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The Discovery of Tyrosinase Enzyme Inhibitors Activity from Polyphenolic Compounds in Red Grape Seeds through *In silico* Study

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ABSTRACT

Tyrosinases are essential metal-containing enzymes in the biosynthesis of melanin, therefore responsible for pigmentation of the skin. The upregulation of tyrosinase enzyme activities leads to hyperpigmentation that will become a health problems and interfere psychosocially. Inhibition of tyrosinase enzyme activity, both competitive and non-competitive become widely developed for most anti hyperpigmentation agent. Natural antioxidants are one of the potential compounds for this purpose. Red grape seeds contain high levels of antioxidant compounds, such as procyanidin, prodelphinidin, and propelargonidin. In this research *in silico* studies, including molecular docking, molecular dynamics simulations, and toxicity predictions, were used to assess the activity of the three molecules of polyphenolic compounds on macromolecules of the tyrosinase enzyme. Molecular docking studies show that the compound propelargonidin has the highest affinity against the macromolecule of the tyrosinase enzyme, with a binding free energy value of −32.87 kJ/mol. These results were confirmed in molecular dynamics simulations that show strong interactions at the macromolecular active site of the tyrosinase enzyme. Toxicity prediction results show that the three polyphenolic compound molecules were classified in the High-Class Category, which shows that safety is not guaranteed, but is likely, not carcinogenic and nongenotoxic. Therefore, the compound propelargonidin is predicted to be able to interact strongly with the tyrosinase enzyme. The results in this research are useful for further study in the development of tyrosinase enzyme inhibitors.

Keywords: tyrosinase enzyme, red grape seeds, polyphenolic compounds, inhibitory pattern, *in silico* study.

INTRODUCTION

Cutaneous pigmentation is a crucial process to protect mammal's DNA against the harmful effect of UV radiation and some environmental challenges [1]. This process runs under some complex biosynthesis reactions regulated by the tyrosinase enzyme [2]. Tyrosinase is Cu2+ or Zn2+ metalloenzymes expressed in the melanocyte and mainly localized within specialized organelles called melanosomes. Tyrosinase plays key roles in melanogenesis, to form the brown pigment of eumelanin and the red pheomelanin pigment [3]. This enzyme is able to catalyze two main reactions of melanogenesis, that was hydroxylation of monophenols (L-tyrosin) to o-diphenols (L-3,4-dihydroxyphenylalanine/L-DOPA) and convert o-diphenols (L-DOPA) compound to o-quinones (L-Dopaquinones) through oxidation reaction [4,5]. This quinone is a reactive compound that will undergo cyclization and rearrange spontaneously to be L-dopachrome in the absence of a thiol compound [4].

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Upregulation of tyrosinase enzyme caused by some factor like excessive of UV radiation, hormonal and stress factor, the side effect of some drugs, and some clinical condition, caused excessive melanin production and accumulation, which lead to hyperpigmentation that may interfere aesthetically, clinically, even psychosocially [2]. Clinically, hyperpigmentation is a marker of several skin diseases such as melasma, freckles, inflammation melanoderma, and Solari's lentigo [2,6]. Psychosocially, hyperpigmentation has a significant impact on the quality of life, where the impacted patient has a negative self-perception, negative social and emotional function especially in a group, also lower self-esteem [7,8].

Inhibition of tyrosinase enzymatic activity both competitive and non-competitively is the most widely used strategy in the development of depigmentation agents, both as cosmeceuticals and or drugs, in the treatment of hyperpigmentation [5]. Competitive inhibitors is usually a compound that can be bound to the active side of tyrosinase enzyme, so that it block the natural ligand from binding to the active site of tyrosinase enzyme. Non-competitive tyrosinase inhibitor is usually a non-enzymatic inhibitor, some of them eliminate or inhibit oxidation reaction in the melanogenesis pathway, or eliminate the triggers that can be upregulated enzymatic activity of tyrosinase [9]. Several compound can have both of these activity simultaneously. A natural antioxidant is a potential compound for these purposes [9]. Red grape seed (*Vitis vinifera)* contains a high level of polyphenol antioxidant compounds [10]. There are three polyphenolic compounds with the highest level of antioxidant in red grape seed, such as procyanidin, prodelphinidin, and propelargonidin [11].

