hydrogen bonds play the most crucial role in docking results because hydrogen bonds have greater strength and stability than other bonds (Parthasarathi & Subramanian, 2006; Mali *et al.*, 2010). Hydrogen bonds can be formed even though the distance between the ligand and receptor is quite far (Lodish *et al.*, 2000). Hydrogen bonds are known to greatly influence the interaction between proteins and ligands so that they can increase the affinity value between that protein and ligands. Thus, if more hydrogen bonds occur due to docking, the strength and stability of the drug-receptor interaction will be substantially higher. However, it does not rule out the possibility that the docking result value is greater for compounds that do not have interactions in the form of hydrogen bonds because it is influenced by the number and strength of other bonds formed (Chen *et al.*, 2016). The interactions in the ligands and receptors used in this study prove that the test compounds can inhibit the target protein tyrosinase. According to a study by Sari et al. (2020), if the amino acid residue

has a binding position like the inhibitor, even though only a few amino acids can interact in the binding site area, the test compound can have inhibitory activity on the receptor.

CONCLUSION

Based on a study that has been carried out, flavonoid derivative compounds such as chalcone, catechin, flavonols, flavanols, flavones, and flavonones that can be synthesized from *M. speciosa* have docking scores of -8.9; -7.7; -7.6; -7.5; -7.5; and 7.4 kcal/mol. Meanwhile, the docking score for kojic acid as a control was -5.5 kcal/mol, so the compounds chalcone, catechin, flavonol, flavanol, flavones, and flavanone have the potential to act as tyrosinase inhibitors and are predicted to be able to prevent hyperpigmentation with in silico methods. The amino acids close to the interaction of chalcone, catechin, flavonol, flavanol, flavone, and flavonone compounds are phenylalanine, proline, aspartame, arginine, and histidine. Further study is needed to identify the content of chalcone, catechin, flavonol, flavanol, flavone, and flavonone compounds in *M. speciosa* fruit. Then, to prove the anti-hyperpigmentation effect and cytotoxicity of the compound, in vitro and in vivo tests are needed on test animals.

REFERENCES

Ahmad, Z. and Damayanti. 2018. *Penuaan Kulit: Patofisiologi dan Manifestasi Klinis*. Berkala Ilmu Kesehatan Kulit dan Kelamin – Periodical of Dermatology and Venereology. Vol. 30(3).

Allgisna, K.N., Hindun, S. and Rantika, N. 2021. *Review: Perbandingan Beberapa Ekstrak Kulit Buah sebagai Anti-hiperpigmentasi*. Jurnal Sains dan Kesehatan. Vol. 3(2).

Arwansyah., Ambarsari, L., and Sumaryada, T. 2014. *Simulasi Docking Senyawa Kurkumin dan Analognya Sebagai Inhibitor Reseptor Androgen pada Kanker Prostat*. Journal Current Biochemistry. Vol 1(1).

Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S.M. and Savidge, T.C. 2016. *Regulation of protein-ligand binding affinity by hydrogen bond pairing*. American Association for the Advancement of Science. Vol. 2(3).

Deri, B., Kanteev, M., Goldfeder, M., Lecina, D., Guallar, V., Adir, N., and Fishman, A. 2016. *The unravelling of the complex pattern of tyrosinase inhibition*. Scientific Reports. Vol. 6(1).

El-Nashar, H.A.S., El-Din, M.I.G., Hritcu, L., and Eldahshan, O.A. 2021. *Insights on the Inhibitory Power of Flavonoids on Tyrosinase Activity: A Survey from 2016 to 2021*. Molecules. Vol. 26(24).

Fatmawaty, A., Aswad, M., Kolobani, M.N., Manggau, M.A., and Alam, G. 2010. *Efektifitas beberapa bahan alam sebagai bahan pemutih kulit: studi in vitro penghambatan aktifitas enzim tirosinase*. Jurnal Bahan Alam Indonesia. Vol. 7(4).

Hastiningsih, I., Pangkahila, W.I., Wiraguna, A.A.G.P., Aman, I.G.M., Pangkahila, J.A., Iswari, I and Tunas, K. 2019. *Ethanol Extract Cream of Jackfruit Tree Bark (*Arthocarpus heterophilus*) is Equally Effective with Hydroquinone Cream on Preventing the Increase of Melanin on Guinea Pigs Skin (*Cavia porcelus*) Exposed to Ultraviolet B Ray*. Journal of Global Pharma Technology. Vol. 11(04).

Jakimiuk, K., Sari, S., Milewski, R., Supuran, C.T., Şöhretoğlu, D., and Tomczyk, M. 2022. *Flavonoids as tyrosinase inhibitors in in silico and in vitro models: basic framework of SAR using a statistical modelling approach*. J Enzyme Inhib Med Chem. Vol.37(1).

Kim, H.D., Choi, H., Abekura, F., Park, J.Y., Yang, W.S., Yang, S.H., and Kim, C.H. 2023. *Naturally-Occurring Tyrosinase Inhibitors Classified by Enzyme Kinetics and Copper Chelation*. Int J Mol Sci. 24(9).

Kunarto, B. and Sani, E.Y. 2020. *Ekstraksi Buah Parijoto (*Medinilla speciosa *Blume) Berbantu Ultrasonik pada Berbagai Suhu, Waktu dan Konsentrasi Pelarut Etanol*. Jurnal Teknologi Pertanian. Vol. 21(1).

Kurniasari, A., Anwar, E., and Djajadisastra, J. 2018. *Potensi Ekstrak Biji Coklat (*Theobroma cacao *Linn) sebagai Inhibitor Tirosinase untuk Produk Pencerah Kulit*. Jurnal Kefarmasian Indonesia. Vol. 8(1).

