International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

An activity of flavonoid derived compounds from *Medinilla speciosa* as anti-hyperpigmentation against tyrosinase proteins with in silico methods

Elshinta Riantica *, Awik Puji Dyah Nurhayati and Noor Nailis Sa'adah

Department of Biology, Faculty of Science and Data Analytics, Sepuluh Nopember Institute of Technology, Surabaya, Indonesia.

International Journal of Science and Research Archive, 2023, 10(02), 1113–1123

Publication history: Received on 14 November 2023; revised on 22 December 2023; accepted on 25 December 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.10.2.1090

Abstract

Parijoto (*Medinilla speciosa*), a typical plant from Indonesia, contains flavonoid compounds as antioxidants. It is able to 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. Currently, there is no further research regarding the existence of flavonoid compound derivatives found in parijoto fruit. The aim of this research is 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 analysis 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.

Keywords: Docking; Flavonoid; Hiperpigmentation; Parijoto; Tyrosinase

1. Introduction

Hyperpigmentation is a condition of inequality pigmentation which is characterized by the appearance of dark spots around the skin area [1]. Hyperpigmentation affects the physical appearance of the skin by providing visible signs of aging, including wrinkling, irregular pigmentation, sagging, and elastosis [2]. Indonesia is a tropical country with exposure to ultraviolet rays from sun throughout the year, so the people are very susceptible to hyperpigmentation [3]. Hyperpigmentation is triggered by formation of free radical (ROS) which is stimulate melanogenesis or melanin pigment formation. If this is not be treated, it will cause a negative impact on health due to the accumulation of free radicals in the body [4]. Melanogenesis is catalyzed by enzyme tyrosinase [5]. When the number of melanocytes produced is uncontrolled, it will cause an abnormal amount of melanin, resulting in hyperpigmentation. One of the preventive measure hyperpigmentation is to inhibit the process of melanin synthesis through inhibiting tyrosinase or the enzyme that controls pigmentation and its activity provides useful information about the melanogenic potential of melanocytes [1].

Inhibition of tyrosinase can be done by utilizing bioactive compounds in plants [6]. Indonesia is mega biodiversity, it has a lot of kind of plants, including herbs, which potential to be used as medicine. A plant that has not been widely

* Corresponding author: Elshinta Riantica

explored pharmacologically is parijoto (*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 the Mount Muria, Kudus, Central Java [7], but nowadays it has begun to be cultivated as an ornamental plant with medicinal purpose [8-9]. *M. speciosa* is usually consumed as a fertilizer and treat various diseases, such as mouth ulcers and diarrhea. Besides of that, it is approved by study as well as an antibacterial, anti-inflammatory, antioxidant, anticancer, anti-hyperlipidemic, anti-obesity and immunostimulant activities [10-13].

Further study on *M. speciosa* natural products, leads to identification of active compounds that have potential in skin depigmentation with fewer negative effects. Based on several previous studies, natural compounds in plants have potential activity as inhibitors of tyrosinase enzyme, such as phenolic and flavonoid compounds [1,6,14]. Based on study by [15], 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 ability to depigment skin by directly inhibiting tyrosinase activity in the melanogenesis process. The bonds that occur between flavonoids and copper (Cu), and their antioxidant effects are reported to play a role in inhibiting the action of tyrosinase enzyme [1]. According to [16], 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, flavanols, catechins, and chalcones [17]. Until now, there has been no study that discusses 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 in silico using 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 [18]. So, this method can be used to predict the most likely activity, position, orientation and conformation between ligands with proteins [19]. The result score from 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 that, the compound is considered to be more potential [20]. If the docking results in this study prove that derivative compounds or flavonoid subgroups can inhibit tyrosinase, then identification and testing for the presence of these compounds in *M. speciosa* fruit can be carried out, followed by in vivo test on animal model 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.

2. Material and 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 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. Starting with separating the protein from its accompanying components using Biovia Discovery Studio 2020 software. It is known that the protein structure which has been downloaded from the Protein Data Bank is a complex structure that contains several components in it. To start the molecular docking, all components in the protein structure have to be removed, 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 have to be prepared. 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\receptor\receptor.pdbqt selected'. The docking process is directed at the active site of the receptor which can bind to the ligand by adjusting the location and dimensions of the gridbox. The amino acids as an active site in this study include: Phe197, Pro201, Asn205, His208, and Arg209. After the gridbox settings on the active side are complete, Autodock Vina can be run. The software will automatically carry out the docking process, 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, visualization of the docking results was carried out 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 used bioinformatics approach and molecular docking analysis for ligands and protein, so the analysis in this research is descriptive. Molecular docking analysis was carried out to see the conformation of the receptor-ligand complex as result from docking with Autodock Vina. The result shown 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, so that, the bonds formed will be stronger. 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.

