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Original Article

DISCOVERING TYROSINASE INHIBITORS FROM *MORUS SP*. PLANTS: AN *IN SILICO* STUDY

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ABSTRACT

Objective: This study aimed to examine *Morus sp.* compounds bonding mode with critical amino acid residues in the binding pocket of the enzyme TRP1 in *In silico* so that it can be used as a support in the design of skin-lightening cosmetics based on *Morus sp.*

Methods: Docking is done using autodock tools software, chem office 2019, ChemDraw professional 12, autodock 4.2, discovery studio 2016.

Results: Isorhamnetin has two hydrogen bonds to amino acids Met215 and Asn205. Other compounds found in *Morus sp.*, which have hydrogen bonds with Asn205, are dihydromorin, kaempferol, quercitrin, rutin, and morusin.

Conclusion: Isorhamnetin has the best potential among other compounds as a tyrosinase inhibitor by hydrogen binding to the amino acid Met215, and Asn205 has a free energy of-6.16 kcal/mol.

Keywords: *Morus sp.*, Melanogenesis, Tyrosinase, Tyrosinase-related protein, *In silico*, Isorhamnetin

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INTRODUCTION

The skin is the body's first line of defense against exposure to the sun's ultraviolet (UV), infrared and visible light, pollution, chemicals, mechanical injury, and internal water loss [1, 2]. UV ray exposure can initiate melanogenesis disruption, one of which is hyperpigmentation. Hyperpigmentation is characterized by the formation of brown color on the skin that is evenly distributed or only inatches due to an excessive amount of melanin. Melanogenesis is the process responsible for the production of melanin. In the regulation of melanogenesis, there are potent enzymes involved, including tyrosinase, tyrosinase-related protein 1 (Tyrp1), and tyrosinase-related protein 2 (Tyrp2). Melanogenesis is initiated by the oxidation of tyrosine by the tyrosinase enzyme to form dopaquinone which is then converted to dopa and dopachrome through an auto-oxidation process [3]. This reaction repeatedly occurs with the help of the enzyme tyrosinase-related protein (Tyrp). The end product of dopachrome oxidation is eumelanin with the help of Tyrp 1. Meanwhile, in the presence of cysteine or glutathione, it will convert dopaquinone into pheomelanin with the help of Tyrp 2 [3, 4]. Tyrp-1, also known as gp75 is a crucial enzyme in controlling the melanogenesis pathway, especially in the production of melanin [4]. The active site of the tyrosinase subdomain of tyrp-1 (PDB ID: 5I3A) is reported to contain two zinc ions, namely Zn301 and Zn302; each metal ion has two hydrogen bonds in Met215 and Gly216, and has two hydrophobic bonds in Val218 and Arg221 (fig. 1) [5].

Several tyrosinase inhibitors are synthesized from plants, including hydroquinone, arbutin, aloesin, and kojic acid, which are currently used as skin-lightening agents [4]. Hydroquinone is a phenolic compound chemically known as 1, 4-dihydroxybenzene. This compound inhibits the enzymatic oxidation of tyrosine, and reduces melanin production by inhibiting the sulfhydryl group and acting as a tyrosinase substrate. The hydroquinone has a covalent bond to histidine and interacts with copper at the tyrosinase active site. This activity does not 'whiten the skin' but gradually suppresses melanin production. Hydroquinone interacts with Bacillus megaterium tyrosinase by forming two hydrogen bonds with Asn205 and Pro219, two hydrophobic contacts with Ala221 and Val218, and one pi-pi interaction with His208. Hydroquinone can act as a substrate for *Bacillus megaterium* and a tyrosinase inhibitor [6].

However, these substances show various kinds of side effects when used for a long time [7-10]. Natural substances derived from plants, bacteria and fungi have recently become increasingly interesting to study as anti-tyrosinase because they produce bioactive compounds. Nature product that can slow down the melanogenesis process are preferred because they are safer with better bioavailability, especially for food, cosmetic and drug applications [2, 4].

