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The Potent of Constituents from Leunca Fruits (*Solanum nigrum* Linn.) as Anti-Skin Aging against TYRP-1: Molecular Docking and ADME-Tox

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Abstract: Leunca fruits are a source of natural antioxidants with compounds that have been reported, namely quercetin, solasodine, solanine, solamargine, and solasonine. However, its effectiveness as an anti-skin aging has not been studied. In the skin, melanin is produced by melanocytes in the deepest layer of the epidermis, thus protecting the skin from damage caused by sunlight radiation. Moreover, pregnant women can also cutaneous changes due to hyperpigmentation by skin lesions. If the formation of abnormal melanin, it is necessary to inhibit the tyrosinase enzyme, TYRP-1. This research was conducted by predicting the interaction between compounds of leunca fruits and the enzyme of TYRP-1 using Audock Tooks 1.5.6. Then, from the molecular docking results, ADMET-Tox was carried out with pre-ADMET to determine transdermal drug delivery and safety when used as a product. The results of in silico molecular docking showed that solasodine and quercetin had a higher binding affinity (ΔG, -8.58 and -7.74 kcal/mol; Ki, 517.15 and 2110 nM) compared to kojic acid (-5.50 kcal/mol; 92720 nM) as a reference. The amino acid His192 becomes a crucial residue when hydrogen interacts based on visualization. While ADME-Tox, solasodine fulfills the safety of the transdermal route. Therefore, this compound can potentially be anti-skin aging from the leunca fruits. Apart from that as a drug topical for pregnant women when melanogenesis in the epidermal melanocytes.

Keywords: ADME-Tox, leunca, molecular docking, TYRP-1

Introduction

Aging is a degenerative process involving all the body's organs, including the skin. Skin aging is caused by physiological factors that are difficult to avoid (Xie et al., 2015). The skin usually becomes dry, scaly, rough, wrinkled, and black spots or spots appear. The aging process is divided into intrinsic, which occurs naturally over time due to biological and extrinsic factors, such as excessive exposure to sunlight, pollution, smoking habits, and unbalanced nutrition (Nisa and Surbakti, 2016).

Indonesia is one of the tropical countries with exposure to ultraviolet rays from the sun throughout the year and is susceptible to skin aging due to long-term exposure (Ahmad and Damayanti, 2018). Open skin areas that are very vulnerable to exposure to ultraviolet rays are the face, neck, and arms. Free radicals attract electrons from molecules, so skin cells experience damage, functional disorders, and even death (Nisa and Surbakti, 2016). Melanin pigment plays a major role in defending the skin from exposure to ultraviolet rays (Maack and Pegard, 2016).

Meanwhile, pregnant women in the third trimester also experience an increase in hormones that stimulate melanogenesis in epidermal melanocytes through induction of the synthesis of melanogenic enzymes, such as tyrosine and tyrosine-related proteins 1 and 2 (TYRP-1 and TYRP-2) (Filoni et al., 2019) results in hyperpigmentation or darkening of the skin color. The reason is, that the melanocyte-stimifying

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hormone (MSH) can regulate pigments that influence skin color. In addition, the hormones estrogen and progesterone trigger excess melanin production on the face, which is called melasma (Putra et al., 2022).

In that case, serious problems occur on the face (Zolghadri et al., 2019) where the tyrosinase enzyme, namely TYRP-1 is the main target in inhibiting the formation of abnormal melanin due to excessive solar radiation (Biswas et al., 2017) and 90% in the pregnant women (Putra et al., 2022). Currently, several methods are used to prevent skin aging on the face such as skin care products and laser technology aimed at tightening the skin. However, this method causes skin problems, so antioxidants sourced from natural ingredients are needed.

One source of natural antioxidants is leunca fruits (*Solanum nigrum* Linn.). The results of phytochemical screening carried out by Khaerunnisa et al. (2022) show that the methanol extract contains flavonoids, alkaloids, saponins, phenolics, tannins, quinones, and steroids. Meanwhile, the ethyl acetate fraction is flavonoids, alkaloids, phenolics, tannins, and steroids, and alkaloids, phenolics, tannins, and steroids for the n-hexane fraction. Most flavonoid secondary metabolite compounds in leunca fruits are quercetin (Husnia and Budiarti, 2021). Apart from that, there are compounds solanine, solamargin, solasonine, and solasodine, which are classified as secondary metabolites of alkaloids (Karmakar et al., 2010).