3. Result

Based on the study that has been done, docking results showed nine 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 value -7.7; -7.6; - 7.5; -7.5; and -7.4 kcal/mol, respectively. The docking score values are in tables I and II.

Table 1 Results of compound docking values

Conformation	**Docking Energy / Binding Affinity (kcal/mol)**						
	Kojic acid	**Flavon**	**Flavonol**	**Flavanol**	**Flavanone**	**Catechin**	**Chalcone**
	E=76.9	**E=187.7**	**E=318.2**	**E=209.1**	**E=214.2**	**E=204.8**	**E=730.7**
5I38_001	-5.5	-7.5	-7.6	-7.5	-7.4	-7.7	-8.9
5I38_002	-5.4	-7.4	-7.5	-7.2	-7.2	-7.6	-8.1
5I38_003	-5.4	-7.3	-7.3	-7.2	-6.8	-7.5	-8.1
5I38_004	-5.3	-7.2	-7.0	-7.0	-6.8	-7.4	-7.8
5I38_005	-5.3	-7.2	-7.0	-6.9	-6.7	-7.3	-7.7
5I38_006	-5.1	-7.1	-6.7	-6.7	-6.6	-7.2	-7.6
5I38_007	-4.8	-6.7	-6.6	-6.6	-6.5	-7.2	-7.3
5I38_008	-4.8	-6.6	-6.3	-6.6	-6.4	-6.7	-6.9
5I38_009	-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. These are the results of the best docking scores from all ligands (Table II).

Table 2 Results of docking value selection

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

The results of the visualization shown the form of interactions between ligands (compounds) and amino acids in protein macromolecules. 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

Ligands and Receptor Target	Amino Acid Visualization	Binding Site
Kojic acid and tyrosinase	His42, His60, **Asn205, His208**, Val217, Val218	Phe197, Pro201, Asn205, His208, Arg209
Flavone and tyrosinase	**Phe197, His208**, Val218, Ala221	
Flavonol and tyrosinase	**His208, Arg209**, Val218, Ala221	
Flavanol and tyrosinase	**Pro201, Asn205, His208**, Val218, Ala221	
Flavanone and tyrosinase	**His208, Arg209**, Val218, Ala221	
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 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.