This research will thoroughly study the interaction and affinity of the three red grape seed polyphenolic compounds to the tyrosinase enzyme receptor using the *in silico* study. The expected outcome of this research is information regarding the interaction and effect of red grape seed polyphenolic molecules on the tyrosinase enzyme. So that it can be a preliminary study of the development of anti-hyperpigmentation agents derived from natural sources.

EXPERIMENT

Macromolecule Enzyme Preparation

Macromolecules used in this research were the tyrosinase enzymes obtained from Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 4P6R [12]. This enzyme macromolecular preparation was performed by removing water molecules and small molecules (natural ligands), adding polar hydrogen atoms, and calculating Kollman's partial charge.

Molecule Ligands Preparation

The ligand molecules used in this research were procyanidin, prodelphinidin, and propelargonidin which had been modeled using ChemOffice (Figure 1). The ligand molecular structure was optimized using Gaussian09 with the Density Functional Theory (DFT) and Becke three-parameter Lee-Yang-Parr (B3LYP) functional on a 3-21G basis set [13]. The partial charge data from the optimized structure was used as input for molecular docking studies.

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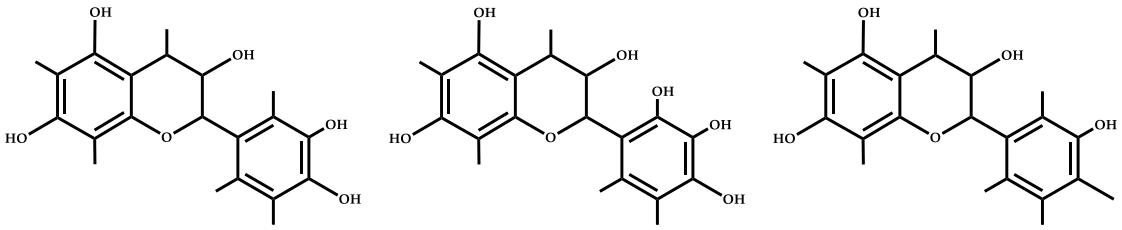

Figure 1. The structure of molecule ligands used in this research.

Molecular Docking Study

The molecular docking studies were conducted by AutoDock 4.2 with MGLTools 1.5.6 [14]. All molecular ligands for this molecular docking study were added with hydrogen atoms, put a partial charge on each atom produced from the DFT method, and set as maximum torsion. A grid map was created by centering the grid at a point 64 × 60 × 60 with a distance of 0.375 Å. The Lamarckian Genetic Algorithm [15] was used with 100 conformations for each molecular ligand. All other molecular docking parameters were set as default. Analysis of the results of molecular docking studies was performed with VMD 1.9.2 [16] and Discovery Studio 2020.

Molecular Dynamic Simulation

Molecular dynamics simulations were conducted for all complexes resulting from molecular docking studies. The simulations were performed by Gromacs 2016 [17–19] and analyzes were accomplished with VMD 1.9.2 [16] and Discovery Studio 2020. The AMBER99SB-ILDN and AMBER general force field (GAFF) [20] were used for macromolecule enzyme parameterization, whereas the molecule ligand was parameterized using ACPYPE [21]. The long-distance electrostatic force was determined by the Particle Mesh Ewald method [22,23]. System neutralization was done by adding Na+ and Cl- ions. The cubic TIP3P water model was used to solvate the complex system. The simulation step includes minimization, heating up to 310 K, temperature equilibrium (NVT), pressure equilibrium (NPT), and production run with timestep 2 fs for 20 ns. System stability was verified by analysis of energy, temperature, pressure, root mean square deviation (RMSD), and root mean square fluctuation (RMSF) of enzyme macromolecules. Analysis of the interaction stability of compound molecules and enzyme macromolecules was accomplished by calculating the value of RMSD and RMSF residues at the binding site of enzyme macromolecules during the simulations.