The content in Mulberry (*Morus sp.*) has the potential as a tyrosinase inhibitor. The flavonoid compounds contained in *Morus sp.* are reported to have a structure-activity relationship on the glucoside, stilbene, and glucoside, 2-arylbenzofuran derivatives, where the resorcinol group is not substituted in the 2'-and 4'-OH in ring B so that it plays a role in tyrosinase inhibition [6]. However, the bonding mode between the compounds contained in the leaf extract of *Morus sp.* has never been explored. Therefore, a study will be conducted to observe molecular mechanisms that will explain the bonding mode of the compounds contained in *Morus sp.* with critical amino acid residues in the binding pocket of the tyrosinase enzyme using *in silico.*

MATERIALS AND METHODS

Hardware was a set of computers with specifications Intel(R) Celeron(R) CPU@1.80 GHz, RAM (Random Access Memory) 2.00 GB. Software consists of Operating System Windows 10 Enterprise Pro 64-bit, x64 based processor, equipped with MGL-Tools program consisting of ADT (Autodock Tools) application, ChemOffice 2010 Program, and ChemDraw Professional 12, AutoDock 4.2, Discovery Studio2016 Client®.

Test components

Eleven compounds (catechin, quercitrin, sanggenon H, dihydromorin, quercetin, kaempferol, sanggenon F, steppogenin, isorhamnetin, rutin, and morusin) from *Morus sp.* leaves as ligands and hydroquinone as a comparison with tyrosinase inhibitors.

Receptors

The 3D crystal structure data for the receptor used for molecular docking analysis were obtained from the Protein Data Bank (PDB ID: 5I3A) RMSD 2.20 a http://www.rcsb.org/pdb/. Three-dimensional (3D) structure of the tyrosinase macromolecule downloaded from https://www.rcsb.org/3d-view/5I3A/1 and has been crystallized by Deri [5].

Protein preparation

The 5I3A tyrosinase receptor preparation (fig. 1) was visualized using the Discovery Studio 2016 Client® program. In this program,

the downloaded receptor is prepared by removing water molecules and their natural ligands. The result is a pure receptor which is then saved in Protein Data Bank (.pdb) format [11].

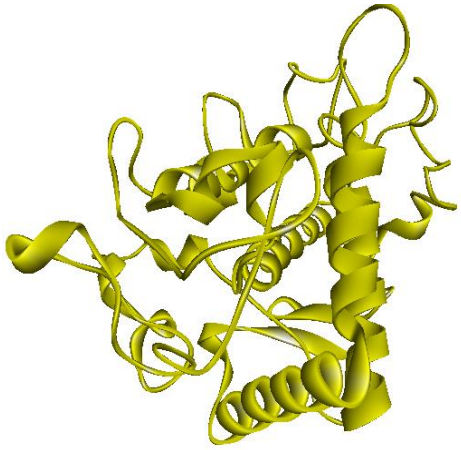


Fig. 1: Tyrosinase receptor (5I3A) [5]

Ligand preparation

The test compound ligands consisted of 11 *Morus sp.* The initial stage is to draw 2D and 3D structures of these compounds using the ChemOffice 2012 application and then save them in (. pdb) format [12].

Method validation

Parameter observation is Root Mean Square Deviation (RMSD) of co-crystal ligand downloaded from PDB (2.20Å) on the selected active site. Then compared with the RMS value of the re-docking results (1.836Å). The smaller the RMSD value of the redocking results indicates that the position is close to the crystallographic results [13].

Docking test compounds with receptors

Docking is done using autodock4 software (run-autodock) [12]. The observed results were Gibbs (ΔG), inhibition constant (Ki), amino acid residues, and the number of hydrogen bonds [14].

Molecular docking analysis and visualization

Docking calculation results are seen in the output in notepad format. Determination of the conformation of the test compound as a result of docking is done by selecting the ligand configuration that has the lowest bond energy (best pose). The position and orientation of these ligands on macromolecules, as well as the amino acids bound to the ligands, were visualized with the Discovery Studio2016 Client® program [11].

RESULTS

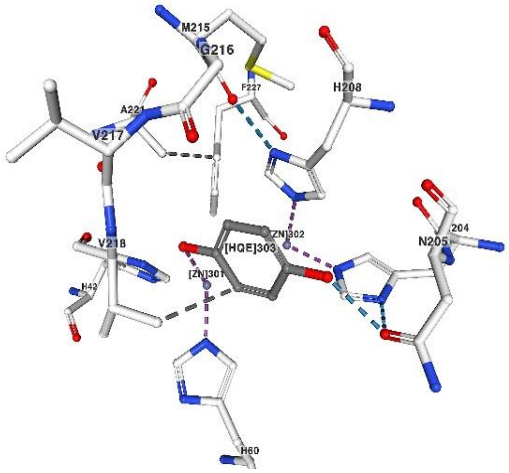


Fig. 2: The active site of *Bacillus megaterium* tyrosinase (PDB ID: 5I3A) crystallized by Deri [5]

Fig. 2 showed the two metal ions ZN301 and ZN302. Each ion shows 2 metal interactions (purple dotted line). The tyrosinase inhibitor labeled with HQE303 (red ash structure) forms two hydrophobic bonds with Val218 and A221 and has two hydrogen bonds with Met215 and Gly216 (downloaded from https://www.rcsb.org/3d-view/ngl/5i3ausing 3D NGL-WebGL).