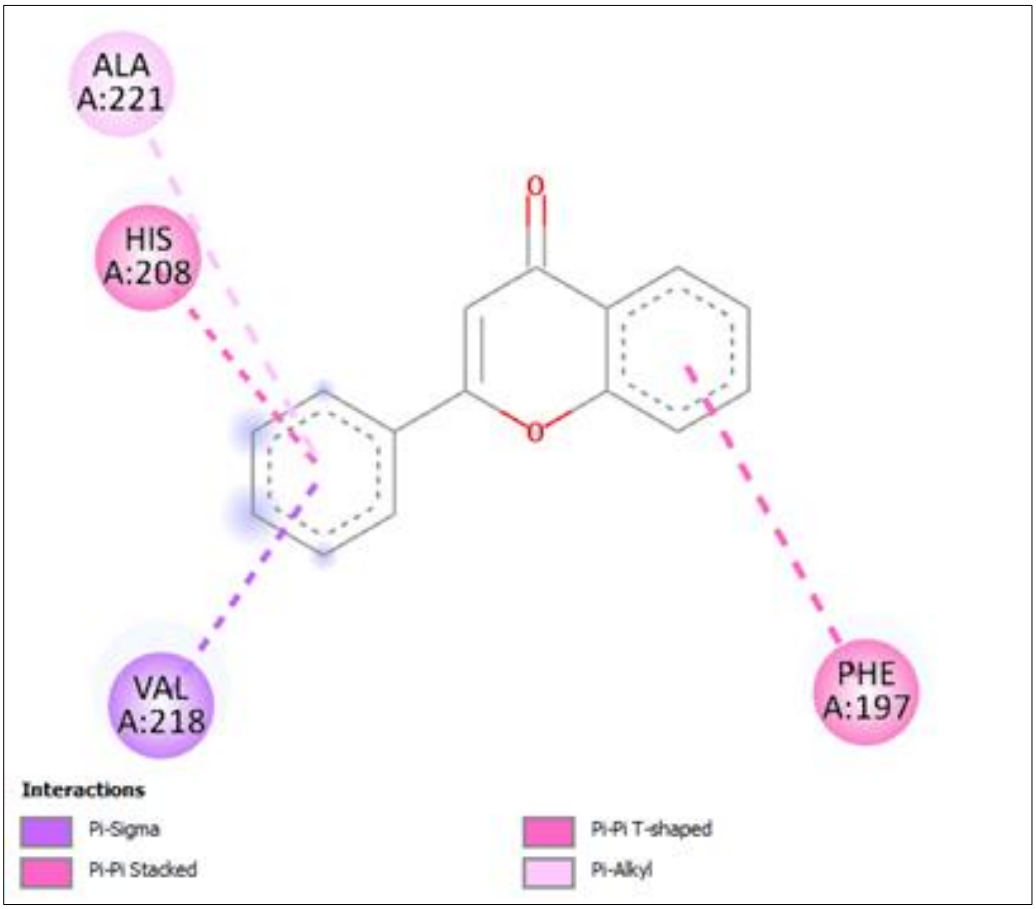

Figure 1 2D visualization of flavone compounds and tyrosinase protein

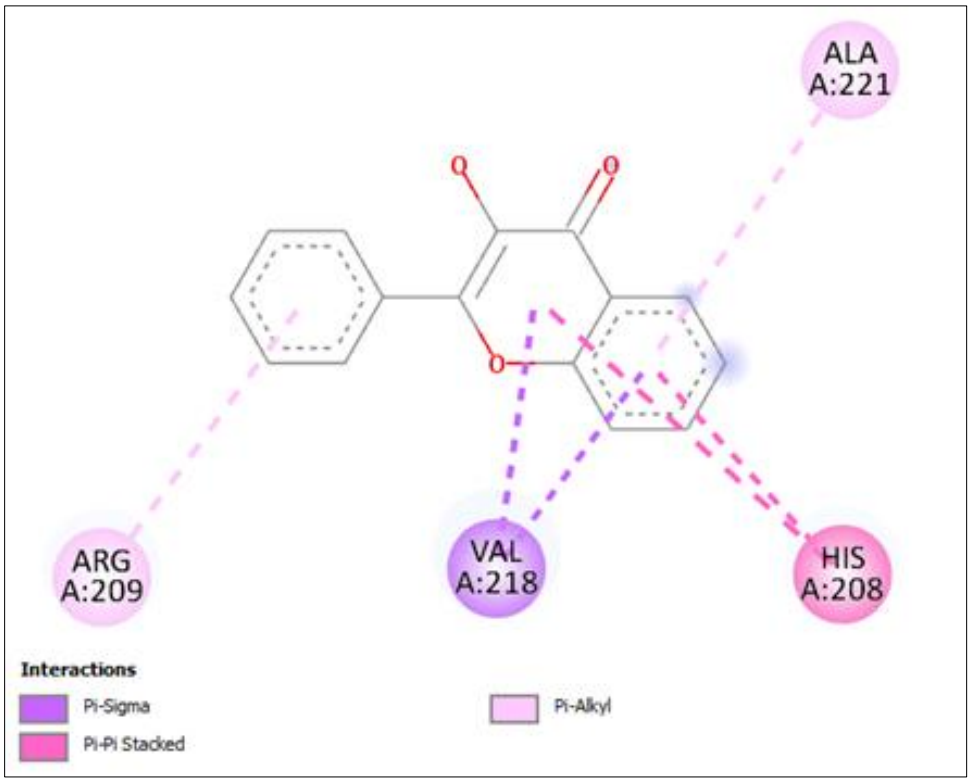

Figure 2 2D visualization of flavonol compounds and tyrosinase protein

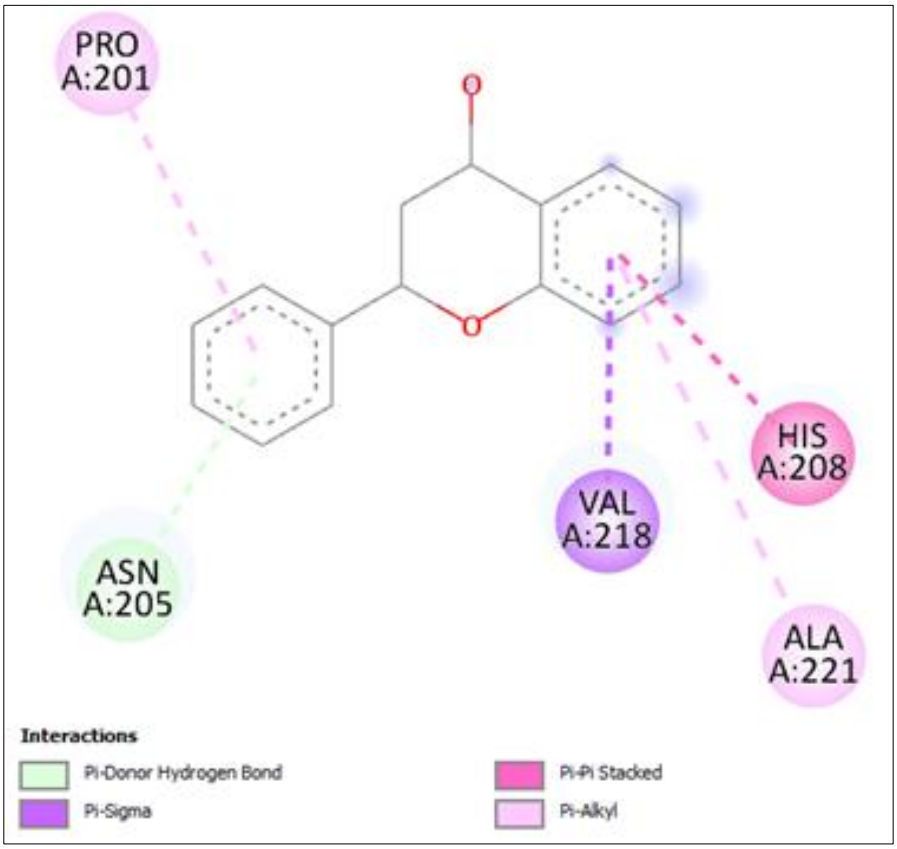

Figure 3 2D visualization of flavanol compounds and tyrosinase protein

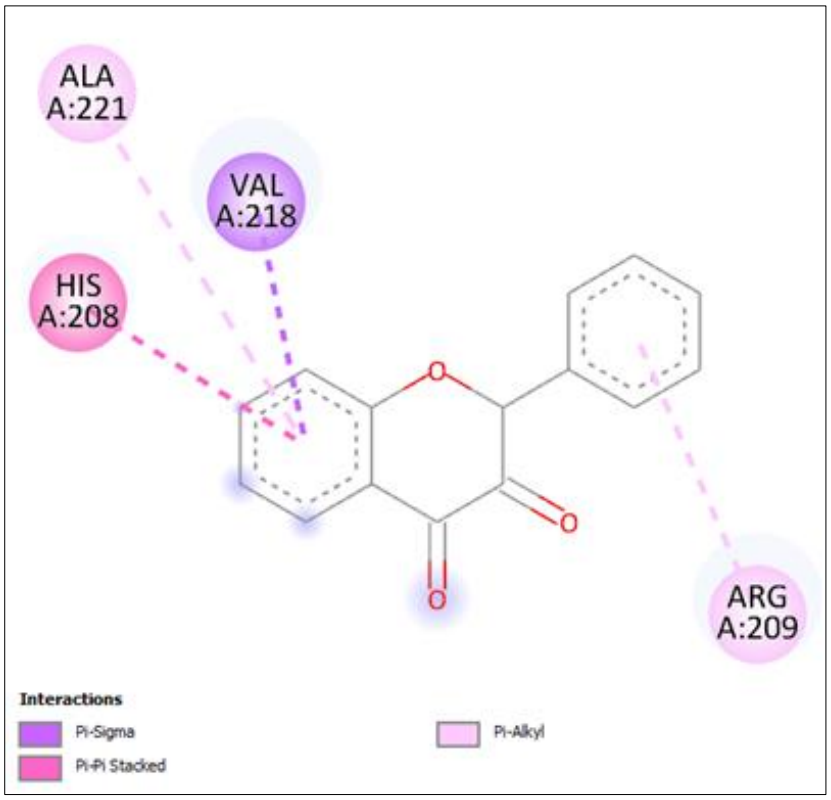

Figure 4 2D visualization of flavanone compounds and tyrosinase protein

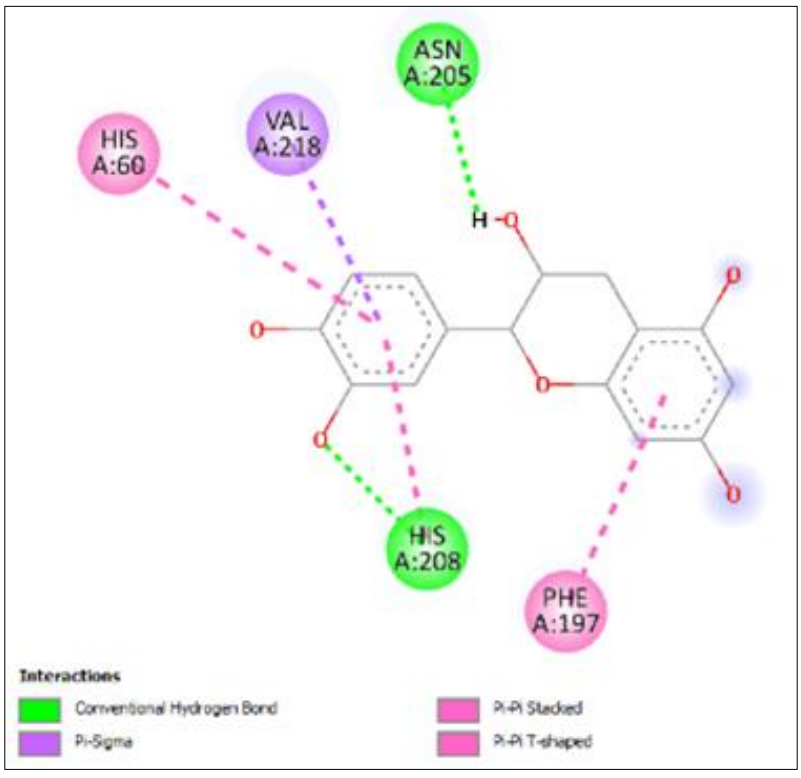

Figure 5 2D visualization of catechin compounds and tyrosinase protein

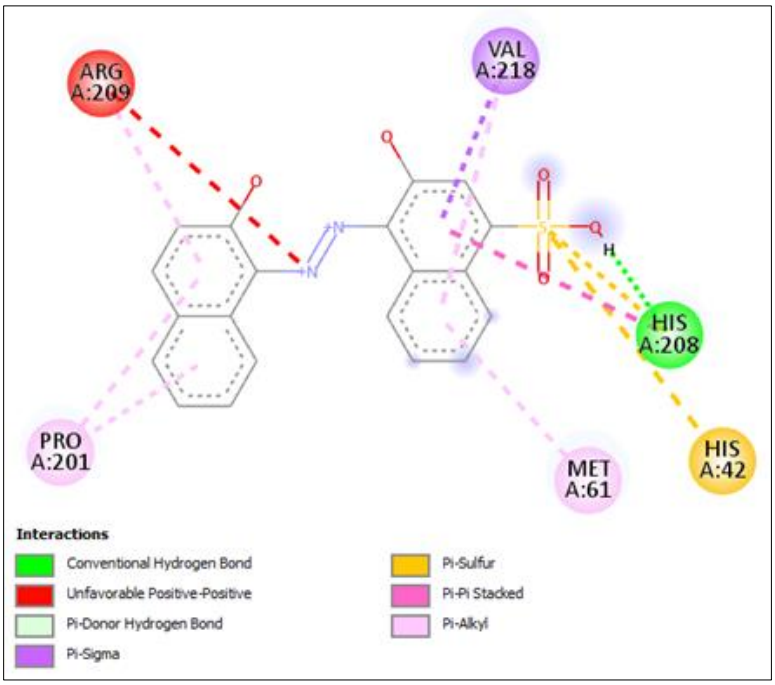

Figure 6 2D visualization of chalcone compounds and tyrosinase protein

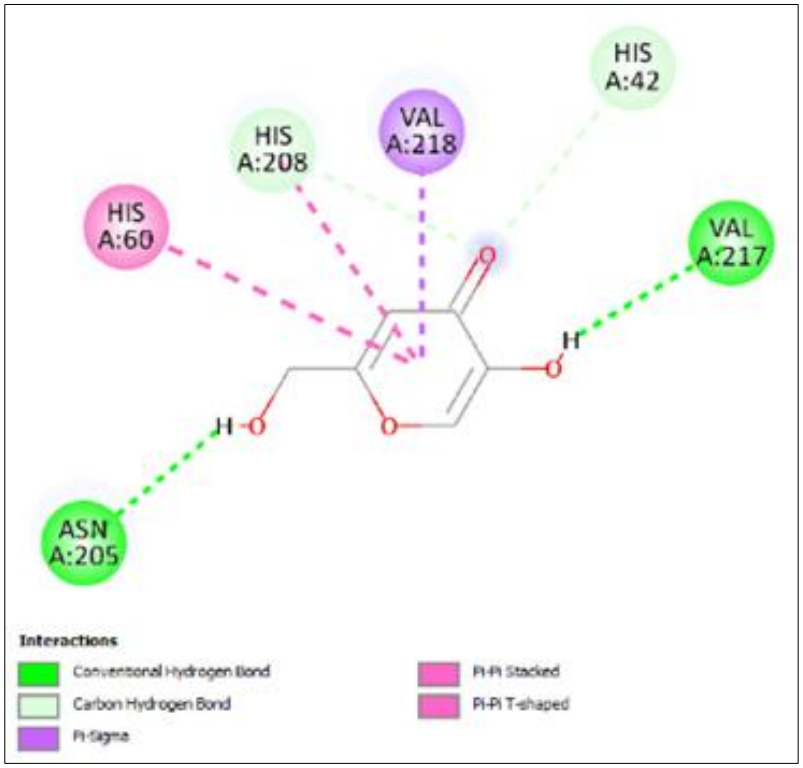

Figure 7 2D visualization of kojic acid compounds and tyrosinase protein

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.

4. Discussion

Based on the method steps, it is known that the process of downloading receptors and ligands used in this study only requires hardware such a computer that connected to the internet network. All files used can be freely accessed on the website or 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 [21]. 