Toxicity Prediction

Toxicity prediction was performed on all ligand molecules using Toxtree v.3.1.0 [24]. The parameters used in this toxicity prediction were Cramer Rules to observe the level of toxicity from the functional group, Kroes TTC decision tree to estimate the threshold for exposure to drug compounds in humans, and Benigni/Bossarulebase (for mutagenicity and carcinogenicity) to find out what these compounds can cause carcinogenicity and mutagenicity.

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RESULT AND DISCUSSION

Molecular Docking Study

The three polyphenolic compounds derived from red grape seeds, namely procyanidin, prodelphinidin, and propelargonidin were docked into the macromolecule of the tyrosinase enzyme. All complexes from the results of the molecular docking study were then selected for further studies using molecular dynamics simulations. All test compound molecules had greater negative binding free energy compared to natural ligands against the active site of the tyrosinase enzyme (Table 1). This phenomenon shows a promising sign that the molecules of these compounds have good affinity with the target enzyme macromolecules. Propelargonidin has the best binding at the active site of the tyrosinase enzyme, with a free energy binding value of −32.87 kJ/mol better than the molecules of other compounds.

Table 1. Binding free energies of the molecule ligands against tyrosinase enzymes.

Ligand	Binding free energy (kJ/mol)
Natural ligand	−23.47
Procyanidin	−30.71
Prodelphinidin	−29.12
Propelargonidin	−32.87

Then identification and evaluation of molecular interactions were formed between the compound molecules and the tyrosinase enzyme macromolecules. Propelargonidin was able to form 22 interactions which include 3 hydrogen bonds (with Arg209 and Met215), 18 hydrophobic interactions (with His42, His60, Met61, Met184, Phe197, Pro201, His204, His208, Arg209, Val218, and Ala221), and 1 electrostatic interaction (with Arg209) (Figure 2). Whereas, procyanidin and prodelphinidin were only able to form 20 interactions on the active site of the macromolecule tyrosinase enzyme.

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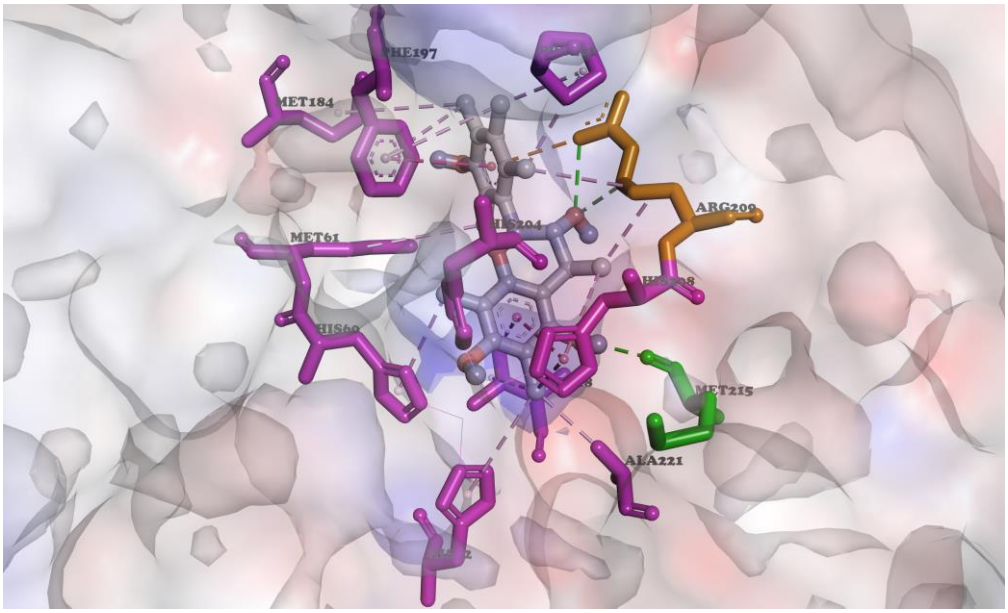

Figure 2. Interaction between propelargonidin molecules on the active site of tyrosinase enzyme macromolecules.

The interaction between the small molecule of the compound and the active site of the target enzyme macromolecule consists mainly of hydrogen bonds, with the compound mainly acting as a hydrogen bond donor and the amino acid residue of the enzyme as a hydrogen bond acceptor. Most of the hydrogen bonds formed were quite strong, with an average bond length of about 3 Å.