The antioxidant test by Halim et al. (2018) on leunca fruit juice showed very high activity with an inhibitory concentration (IC_{50}) value of 206.5542 ± 9.3771 mg/L. This has the potential to inhibit the enzyme of TYRP-1 which causes hyperpigmentation on the skin. The use of leunca fruits as anti-skin aging has not been reported, so research must be carried out using a structure-based drug design (SBDD) approach, namely in-silico molecular docking as an initial stage (Meng et al., 2011). This approach aims to identify the similarity of active molecules to specific target proteins with binding affinity as an important value that needs to be considered (Kelutur, 2023). Apart from that, predictions of transdermal drug delivery and drug safety with Pre-ADMET were also carried out.

Materials and Methods

Data Preparation

Test ligands in the form of flavonoids (quercetin) and alkaloids (solanine, solamargine, solasonine, and solasodine) from the leunca fruits were obtained from research that has been carried out (Husnia and Budiarti, 2021; Karmakar et al., 2010). Then, download these secondary metabolites in PubChem (https://pubchem.ncbi.nlm.nih.gov/) with 2D structures in SDF format. Kojic acid as a reference ligand (native ligand) and TYRP-1 protein (Figure 1) were obtained from the Protein Data Bank (https://www.rcsb.org/) with the PDB ID code 5M8M.

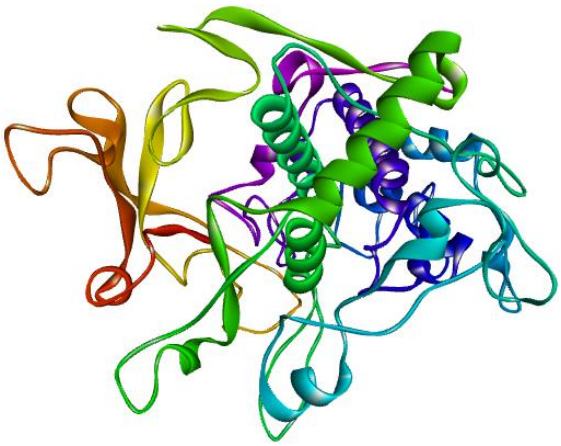


Figure 1. The enzyme of TYRP-1

Procedures

Enzyme and ligands

The water molecule and native ligand were separated from the enzyme of TYRP-1 using Discovery Studio 3.5 Visualizer® (DS). The results obtained are pure enzyme and saved in PDB format (.pdb) (Wibowo et al., 2019). Then, the enzyme was re-prepared using Autodock Tools® 1.5.6 (ADT) by adding Kollman charges and hydrogen polar only then saved in pdbqt format (Arba et al., 2018; Morris et al., 2009). Meanwhile, the native ligand (kojic acid) is obtained when separated from the enzyme using DS by selecting the script menu, selection then selecting protein chains and saving in .pdb format (Wibowo et al., 2019) as well as the test ligand downloaded from PubChem. After that, native and test ligands added

Gasteiger, all hydrogen, and non-polar merge using ADT and saved in pdbqt format format (Kelutur and Mustarichie, 2020).

Identification of active pocket from the enzyme of TYRP-1

Grid box settings to determine the position of the active pocket, namely the size of the grid box and the center coordinate values (x, y, z) using ADT. The grid box values obtained are used as a reference for the process of molecular docking test ligands to the TYRP-1. Meanwhile, the docking parameters are based on the Lamarckian Genetic algorithm (100 times) with the algorithm generation value being 2,500,000 and 150 for the population as well as other values are left at defaults (Hariyanti et al., 2021; Opo et al., 2021).

Molecular docking process

Docking between native ligand and enzyme of TYRP-1 using ADT program (run-autogrid and run-autodock). Then edit the cmd directory (autogrid4 -p dock.dpf -l dock.dlg &) for autogrid and autodock (autodock4 -p dock.dpf -l dock.dlg &) as well as click launch, which indicates the molecular docking process has started (Kelutur, 2022).

Method validation based on the RMSD

Method validation was carried out by evaluating the root-mean standard deviation (RMSD) value. To be more accurate, it is visualized by the overlap between the native ligand crystallography and *re*-docking (Kelutur, 2023).

Molecular docking of active compounds from leunca fruits to TYRP-1 and visualization

The in silico molecular docking process for the test ligands was carried out in the same way as the validation process, which used parameters of grid and docking from the validation results (Kim and Skolnick, 2008). The molecular docking process uses ADT, where the results obtained are opened with Notepad++® to obtain the binding affinity (ΔG and Ki) based on histogram clustering. In addition, visualization of amino acid residues (hydrogen bond, HB and Van der Waals, VdW) was carried out with DS (Kelutur et al., 2021; Wibowo et al., 2019).

