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Exploration of Indonesian Plants as Skin Lightening Against Tyrosinase: A Virtual Screening

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Abstract

Aesthetic is nowadays becoming as one of the important things for people to increase self-confidence. Flawless skin could be one of the desired looks especially for females which is a valuable cosmetics market. Skin browning is often associated with dermatological problems which may lead to hyperpigmentation due to the increase of the melanin biosynthesis. Skin browning could be caused by the overproduction of melanin which is accelerated by the presence of human tyrosinase. Therefore, the inhibition of this kind of enzyme is associated with skin lightening. In this present study, we perform in silico virtual screening against human tyrosinase by utilizing a natural product database from Indonesia Herbal Database (http://herbaldb.farmasi.ui.ac.id/v3/) to select 20 hit compounds. The simulation was carried using AutoDock Vina via molecular docking. The result shows that from 200 compounds of the database, those hits have a free energy of binding range from -12.1 to -9.2 kcal/mol predicting the affinity of the corresponding compounds towards human tyrosinase associating with its potential as a skin whitening agent. The hits correspond to their presence in Indonesian local plant that could be potential as resources for skin whitening.

Keywords: Indonesian plant, molecular docking, skin lightening, tyrosinase, virtual screening

Eksplorasi Tanaman Indonesia Sebagai Pencerah Kulit Melawan Tirosinase: Penapisan Maya

Abstrak

Estetika saat ini menjadi salah satu hal penting bagi orang untuk meningkatkan kepercayaan diri. Kulit yang putih dan sehat bisa menjadi salah satu penampilan yang diinginkan terutama untuk wanita yang merupakan pasar kosmetik yang berharga. Penggelapan kulit dapat disebabkan oleh peningkatan melanin yang dipercepat oleh aktivitas tirosinase manusia. Oleh karena itu, penghambatan enzim ini dikaitkan dengan pencerahan kulit. Dalam penelitian ini, kami melakukan penapisan maya terhadap tirosinase manusia dengan memanfaatkan basis data produk alami dari Database Herbal Indonesia untuk memilih 20 senyawa hit. Simulasi dilakukan dengan menggunakan AutoDock Vina melalui penambatan molekuler. Hasil menunjukkan bahwa dari 200 senyawa dari database, diperoleh 20 senyawa hit memiliki energi bebas ikatan berkisar dari -12,1 hingga -9,2 kkal / mol menunjukkan afinitas yang baik terhadap tirosinase manusia yang berkaitan dengan potensinya sebagai agen pemutih kulit. Hit tersebut kemudian dihubungkan dengan keberadaan mereka di tanaman lokal Indonesia yang dapat berpotensi sebagai sumber daya untuk pemutihan kulit.

Kata Kunci: Tanaman Indonesia, penambatan molekuler, tirosinase, penapisan maya.

1. Introduction

Healthy and beautiful skin is pursued especially in females which has been a potential market for cosmetic industries.[1] Nowadays, people start to beautify themselves no matter how expensive they have to expend their money for cosmetics.[2] Skin whitening agent has been attracting women for a long time to manipulate their dark skin to be more flawless.[3] Unfortunately, further study shows that some whitening agent is no longer acceptable to be applied due to their toxicity such as skin cancer.[4] As reported, mercury and hydroquinone have been banned in the market as they showed carcinogenesis and irritating properties, respectively.[5]

Skin browning is often associated with the dermatological problem which may lead to hyperpigmentation due to the increase of the melanin biosynthesis.[6] At the molecular stage, this condition is catalyzed by an enzyme called as tyrosinase which facilitates the hydroxylation of L-tyrosine (tyrosine monophenol) to 3,4-dihydroxy-phenylalanine (L-DOPA) as well as the oxidation of L-DOPA to give dopaquinone.[7] There have been two current tyrosinase inhibitors available in the market i.e., kojic acid and hydroquinone[8], but as previously mentioned, hydroquinone has been discouraged to be used as depigmentation due to its skin irritating properties.[9] Other compounds such as benzylidene aniline[10], biphenyl analogue[11], dialkylphosphoryl hydrazone[12], metimazole[13], benzaldehyde thiosemicarbazone[14], benzylidene thiobarbiturate[15], N-benzylbenzamide[16], hydroxynaphthyl benzenetriol[17], and many more[18-20] have been reported as synthetic tyrosinase inhibitors.