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 9 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, flavanols, flavones, and flavonones which have value -7.7; -7.6; -7.5; -7.5; and 7.4 kcal/mol, respectively (Table 1-2). The docking score is a parameter of binding affinity strength of the ligands and receptor. The more stable ligand-protein interaction is reflected by the lower of docking score (minus). The comparison of these scores explains whether a compound is potent or not to inhibiting the performance of the specific protein being targeted. The smaller docking result means the compound is considered to be more potent in inhibiting protein activity [20]. The docking score from the results of this study showed that flavonoid derivative compounds such as chalcone, catechin, flavonol, flavanol, flavones and flavonone have potential to inhibit tyrosinase activity which can cause hyperpigmentation. Of the six test ligands used, chalcone showed the greatest affinity value, which means it was predicted to be the most effective compound in inhibiting tyrosinase compared to others.

The mechanism of melanin production through melanogenesis pathway begins with 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 to produce the final product DOPA-quinone. Tyrosinase, along with tyrosinase-related protein (TRP), catechol oxidase, and hemocyanin, belongs 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 a His residue in the catalytic site of tyrosinase enzyme. If tyrosinase is inhibited by an inhibitor, melanin production will also be inhibited. Human tyrosinase inhibitors are very useful in pharmaceutical and cosmetic fields. Historically, a representative whitening agent is L-ascorbic acid (vitamin C) because it has been shown to play a role in inhibiting melanin synthesis through the reduction of 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 as tyrosinase inhibitors, including kojic acid which was used as a control in this study, although in certain doses it is cytotoxic to normal cells and cannot be penetrated into 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 [22].

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 is able to imitate tyrosine substrates so that it can 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 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 conjugated to saccharides or organic acids [22]. This is also possible for the chalcone, catechin, flavonols,

flavanols, flavones and flavonones compounds, because they 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 [23-24]. However, to confirm the results of this test, in-vitro and in-vivo tests are needed on animal model to see the biological activity and possible toxicity and other side effects if the ingredient is used as a skin care product.

The results of the visualization showed interactions between ligands (compounds) and amino acids in protein macromolecules. The amino acid residues that interact with the ligand will determine the type of bond that occurs between ligands and protein. Based on the results of visualization, it showed that the docking and visualization process of chalcone, catechin, flavonol, flavanol, flavone and flavonone compounds has been proven to be able 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 own characteristics, where these characters can determine the type of interaction that occurs with the compound. The active site or binding site in tyrosinase consists of four types of amino acids with different structures. The four types include: Phenylalanine, Proline, Asparagine, Arginine, and Histamine [25]. A binding site is a protein binding area for ligands which will influence the conformation and function of the protein. This area also shows amino acid residues which play a role in forming interactions between receptor and ligands [26]. In general, amino acids consist of an amino group, a carboxyl group, a hydrogen group and a side chain (R group) [27]. According to [25] the possibility of compound movement, which in this case uses kojic acid, in the active site can be shown in two positions: the peripheral site and the active site. 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), similar to 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 [22].