Prediction of pharmacokinetic properties and toxicity

Test and native ligands that have the potential as drug products are drawn and uploaded in the Mol file format (*.mol) for prediction of transdermal drug delivery, mutagenic, and carcinogenic using Pre-ADMET (https://preadmet.webservice.bmdrc.org/adme/).

Results and Discussion

Results

Validation Method

In this study, the RMSD value obtained was 2.328 Å, as shown in Figure 2 when overlapping. Meanwhile, the coordinates of center values for the TYRP-1 protein are x = -31.471, y = -3.610, and z = -24.849, respectively, with grid points of 40×40×40 as well as a spacing of 0.375 Å. The algorithm method used is Lamarckian Genetic, with a GA run value of 100 times.

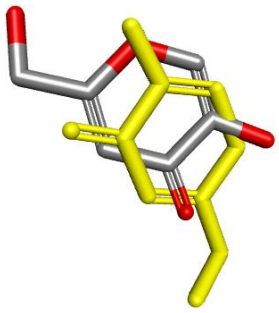


Figure 2. Overlap native ligand between crystallography (yellow) and *re*-docking (gray)

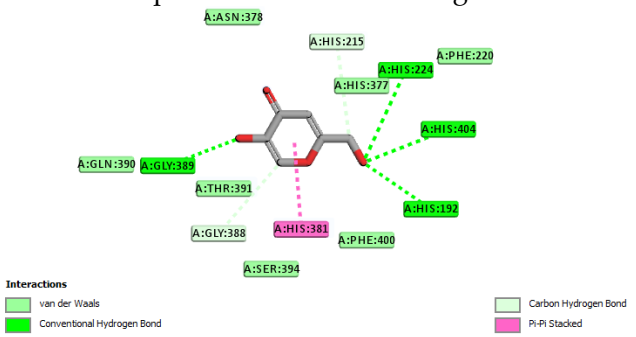
Interpretation of Ligand-Enzyme Interactions and Visualization

The results of in silico molecular docking obtained binding affinity and interactions, shown in Table 1.

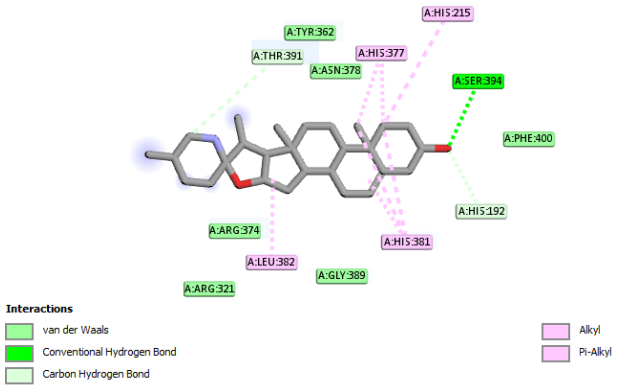
Table 1. The Results of Molecular Docking to TYRP-1

Compounds	Amino Acid Residue Interactions		ΔG (kcal/mol)	Ki (nM)
	Hydrogen Bonds	Van der Waals Bonds (Hydrophobic)		
Test ligands (flavonoid)				
Quercetin	His192, His377, Thr391, His381, Asn378, Arg374, and Arg321	Gln390, Gly388, Phe400, His215, and Gly389	-7.74	2110
Test ligands (alkaloids)				
Solasodine	His192, Ser394, and Thr391	Tyr362, Asn378, Arg374, Arg321, Gly389, Phe400	-8.58	517.15
Solanine	Thr391	Gly388, Leu379, Phe220, Ile399, and Val401	+951.97	-
Solamargine	-	-	+197.10	-
Solasonine	Tyr362, Thr391, and Ser394	Asn378, Pro395, Gly389, Gly388, Gln390, and Arg321	+975.47	-
Reference ligand				
Kojic acid	His192, Gly389, His224, His404, Gly 388, and His215	Asn378, Gln390, Thr391, Ser394, Phe400, His377, and Phe220	-5.50	92720