Instead of synthetic compounds which have been used in depigmentation, natural product is also an abundant resource to serve the same activity by inhibiting tyrosinase[21]. As reported, genus Piper, *Strypnodendron barbatimao*, *Portulaca pilosa*, *Cariniana braziliensis*, etc.[22-24] demonstrate good activities to inhibit tyrosinase. The interesting compound isolated from L. molleoides .i.e., (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol is 37 times more active than kojic acid.[25]

Indonesia is included in the top mega biodiversity of the world[26] that must be rich in the natural product compounds serving as tyrosinase inhibitor resources. In this present study, we perform virtual screening to have a short list of 20 compounds from 200 natural product compounds in the database which are in silico active against human tyrosinase via molecular docking simulation. These compounds correspond to the local Indonesian plant which could be suggested as the resource of tyrosinase inhibitors.

2. Methods

2.1. Equipment

HP laptop with Intel i3-500 5U Quad Core @2.0GHz with Windows 10, 4GB RAM and 500GB HDD.

2.2. Materials

The soft files used in this work are PDB 5M8S from protein data bank (www.rcsb.org), Indonesian Herbal Database (http://herbaldb.farmasi.ui.ac.id/v3/), and compounds collected from references.[27-29]

The software used for constructing and energy minimization of 2D chemical structure is Marvin Sketch (https://chemaxon.com), whereas Discovery Studio 4.1 (www.accelrys.com) is used to convert them into 3D structure. The virtual screening uses the molecular docking protocol in AutoDock Vina (www.scripps.edu) and the output is visualized using Discovery Studio 4.1 (www.accelrys.com).

2.3. Procedures

2.3.1. Control Docking

The crystal structure was downloaded from protein data bank with PDB ID 5M8S (www.rcsb.org) consisting of human tyrosinase with phenylthiourea used as the control ligand located at the pocket site. The tyrosinase is presented in tetramer, therefore, only a monomer used for molecular modeling. The co-crystal ligand (phenylthiourea = URS) is separated from human tyrosinase using Discovery Studio 4.1 and total hydrogens were added, and then saved as pdb file. Human tyrosinase was prepared using the

same program used to give total hydrogens and saved as pdb file. The grid box was automatically defined by PyRx program (exhaustiveness = 32; size 20.10, 20, 20 and center x = 121.762, y= 139.470, z= 216.382) and the docking was run using AutoDock Vina[30] embedded in PyRx program. The docking parameter is defined as valid if giving the RMSD value less than 2Å.[31]

2.3.2. External Docking

There are several compounds published as inhibitors for tyrosinase[27-29] that could be used for external validation before the docking parameters used for virtual screening. The compounds having the activity within 0.15 – 38.0 µM are sketched and energetically optimized (MM+ force field) using Marvin Sketch program. The compounds were then docked to the human tyrosinase using the same parameter with control docking. The observed free energy of binding was then collected and ranked from the lowest to highest binding affinity and calculated its coefficient correlation against experimental IC_{50}. The extrapolation of binding affinity vs log IC_{50} was conducted using Microsoft Excel 2016.

2.3.3. Virtual Screening

The natural product compounds (200) were downloaded from Indonesia Herbal Database (http://herbaldb.farmasi.ui.ac.id/v3/), then prepared to pdb file using Discovery Studio 4.1. The virtual screening was then performed using the same protocol with control docking as defined. The output was then collected in csv file and the compounds were ranked according to the free energy of binding. There are 20 compounds with the lowest energy binding shortlisted as the in silico hits compounds.