Kusumawati, I., Kurniawan, K. O., Rullyansyah, S., Prijo, T. A., Widyowati, R., Ekowati, J., Hestianah, E. P., Maat, S., and Matsunami, K. 2018. *Anti- Aging Properties of* Curcuma heyneana *Valeton & Zipj: A Scientific Approach to Its Use in Javanese Tradition*. Journal of Ethnopharmacology. Vol. 225.

Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D. and Darnell, J. 2000. *Molecular Cell Biology*. Online website from NCBI https://www.ncbi.nlm.nih.gov/bookshelf Diakses 9 September 2023.

Mali, K.S., Lava, K., Binnemans, K., and Feyter, S.D. 2010. *Hydrogen Bonding Versus van der Waals Interactions: Competitive Influence of Noncovalent Interactions on 2D Self-Assembly at the Liquid–Solid Interface*. Chem. Eur. J. Vol. 16.

Maria, C., Buta, E., and Hort D. 2014. *Medinilla: an exotic and attractive indoor plant with great value.* Journal of Horticulture. Forestry and Biotechnology. Vol. 16(2).

Melinda, S., Annisa', E., and Sasikirana, W. 2021. *Potensi Sitotoksik Ekstrak Buah Parijoto (*Medinilla speciosa*) Terpurifikasi pada Sel Kanker Serviks Hela*. Generic: Journal of Research in Pharmacy. Vol. 1(2).

Milanda, T., Lestari, K. and Tarina, N.T.I. 2021. *Antibacterial Activity of Parijoto (*Medinilla speciosa *Blume) Fruit Against* Serratia marcescens *and* Staphylococcus aureus. Indonesian Journal of Pharmaceutical Science and Technology. Vol. 8(2).

Motiejunas, D. and Wade, R. 2006. *Structural, energetics, and dinamic aspects of ligand-receptor interaction* In J B. Taylor & D. J Triggle (Eds) Comprehensive medicinal chemistry II vol 4: Computer-assisted drug design. Elsevier.

Nautiyal, A. and Wairkar, S. 2021. *Management of Hyperpigmentation: Current Treatments and Emerging Therapies*. Pigment Cell & Melanoma Research. Vol. 34(6).

Panche, A. N., Diwan, A. D., and Chandra, S. R. 2016. *Flavonoids: an overview*. Journal of Nutritional Science. Vol. 5.

Parthasarathi, R. and Subarmanian, V. 2006. *Characterization of Hydrogen Bonding: From Van Der Waals Interactions to Covalency*. Springer : 1-50.

Priani, Sani and Muhammad Fakih, Taufik. 2021. *Identifikasi Aktivitas Inhibitor Enzim Tirosinase Senyawa Turunan Flavonoid pada Kulit Buah Cokelat (*Theobroma cacao *L) secara In Silico*. ALCHEMY Jurnal Penelitian Kimia. Vol.17(168).

Purnomo, H. 2011. *Kimia Komputasi: Molecular Docking Plants Penambatan Molekul Plants [Protein-Ligand AntSystem] (Ilmu Semut).* Yogyakarta: Pustaka Pelajar.

Quiroga, R. and Villarreal, M. A. 2015. *Vinardo: A Scoring Function Based on Autodock Vina Improves Scoring, Docking, and Virtual Screening*. Plos One. Vol. 11(5).

Ravi, L. and Krishnan, K. 2016. *A Handbook on Protein-Ligand Docking Tool : Autodock4*. Innovare Journal of Medicinal Sciences. Vol. 4(3).

Sa'adah, N., Nurhayati, A.P.D., and Purwani, K.I. 2018. A*ntihyperlipidemic and anti-obesity effects of the methanolic extract of parijot*o (Medinilla speciosa). AIP Conference Proceedings 2002. Vol. 1.

Sa'adah, N., Purwani, K.I., Nurhayati, A.P.D., and Ashuri, N.M. 2017. *Analysis of lipid profile and atherogenic index in hyperlipidemic rat* (Rattus norvegicus Berkenhout, 1769) *that given the methanolic extract of Parijoto* (Medinilla speciosa). AIP Conference Proceedings 1854, Vol. 1.

Sa'adah, N., Indiani, A.M., Nurhayati, A.P.D., and Ashuri, N.M. 2019. *Anthocyanins content of methanol extract of parijoto (*Medinilla speciosa*) and its effect on serum malondialdehyde (MDA) level of hyperlipidemic rat*. Nusantara Bioscience. Nusantara Bioscience. Vol. 11(1).

Sa'adah, N., Indiani, A.M., Nurhayati, A.P.D., and Ashuri, N.M. 2019. *Anthocyanins content of methanol extract of parijoto* (Medinilla speciosa) *and its effect on serum malondialdehyde (MDA) level of hyperlipidemic rat. Nusantara Bioscience.* Nusantara Bioscience. Vol. 11(1).

Sari, I.W., Junaidin, and Pratiwi, D. 2020. *Studi Molecular Docking Senyawa Flavonoid Herba Kumis Kucing (Orthosiphon stamineus B.) pada Reseptor Alpha-Glukosidase sebagai Antidiabetes Tipe 2*. Jurnal Farmagazine. Vol. VII(2).

Simamora, A. 2015. *Asam Amino, Peptida dan Protein. Buku Ajar Blok 3 Jilid 1*. Jakarta : Universitas Kristen Krida Wacana.

Yuwono, T. 2005. *Biologi Molekuler*. Erlangga : Jakarta