Eleven compounds, including catechin, quercetin, sanggenon H, dihydromorin, quercetin, kaempferol, sanggenon F, steppogenin, isorhamnetin, rutin, and morusin, have good physicochemical and bioavailability profiles based on Lipinski's rule of five predictions (table 1).

Table 1: Lipinski's rule of five prediction result

No	Compound	MW (g/mol)	Log P	Donor H+	Acceptor H-
1.	Hydroquinone	142.11	-2.45	3	3
2.	Dihydromorin	304.25	0.58	6	7
3.	Isorhamnetin	396.33	0.73	4	9
4.	Kaempferol	286.24	0.74	5	5
5.	Catechin	290.27	1.5	5	6
6.	Quercitrine	448.38	-0.54	8	10
7.	Quercetine	302.24	0.35	6	6
8.	Rutin	610.52	-2.28	11	15
9.	Morucin	420.45	3.99	3	5
10.	Sanggenon F	354.35	2.25	4	6
11.	Sanggenon H	354.35	2.25	4	6
12.	Steppogenin	288.25	1.24	5	6

The structure of eleven compounds: catechin, quercitrin, sanggenon H, dihydromorin, quercetin, kaempferol, sanggenone F, steppogenin, isorhamnetin, rutin, and morusin contained in the leaves of *Morus sp.* It can be seen in table 2.

Docking control experiments were carried out to validate the docking parameters, which will later be used in screening compounds in *Morus sp.* The best compound docking position was selected based on the densest cluster, followed by the intermolecular interaction between the ligand and receptor, and then the lowest binding energy was taken. The simulation results are shown in table 3.

Table 2: Structure of 11 compounds in *Morus sp.* leaves

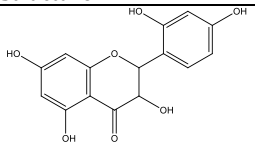
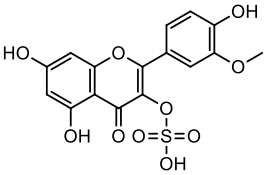
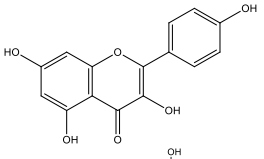
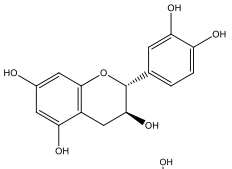
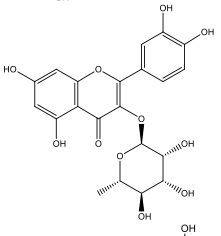
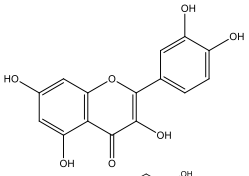
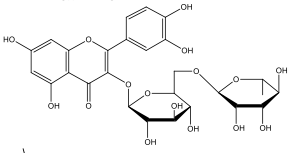
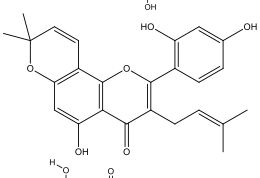
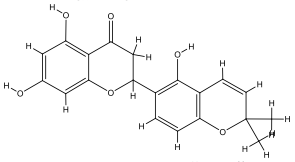
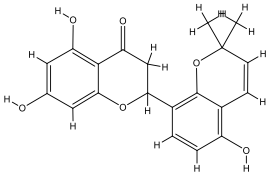
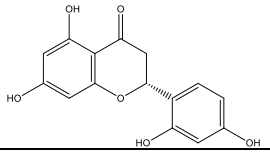
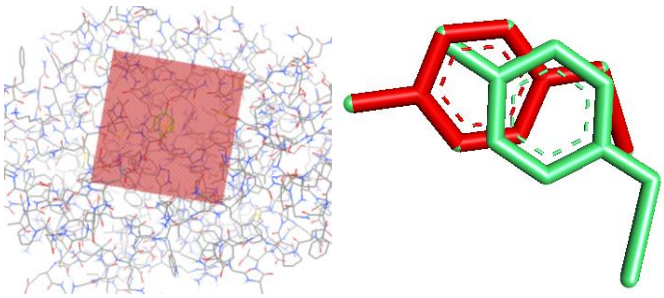
No	Compounds	Molecule structure	Structure
1.	Dihydromorin	$C_{15}H_{12}O_7$	
2.	Isorhamnetin	$C_{16}H_{12}O_{10}S$	
3.	Kaempferol	$C_{15}H_{10}O_6$	
4.	Catechin	$C_{15}H_{14}O_6$	
5.	Quercitrin	$C_{21}H_{20}O_{11}$	
6.	Quercetin	$C_{15}H_{10}O_7$	
7.	Rutin	$C_{27}H_{30}O_{16}$	
8.	Morusin	$C_{25}H_{24}O_6$	
9.	Sanggenon F	$C_{20}H_{18}O_6$	
10.	Sanggenon H	$C_{20}H_{18}O_6$	
11.	Steppogenin	$C_{15}H_{12}O_6$	