3. Results

The control docking pose is found at the same binding site with the initial pose with RMSD lower bound and upper bound are 1.58 Å and 2.12 Å as shown in Figure 1 The linear regression is generated to see whether the docking parameter able to predict the nine ligand binding affinities which correlates with its log IC_{50}. Interestingly, the correlation coefficient (R^2) is equal to 0.7089 as shown in Figure 2. The virtual screening of 200 ligands collected from Indonesia Herbal Database is then conducted to shortlist 20 hits based on the rank of binding affinities (kcal/mol). The binding affinities as shown in Table 1 range from -12.1 to -9.2 kcal/mol

Eighteen out of 20 ligands are docked at the same binding site with URS as well as nine external ligands. Two ligands which bound to

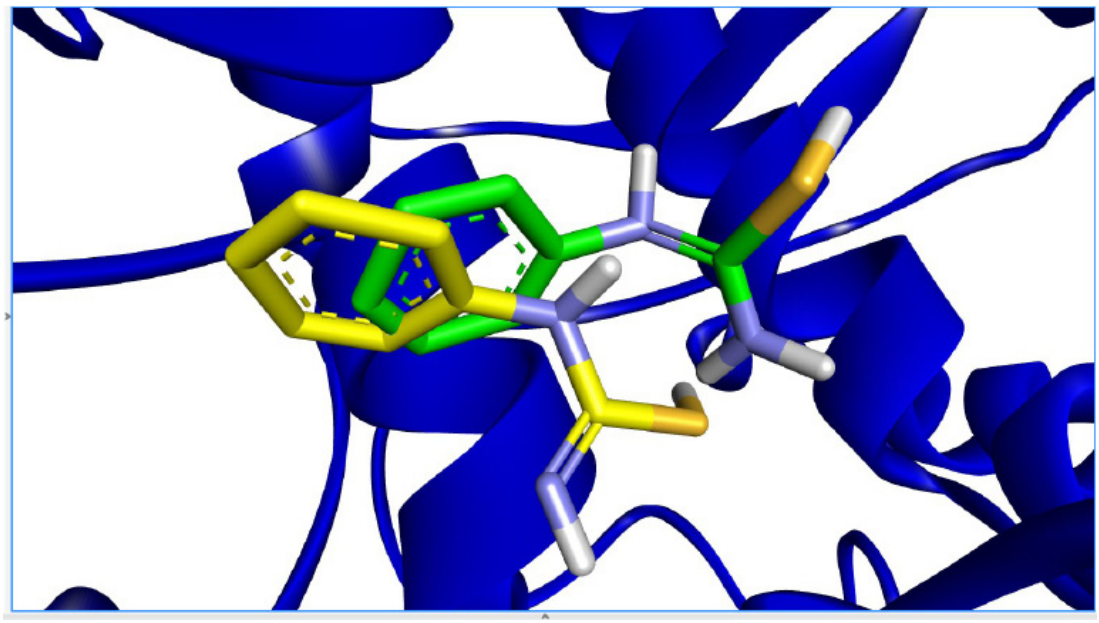

Fig 1. The superposition of initial pose (yellow carbon) and control docking pose (green carbon) of URS into human tyrosinase binding site.