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 in the form of noncovalent interactions, such as: hydrogen bonds, ionic bonds, van der Waals interactions, and hydrophobic interactions [28]. 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 flavonone 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 are the type of bond that plays the most important role in docking results because hydrogen bonds have greater strength and stability than other bonds [29-30]. It is because hydrogen bonds can be formed even though the distance between the ligand and receptor is quite far [31]. Hydrogen bonds are known to have a big influence on the interaction between proteins and ligands, so they can increase the affinity value between that protein and ligands. Thus, if there are more hydrogen bonds occurring as a result of 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 [32]. The interactions that occur in the ligands and receptors used in this study prove that the test compounds are able to inhibit the target protein tyrosinase. According to study by [33], if the amino acid residue has a binding position similar to the inhibitor, even though only a few amino acids can interact in the binding site area, this allows the test compound to have inhibitory activity on the receptor.

5. Conclusion

Based on 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 that the compounds chalcone, catechin, flavonol, flavanol, flavones and flavonone have the potential to act as tyrosinase inhibitors and are predicted to be able to prevent hyperpigmentation with in silico methods. The types of amino acids that are 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.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] K.N. Allgisna, S. Hindun, and N. Rantika, Review: Comparison of Several Fruit Peel Extracts as Anti-hyperpigmentation, Journal of Science and Health, vol, 3(2), 2021, pp.335-342.

[2] A. Nautiyal, and S. Wairkar, Management of Hyperpigmentation: Current Treatments and Emerging Therapies, Pigment Cell & Melanoma Research, vol. 34(6), 2021, pp.1000-1014.

[3] Z. Ahmad and Damayanti, Skin Aging: Pathophysiology and Clinical Manifestations, Periodical of Skin and Venereology Health Sciences – Periodical of Dermatology and Venereology, vol. 30(3), 2018, pp.208-215.

[4] I. Hastiningsih, W.I. Pangkahila, A.A.G.P. Wiraguna, I.G.M. Aman, J.A. Pangkahila, I. Iswari, K. Tunas, 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), 2019, pp.280-285.

[5] I. Kusumawati, K. O. Kurniawan, S. Rullyansyah, T. A. Prijo, R. Widyowati, J. Ekowati, E. P. Hestianah, S. Maat, and K. Matsunami, Anti- Aging Properties of Curcuma heyneana Valeton & Zipj: A Scientific Approach to Its Use in Javanese Tradition, Journal of Ethnopharmacology, vol. 225, 2018, pp.64-70.

[6] A. Fatmawaty, M. Aswad, M.N. Kolobani, M.A. Manggau, and G. Alam, Effectiveness of several natural ingredients as skin whitening agents: in vitro study of inhibition of tyrosinase enzyme activity, Indonesian Journal of Natural Materials, vol. 7(4), 2010, pp.219-223.

[7] T. Milanda, K. Lestari, and N.T.I. Tarina, Antibacterial Activity of Parijoto (*Medinilla speciosa* Blume) Fruit Against Serratia marcescens and Staphylococcus aureus, Indonesian Journal of Pharmaceutical Science and Technology, vol. 8(2), 2021, pp.76-85.

[8] C. Maria, E. Buta, and D. Hort, Medinilla: an exotic and attractive indoor plant with great value. Journal of Horticulture, Forestry and Biotechnology, vol. 16(2), 2014, pp.9-12.

[9] B. Kunarto and E.Y. Sani, Ultrasonic Assisted Extraction of Parijoto Fruit (Medinilla speciosa Blume) at Various Temperatures, Times and Ethanol Solvent Concentrations, Journal of Agricultural Technology, vol. 21(1), 2020, pp.29-38.

[10] A. S. Hanum, E. Prihastanti and Jumari, Ethnobotany of utilization, role, and philosopical meaning of parijoto (Medinilla, spp) on Mount Muria in Kudus Regency, Central Java, AIP Conference Proceedings 1868, vol. 1, pp. 090018-1 – 090018-6, 2017.

[11] N. Sa'adah, K.I. Purwani, A.P.D. Nurhayati, and N.M. Ashuri, 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, 2017, pp.020031-1 – 020031-8.