Molecular Dynamic Simulation

Further identification was performed on the three molecular complexes of the polyphenol compound and target enzyme macromolecules to examine the effect of compound molecular bonds on the target amino acid macromolecular residues, especially, in the binding site area. The dynamics of molecular interactions between the molecules of these compounds and their target macromolecules were studied using molecular dynamics simulations with explicit solvents. Strong affinity tends to decrease the movement of the atoms it binds to and generally stabilizes the binding region of the tyrosinase enzyme. This phenomenon was analyzed by calculating the root mean square deviation (RMSD) of the atomic binding site of macromolecular enzymes during a 20 ns simulation. In addition, the overall fluctuation of each amino acid residue at the binding site was also analyzed by calculating the root mean square fluctuation (RMSF).

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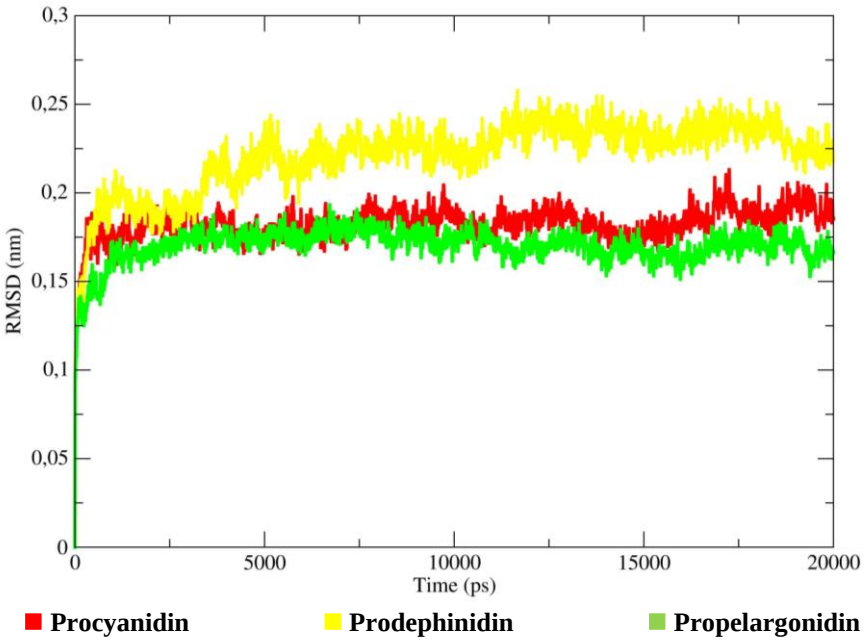

Figure 3. Variation of RMSD graphic from the complex of tyrosinase enzyme macromolecules and red grape seed polyphenolic compounds.

During the molecular dynamic simulations, procyanidin and propelargonidin molecules were able to stabilize the macromolecule tyrosinase enzyme. While the prodelphinidin molecule has a significant RMSD value from the beginning to the end of the simulations, with an average value above 2 Å (Figure 3). This phenomenon proves that these three-polyphenol compound can work as tyrosinase enzyme inhibitors by binding strongly to the active site of enzyme macromolecules and competing with their natural ligands. Moreover, it was able to stabilize the structure of enzyme macromolecules and prevent the conformational changes needed by enzyme macromolecules to catalyze a reaction.

It can be stated that these three antioxidant compounds from red grape seeds have a double action. First as a non-competitive inhibitor related to the strong antioxidant activity they have [10,11]. One of the things that plays a role in preventing hyperpigmentation is reducing the trigger which can upregulate tyrosinase enzyme activity, one of which is an oxidation reaction. Secondly, as a competitive inhibitor, because these three polyphenol compounds are proven to have better affinity and can be bound more strongly than the natural ligand of the tyrosinase enzyme and form a stable enzyme conformation. So that, when these compounds bind to the tyrosinase enzyme, it will immediately block its natural ligand to bind to the enzyme and terminate the melanogenesis reaction sequence, at the end of the reaction the formation of skin color pigment (eumelanin) is inhibited.