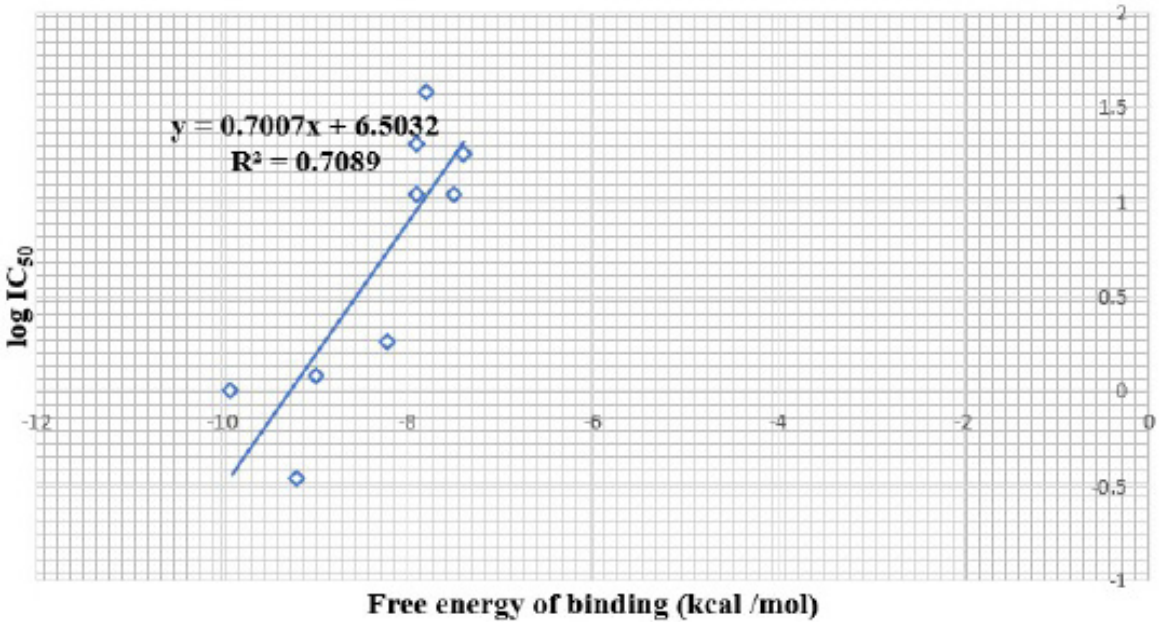

Fig 2. The graph extrapolating free energy of binding/ binding affinities (kcal/mol) vs log IC_{50}

extra binding sites are digoxin and digitoxin. These could be a potential allosteric site which able to change active site conformation through signal transduction from ligand-allosteric site binding. However, we do not study this assumption but suggest to carry out this for further study. Sequioaflavone binds the active site of human tyrosinase with binding affinity = -10.4 kcal/mol by interacting with TYR69 and ARG114 as shown in Figure 4.

4-prenylresveratrol interacts with PRO242 at human tyrosinase's main binding

Table 1. The free energy binding of 20 hit compounds alongside with their binding energy values as well as their plants where the ligand is deposited in.

Ligands	Binding Energy (kcal/mol)	Plants (in Latine)
Digitoxin	-12.1	*Cordylin fructicose*
Gitoxin	-12.1	*Cordylin fructicose*
Digoxin	-11	*Cordylin fructicose*
Beta-citraurin	-10.7	*Capsicum annuum*
Sequioaflavone	-10.4	*Elateriospermum tapos*
Poncirin	-10.3	*Citrus aurantifolia*
Glyceollin I	-10.1	*Glycine Soja*
Ophiopogonin C	-9.9	*Parkia javanica*
Chlorogenic acid	-9.7	*Mimosa pudica*
Apigetrin	-9.7	*Sambucus nigra*
Asiaticoside F	-9.5	*Centella asiatica*
4-prenylresveratrol	-9.5	*Arachis hypogaea*
Sappanone B	-9.4	*Caesania sappan*
Hydroxyanthraquinone	-9.4	*Rhamnus purshiana*
Fargesin	-9.3	*Magnolia coco*
Betanine	-9.3	*Beta vulgaris*
2'-O-methylvestitol	-9.3	*Gliricidia maculata*
Liquiritigenin	-9.3	*Jasminum sambac*
Euxanthone	-9.2	*Caesania sappan*
Apoviolaxanthinal	-9.2	*Citrus sinensis*