Table 3: 5I3A receptor validation with hydroquinone co-crystal ligand

Compound	Interaction with amino acids		RMSD (Å)	Ki (µM)	(ΔGbind) (kcal/mol)
	Hydrogen bonding	Van der walls (Hydrophobic)			
Hydroquinone	His204, His69, His60, His31	Phe227, Gln214, Gly216, Val217	1.836	94.77	-5.49

Determining the grid layout involves setting the parameters and *sp.* ecifying the grid box. The results of the 5I3A receptor grid box have active side coordinates: center x = 23.216 y = 101,846 z = 48,198 with a distance of 0.375 Å. 5I3A receptor visualization results using Autodock 4.2 can be seen in fig. 3.

The binding of 11 compounds to the receptor was carried out to obtain the lowest free energy binding. The results showed that the Isorhamnetin compound produced the lowest free energy with a value of-6.15 kcal/mol and an inhibition constant of 31.10 µM compared to acid-4.26 kcal/mol of inhibition constant of 756.63 µM. The simulation results of the docking of hydroquinone molecules and 11 compounds contained in the leaves of *Morus sp.* against tyrosinase (5I3A) can be seen in table 4.



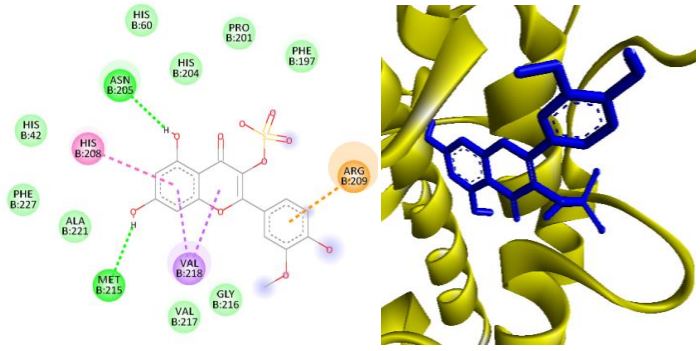
A **B**

Fig. 3: (A) Preparation of the 4HJO receptor with *Autodock 4.2* (B) Overlapping the position of the hydroquinone ligand with crystallographic results (Red = crystallographic results, Green = redocking pose)

Table 4: Molecular docking parameters for *Morus sp.*

No.	Compound	ΔG kcal/mol	KI (uM)	Interaction with amino acids	
				Hydrogen bonding	Van der wals hydrophobic bonding
1	Hydroquinone	-4.26	756.63	Met215	His42, Phe227, Val217, Gly216
2	Dyhidromorin	-5.87	49.62	Asn205	Hiw69, His60, His231, His204, Phe65, Pro201, Gly216, Val217, Met215, Phe227
3	Isorhamnetin	-6.15	31.10	Met215, Asn205	His60, His204, Pro201, Phe197, Gly216, Val217, Ala221, Phe227, His42
4	Kaempferol	-5.58	81.43	Val218, Asn205	His60, His204, His42, Pro201, Pro222, Val217, Ala221, Phe227, Arg209, Met184, Met215
5	Catechin	-6.04	37.55	Gly216, Gly215	Gln214, Val217, Met61, Phe197, Pro201, Asn205, His204, Phe65, His69, His60, His231, Phe227
6	Quercitrin	-5.32	125.09	Met184, Asn205, Val218, Gly216	Met61, PHE197, Glu195, HIS60, His204
7	Quercetine	-5.65	71.85	Asn205, His69, His231, His60	Pro201, Arg208, Phe65, His204, Phe227, His42, Pro222, Gly216, Val217
8	Rutin	-3.91	1.37	Asn205, Arg209	His231, His204, His42, Phe227, Asn52, Met184, Phe197, Gly216, Pro219, Met215, Ala221
9.	Morusin	-5.15	168.08	Val218, Asn57, Asn205	Gly46, Glu195, His204, Val217, Gly216
10.	Sanggenon F	-5.66	71.51	His204	Pro222, Phe227, Val217, His231, His69, Phe65, Glu195, Met61, Asn205, Phe197
11.	Sanggenon H	-5.86	50.81	Glu195	Phe197, Met61, His60, Asn205, His208, Met215, Pro219, Gly216
12.	Steppogenin	-5.80	56.33	Gly216, His60	Val217, Met215, Phe227, His69, Phe65, His204, Asn205, Pro201