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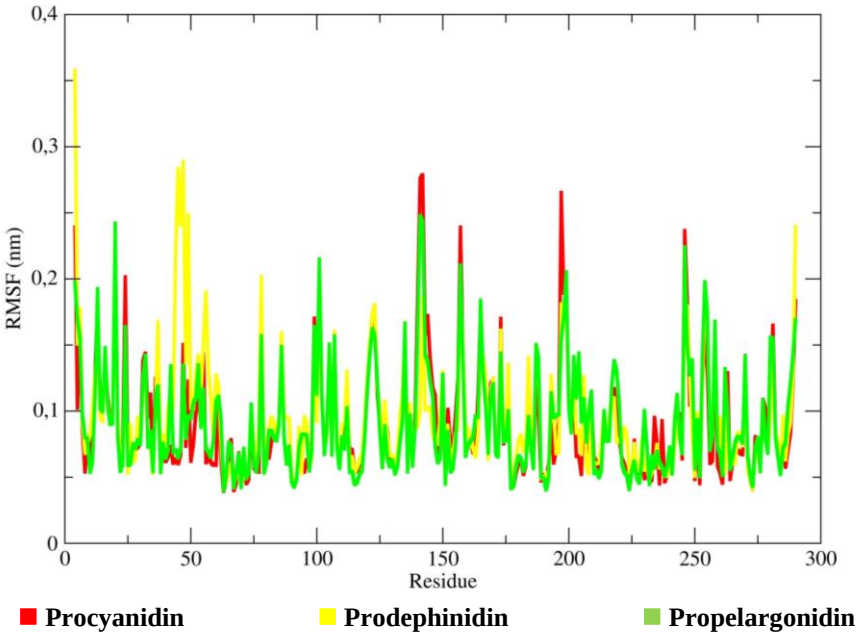

Figure 4. RMSF amino acid residues in the active site of the tyrosinase enzyme macromolecules when complexed with red grape seed polyphenolic molecule compounds.

The RMSD data was also supported by identifying the RMSF values of amino acid residues found at the binding sites of enzyme macromolecules (Figure 4). Propelargonidin compound molecules were able to bind strongly to amino acid residues in the active site of enzyme macromolecules, as can be observed at low RMSF values of these amino acid residues compared to RMSF values of the compound molecules of procyanidin and prodelphinidin. Some of these low-fluctuating amino acid residues were included in amino acid residues that were responsible for binding with propelargonidin molecules, namely His42, His60, Met61, Met184, Phe197, Pro201, His204, His208, Arg209, Met215, Val218, and Ala221.

Toxicity Prediction

The toxicity properties of the three test compound molecules were predicted using Toxtree software based on Cramer Rules, Kroes TTC decision tree, and Benigni/Bossarulebase. According to Cramer rules, molecular compounds were classified as Class III High Toxicity, which means that at high concentrations, these compounds have a risk of causing toxic effects. This phenomenon is caused by functional groups of compounds that are similar to many known poisons, such as cyclic, heterocyclic, and heteroaromatic functional groups.

The TTC Kroes decision tree concluded that the three compound molecules were predicted to have carcinogenic properties at doses of more than 0.15 μg/day. However, there is an 86-97% chance of risk reduction if a dose of less than 0.15 ug/day is used. Then, based on Benigni-Bossarulebase, some compound molecules were predicted to have genotoxic carcinogenic properties because they have functional groups that are identical to hydrazine. Although the toxicity prediction concludes that the compound molecules exhibit possible toxic or carcinogenic properties, this is based on statistical analysis, and laboratory toxicity tests need to be performed to ensure further safety.

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CONCLUSION

Based on the results of molecular docking studies, it is proven that the propelargonidin molecule was able to bind strongly to the active site of the macromolecule of the tyrosinase enzyme, with the binding free energy value of −32.87 kJ/mol. Interestingly, these compound molecules can also form very stable interactions with the active site of the tyrosinase enzyme during molecular dynamics simulations of 20 ns. The results of the research indicate that the compound propelargonidin molecule has the potential to be further developed as an inhibitor of the tyrosinase enzyme.

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