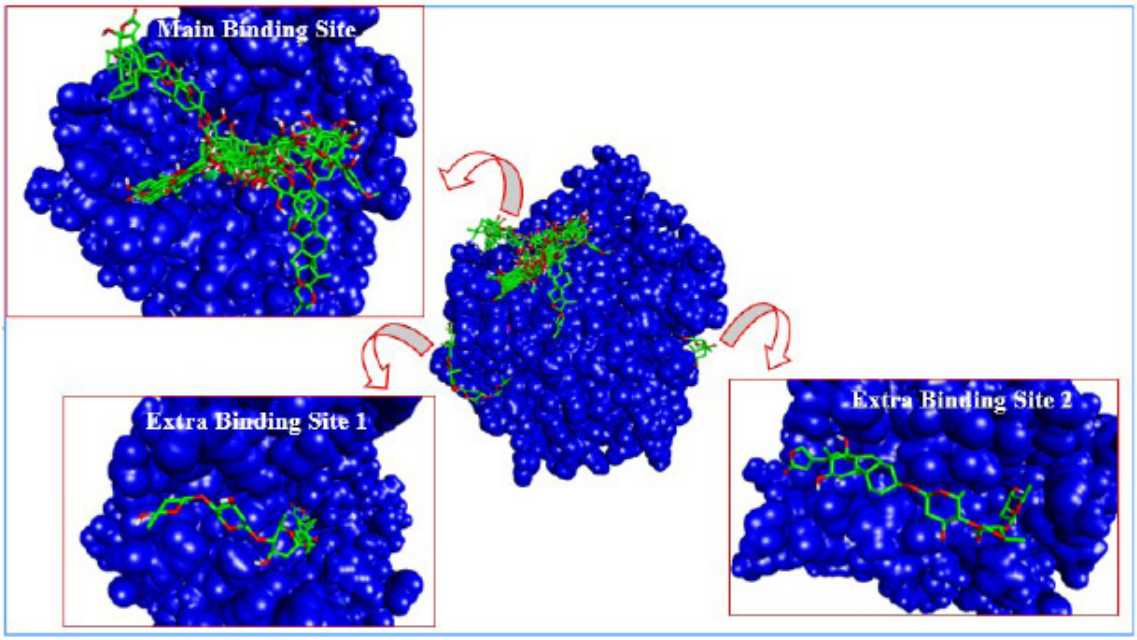

Fig 3. The superposition of 20 hits docking pose into human tyrosinase binding site. Digoxin is bound to the extra binding site 1, whereas digitoxin is bound to the extra binding site 2.

site with binding affinity = -9.5 kcal/mol as shown in Figure 5.

4. Discussions

The result of control docking showed the RMSD lower bound and upper bound are 1.58 Å and 2.12 Å, respectively, indicating the docking parameterization is acceptable for designing new ligands. The deviation of docking and the initial pose is due to the tautomeric state being caused by the protonation of nitrogen atom. This is still our preliminary prediction but it still requires further molecular experiments to confirm these phenomena.

External docking result reflects considerably good docking parameters in predicting the activity of these ligands and the docking parameters can be used to predict the binding affinities of 200 ligands from Indonesian Herbal Database via virtual screening. 20 hit compounds have been discovered from virtual screening and having binding affinities range from -12.1 to -9.2 kcal/mol associating with the capabilities of the hits to strongly interact with human tyrosinase. To

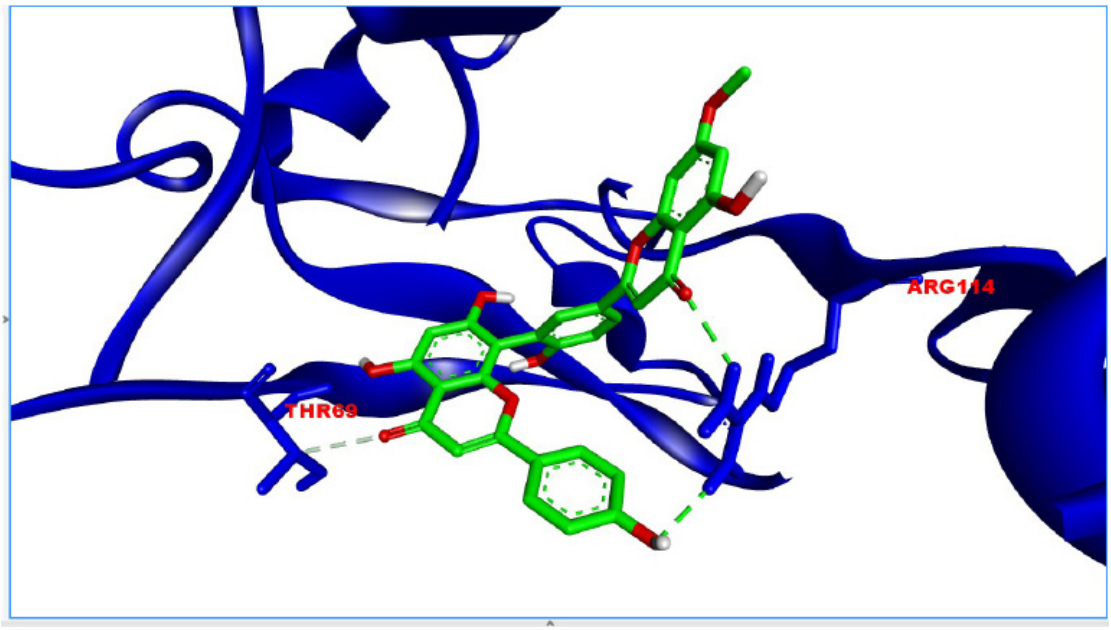

Fig 4. The docking pose of sequioaflavone into human tyrosinase binding site.