Isorhamnetin binds to amino acid residues in the form of Met215 and Asn205. Visualization of the results of docking isorhamnetin compounds with receptors can be seen in fig. 4.



A. **B.**

Fig. 4: Isorhamnetin binds to the amino acid residues Met215 and Asn205 (A) 2D visualization (B) 3D visualization

DISCUSSION

The 3D crystal structure of the macromolecule used in this study is the tyrosinase receptor (PDB ID: 5I3A), with a resolution value of 2.20 Å. The analysis used to evaluate the validation results is the RMSD value, the binding site, and the parameters used. The analysis is declared valid if the results obtained are RMSD ≤ 2 Å. Based on the validation results, the RMSD value was obtained at 1,836 Å. RMSD values meet the validation requirements so that the parameters can be used for molecular docking simulations.

The molecular docking results were analyzed on the value of free energy, inhibition constant, and how many interactions between the amino acids of the ligand and the receptor protein. Isorhamnetin is the best value with the lowest free energy with a weight of-6.15 kcal/mol and inhibition constant of 31.10 uM compared to Hydroquinone-4.26 kcal/mol with an inhibition persistent of 756.63 uM. The smaller the free energy value indicates a strong bond with the receptor. In addition, isorhamnetin binds more amino acids than its comparison (hydroquinone).

Potential isorhamentin (fig. 4) has hydrogen bonds, namely Met215 and Asn205, which is indicated by bright green color and has van der walls bonds, namely His60, His204, Pro201, Phe197, Gly216, Val217, Ala221, Phe227, His42. Isorhamentin and hydroquinone bind to the same amino acid, namely Met215. The active site of *B. megaterium* tyrosinase (fig. 2) has two hydrogen bonds with Met215 and Gly216 [5]. In this interaction, the amino acid Met215 is an amino acid residue that provides activity as a tyrosinase inhibitor for anti-hyperpigmentation, allowing isorhamnetin with natural ligands to have biological activity with receptors on the active site [15]. Other compounds found in *Morus sp*., which have hydrogen bonds with Asn205, are dihydromorin, kaempferol, quercitrin, rutin, and morusin. Asn205 has significant potential in tyrosinase activity through activating water molecules 35,56. The interaction of Asn205 with isorhamnentin can prevent tyrosinase activation and thus inhibit tyrosinase activity [5]. Prediction of the melanogenic activity of isorhamnetin has been successfully carried out from *Vernonia anthelmintic* (L.) through the admetSAR and SDTNBI methods from tissue pharmacology analysis, significantly increasing tyrosinase activity, MITF protein expression, and melanin-biosynthetic gene mRNA expression (Mc1r, Mitf, Tyr)., TYRP1 and DCT) [14, 15]. The discovery of new compounds in *Morus sp.*, such as isorhamnetin, adds new knowledge to developing natural materials in melanogenesis.

Compounds isolated from the twigs of *Morus sp*. like steppogenin, 2,4,2′,4′-tetrahydroxychalcone, morachalcone A, oxyresveratrol, and moracin M are significant tyrosinase inhibitory activity fields [5]. These substances are the main components responsible for the strong tyrosinase inhibitory activity. *Morus sp.* can be a promising source in nutraceuticals and cosmeceuticals to inhibit tyrosinase activity in food products or be used in cosmetics as skin whitening agents.

CONCLUSION

Based on the validation results, the RMSD value was 1.836 Å; analysis of free energy parameters and interactions between amino acids isorhamnetin is a new compound that has the best potential among other compounds as a tyrosinase inhibitor by hydrogen binding to the amino acid Met215 and Asn205 has a free energy of-6.16 kcal/mol.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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