[12] N. Sa'adah, A.P.D. Nurhayati, and K.I. Purwani, Antihyperlipidemic and anti-obesity effects of the methanolic extract of parijoto (*Medinilla speciosa*), AIP Conference Proceedings 2002, vol. 1. 2018, pp.020046-1 – 020046-8.

[13] N. Sa'adah, A.M. Indiani, A.P.D. Nurhayati, and N.M. Ashuri, 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), 2019, pp.112-118.

[14] A. Kurniasari, E. Anwar, and J. Djajadisastra, Potential of Cocoa Bean Extract (Theobroma cacao Linn) as a Tyrosinase Inhibitor for Skin Lightening Products, Indonesian Pharmaceutical Journal, vol. 8(1), 2018, pp.34-43.

[15] O.V. Febrilian, and E. Pujiastuti, Test of the Effectiveness of Parijoto Fruit Extract (Medinilla speciosa Blume) on Blood Glucose Levels in White Wistar Rats Loaded with Sucrose, Proceedings of Hefa (Health Events for All), 2017, pp. 341-346

[16] H.A.S. El-Nashar, M.I.G. El-Din, L. Hritcu, and O.A. Eldahshan, Insights on the Inhibitory Power of Flavonoids on Tyrosinase Activity: A Survey from 2016 to 2021, Molecules, vol. 26(24), 2021, pp.1-22

[17] A.N. Panche, A.D. Diwan, and S.R. Chandra, Flavonoids: an overview, Journal of Nutritional Science, vol. 5, 2016, pp.1-15.

[18] D. Motiejunas, and R. Wade, Structural, energetics, and dinamic aspects of ligand-receptor interaction In J B. Taylor and D. J Triggle (Eds) Comprehensive medicinal chemistry II vol 4: Computer-assisted drug design, Elsevier, 2006, pp.193-214

[19] R. Quiroga, and M.A. Villarreal, Vinardo: A Scoring Function Based on Autodock Vina Improves Scoring, Docking, and Virtual Screening, Plos One, vol. 11(5), 2015, pp.1-18.

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

[21] L. Ravi and K. Krishnan, A Handbook on Protein-Ligand Docking Tool: Autodock4, Innovare Journal of Medicinal Sciences, vol. 4(3), 2016, pp.1-6.

[22] H.D. Kim, H. Choi, F. Abekura, J.Y. Park, W.S. Yang, S.H. Yang, and C.H. Kim, Naturally-Occurring Tyrosinase Inhibitors Classified by Enzyme Kinetics and Copper Chelation, Int J Mol Sci. 24(9), 2023, pp.1-12.

[23] S. Priani, and T.M. Fakih, Identification of Tyrosinase Enzyme Inhibitory Activity of Flavonoid Derived Compounds in Chocolate Fruit Skin (Theobroma cacao L) in Silico, ALCHEMY Journal of Chemical Research, vol.17(168), 2021, pp.168-176.

[24] K. Jakimiuk, S. Sari, R. Milewski, C.T. Supuran, D. Şöhretoğlu, and M. Tomczyk, 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), 2022, pp.421-430.

[25] B. Deri, M. Kanteev, M. Goldfeder, D. Lecina, V. Guallar, N. Adir, and A. Fishman, The unravelling of the complex pattern of tyrosinase inhibition, Scientific Reports, vol. 6(1), 2016, pp.1-10.

[26] Arwansyah, L. Ambarsari, and T. Sumaryada, Docking Simulation of Curcumin Compounds and Their Analogs as Androgen Receptor Inhibitors in Prostate Cancer, Journal Current Biochemistry, vol 1(1), 2014, pp.11-19.

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

[28] T. Yuwono, Biologi Molekuler, Erlangga: Jakarta, 2005.

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

[30] K.S. Mali, K. Lava, K. Binnemans, and S.D. Feyter, 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, 2010, pp.14447-14458.

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

[32] D. Chen, N. Oezguen, P. Urvil, C. Ferguson, S.M. Dann, and T.C. Savidge, Regulation of protein-ligand binding affinity by hydrogen bond pairing, American Association for the Advancement of Science, vol. 2(3), 2016, pp.151-169.

[33] I.W. Sari, Junaidin, and D. Pratiwi, Molecular Docking Study of Flavonoid Compounds of Cat's Whisker Herb (Orthosiphon stamineus B.) on Alpha-Glucosidase Receptors as Type 2 Antidiabetes, Journal of Farmagazine, vol, vII(2), 2020, pp.54-61.