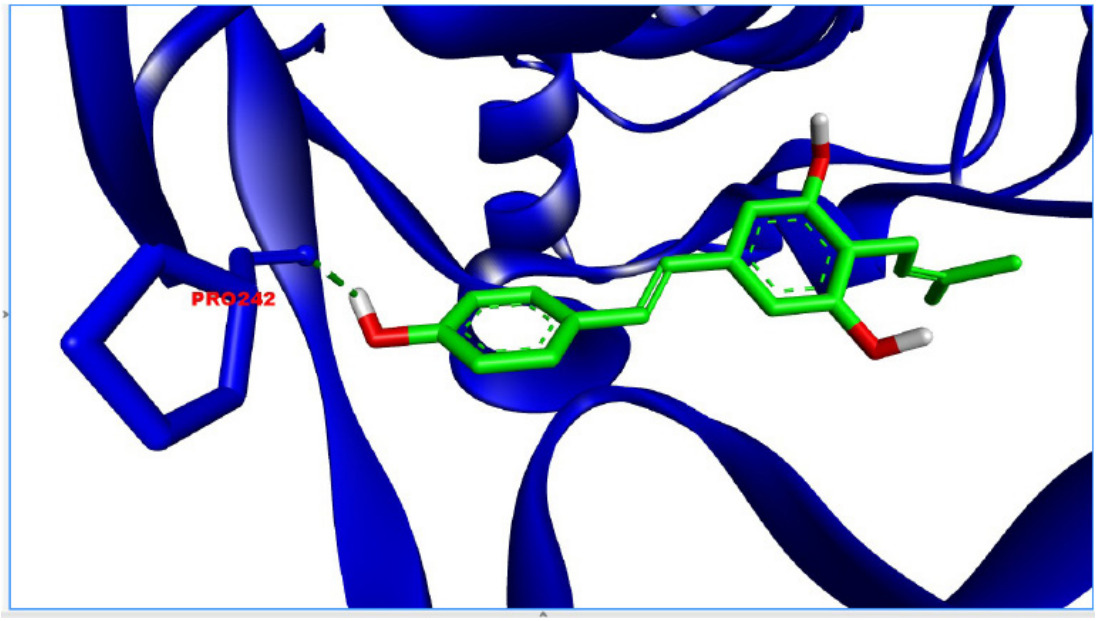

Fig 5. The docking pose of 4-prenylresveratrol into human tyrosinase binding site.

be a drug candidate, ligand should meet the requirement by the Lipinski rule of five. This rule defines a drug should have a molecular weight (MW) not greater than 500, partition coefficient (logP) not greater than 5, number of hydrogen bond donor (HBD) not greater than 5 and the number of hydrogen bond acceptor (HBA) up to 10. According to this rule, we can narrow down to select only two ligands that roughly meet the requirement of Lipinski rule of five. They are sequioaflavone (MW = 552.91; logP = 5.4; HBD = 5 and HBA = 10.) and 4-prenylresveratrol (MW = 296.36; logP = 5.1; HBD = 3 and HBA = 4) which are contained in Elateriospermum tapos and Arachis hypogaea, A relevant prove that strengthen 4-prenylresveratrol activity is the activity of resveratrol, a chemical substance contained in grapes and has been used as anti-aging due to its capability to inhibit sirtuin activity. It is believed that sirtuins play a key role during cell response to a variety of stresses, such as oxidative or genotoxic stress and are crucial for cell metabolism.[32] It is, therefore, can be said that resveratrol has a double activity, could be on one hand acts on sirtuin and on the other hand, inhibit the human tyrosinase which gives a synergistic activity.

5. Conclusion

There have been conducted virtual screening by using molecular docking to select 20 hits from 200 natural product compounds in the database. The 20 top hits were then further selected its properties should meet the requirement as defined by the Lipinski rule of five. As evaluated, two ligands i.e sequioaflavone and 4-prenylresveratrol have been further selected as a potential inhibitor for human tyrosinase associating with skin lightening activity. The plants belong to sequioaflavone and 4-prenylresveratrol are respectively *Elateriospermum tapos* and *Arachis hypogaea*.

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