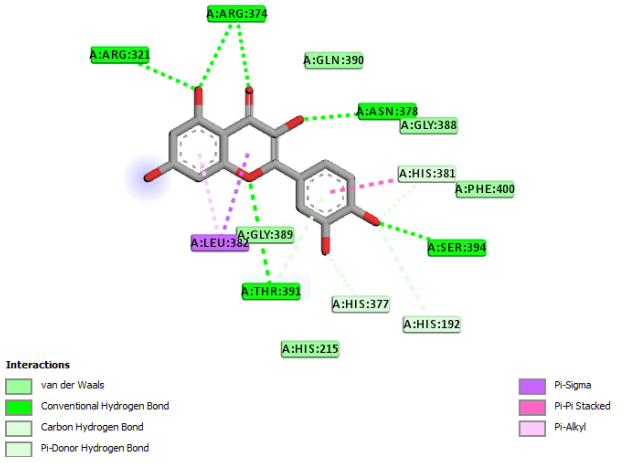
The interaction between the active compounds from leunca fruits and the amino acid residues of the TYRP-1 protein can be seen in Figure 3.



(**a**)



(**b**)



(**c**)

Figure 3. Visualization of molecular docking results of kojic acid (**a**), solasodine (**b**), and quercetin (**c**)

ADME-Tox

The prediction results of the transdermal route and toxicity are shown in Table 2.

Table 2. The Results of ADME-Tox

Compounds	ADME	Toxicity	
	Skin Permeability (cm/hours)	Mutagenic	Carcinogenic
Quercetin	-4.433	+	-
Solasodine	-3.377	-	-
Kojic acid	-4.785	+	-

Discussion

The validation method is declared valid because the RMSD value is ≤ 3.00 Å (Jain and Nicholls, 2008). It shows that the docking software is ready to test ligands. If the bond value between the ligand and enzyme is low, the bond is stronger due to the stability and strength of non-covalent interactions. Binding affinity can be evaluated from the free energy of binding (ΔG) and inhibition constant (Ki) values (Kelutur, 2023). The smaller or minus the ΔG value indicates increased activity so that less energy is needed to form stronger bonds (Umamaheswari et al., 2013).

ΔG, also called Gibbs free energy, aims to predict whether a reaction can proceed spontaneously or not. A value of $\Delta G < 0$ means the reaction can occur spontaneously, $\Delta G = 0$ shows the reaction takes place in equilibrium, and $\Delta G > 0$ means the reaction is not spontaneous (Chagas et al., 2018). Experimentally ΔG is closely related to Ki so that the equation is obtained :

$$\Delta G = -RT \ln Ki$$

where the ΔG value is to estimate the interaction ability between active compounds from leunca fruit and macromolecules, namely TYRP-1 (Kartasasmita et al., 2009). Ki explains the inhibitory ability of compounds to the target. So, the smaller the Ki value, the stronger the inhibitory force (Umamaheswari et al., 2013).

Binding interactions between the ligand and amino acid residues indicate binding to the active pocket of TYRP-1 (Kelutur, 2023). The most important types of bonds in biological systems are HB and VdW. HB is a non-covalent interaction and also represents a good electrostatic interaction. This bond plays a crucial role when the ligand-enzyme binds to each other, which functions as a proton donor and acceptor with electrostatic properties. Although the strength of HB is weaker than ionic (covalent) bonds, it is very dominant in the specificity of molecular recognition and the conformation of some macromolecules (Muchtaridi et al., 2018).

VdW is a hydrophobic interaction that is very important for drug-enzyme interactions. The strength of the hydrophobic interaction depends largely on the quality of the steric effect between the two molecules (Muchtaridi et al., 2018). His192 becomes a crucial amino acid residue when active compounds from leunca fruits and drugs as a reference ligand to TYRP-1 as an anti-aging skin target.

Skin permeability is an important consideration for product efficacy and the development of drug delivery systems by the transdermal route. A compound is said to have potential as a drug if it has a relatively low skin permeability value, namely log Kp > -2.5 cm/hours (Abdullah et al., 2021). Toxicity in the form of mutagenic and carcinogenic is carried out to determine the characteristics of compounds that can cause cell or organ damage (Kelutur et al., 2022).

Based on the results of a review conducted by Putra et al., 2022 it is explained that kojic acid is one of the drug topical products used for hyperpigmentation disorders in pregnant women, it has a low absorption system, no fetomaternal risk in animals, and no data available on the system reproductive human, but not approved by the US FDA. Alpha hydroxyl acid (AHA) is a drug that has good efficacy because of lower toxicity when given to pregnant women.

Conclusions

Based on in silico molecular docking as well as predictions of skin permeability and toxicity, it can be concluded that only the alkaloid secondary metabolite, solasodine, from leunca fruits, has the potential to inhibit the formation of abnormal melanin, namely TYRP-1. Moreover, it has the potential to be used as a topical product for cutaneous changes in pregnant women.

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