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Research Article

IN-SILIC0 STUDY OF CURCUMIN, DEMETHOXYCURCUMIN AND XANTHORRIZOL AS SKIN WHITENING AGENTS

Resmi Mustarichie*, Jutti Levita and Dini Febriani

Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor – Indonesia

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ABSTRACT

Curcumin, demetoxycurcumin and xanthorrhizol are an active compounds contained in temulawak (Curcuma xanthorrhiza) which have activity in inhibiting the tyrosinase enzyme and α-melanocyte stimulating hormone (α-MSH) in vitro. Docking simulation was done to determine and visualize the interaction of the three compounds with the tyrosinase enzyme and α-MSH. The results of docking simulations showed that the three compounds can interact spontaneously with the tyrosinase enzyme and α-MSH. On the tyrosinase enzyme, xanthorrhizol interact most easily through the formation of hydrogen bonds with Asn205. On the α-MSH, demethoxycurcumin interact most easily through the formation of two hydrogen bonds with His3 and Arg5. With the inhibitory effect on the enzyme tyrosinase and α-melanocyte stimulating hormone (α-MSH) means preventing the formation of skin coloring pigment melanin, indicating that the three compounds studied can be applied as skin whitening agents.

Keywords *: Curcuma xanthorrhiza,* Tirosinase enzyme, α-MSH, Docking simulation, skin whitening agent

INTRODUCTION

Bright white skin and coveted by many women because of the assumption that synonymous with beautiful white and bright. As a result, the use of cosmetics aimed at increasing skin whitening, bleaching agents used although not necessarily safe for the skin. Each human skin color varies depending on the levels of melanin in the skin. Basically, melanin is a brown pigment that protects the skin from the handy exposure to sunlight [1]. Excessive sun exposure can cause a buildup of melanin that would cause dark spots and discoloration of skin become darker.

To get a whiter and brighter skin, use a variety of cosmetic whitening agent that is able to inhibit the enzyme tyrosinase in order to suppress excessive production of melanin in the epidermis. The enzyme tyrosinase is an enzyme involved in the biosynthesis of melanin pigment (melanogenesis) wherein the substrate is a precursor amino acid tyrosine [2,3,4]. Melanin production is also influenced by the hormone α-melanocyte stimulating hormone (α-MSH), which is a hormone that triggers the production of melanin. In vitro studies conducted by Batubara *et al*. [5] showed that the extract of turmeric (*Curcuma xanthorrhiza*) has activity in inhibiting the enzyme tyrosinase with IC_{50} of 267.3 ± 6.1 mg/mL. Lee *et al.* [6,7] showed that curcumin, a compound found in ginger rhizome can inhibit the activity of the enzyme tyrosinase by pressing α-melanocyte stimulating hormone (α-MSH) in B16F10 melanoma cells.

Development of curcumin, demethoxycurcumin, and xanthorrizol as tyrosinase enzyme inhibitors and α-melanocyte stimulating hormone (α-MSH) can be seen by its ability to bind to the enzyme tyrosinase and α-MSH can be predicted by docking simulations using an approach based on ligand based. Principle of docking is algorithms and scoring functions [7], therefore, the information about the relative position of ligand docking to the receptor, the chemical bonds involved in ligand-receptor interactions can be used to predict the activity of curcumin, demethoxycurcumin, and xanthorrizol as a whitening agent compounds through their bonding to the enzyme tyrosinase and α-MSH.

MATERIALS AND METHODS

Tools: Hardware used for calculations, molecular modeling, and docking molecule includes a personal computer (personal computer) equipped

Corresponding Author Address: Resmi Mustarichie, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor – Indonesia,
E-mail: resmi.mustarichie@unpad.ac.id

with Intel Core Duo processor T6500 2.1 GHz, 800 MHz FSB, operating system Windows VistaTM Home Basic, a hard disk capacity of 320 GB, and RAM memory 2 GB.

The software used in this study was SwissPDBViewer v.4.01 which was downloaded from http://www.expasy.org. ChemOffice 2004 software (by Cambridge Soft Corporation 2003 downloaded from www.cambridgesoft.com). Portable HyperChem Release 8.0.7 (by Hypercube Incorporation downloaded from http://www.hyper.com 2007). Ligand Explorer Viewer v.3.8 application was data on line from (http://www.pdb.org/pdb/explore). Program v.4.0.1 ArgusLab downloaded from http://www.arguslab.com and AutoDockTools v.3.05 software package program in MGLTools v1.5.4 (Molecular Graphics Laboratory, The Scripps Research Institute in 2009 downloaded from http://mgltools . scripps.edu).

Materials: Three-dimensional structure of the enzyme tyrosinase which crystallized with kojik acid with 2.3 Å resolution (PDB code: 3NQ1) by Sendovski *et al*. [8] and α-MSH by Giblin *et al*. [9] (PDB code: 1B0Q) . Both the 3D structure was obtained from the data base on line Protein Data Bank (www.pdb.org). Two-and three-dimensional structure of curcumin, demethoxycurcumin, xanthorrizol and acid kojik drawn using the program package ChemOffice ChemDraw Ultra 2004 that Chem3D Ultra 8.0.3 and 8.0.3.

Method: The study began with the preparation of ERα (PDB code: 1A52) obtained from the Protein Data Bank (www.pdb.org). This was done by downloaded data from the Protein Data Bank Erα, reduction ERα chains into monomeric form using SwissPDBViewer v4.01, analysis binding pocket by Ligand Explorer Viewer bond in the Protein Data Bank (www.pdb.org) and Q-SiteFinder, analysis of ligand-receptor interactions with the Ligand Explorer Viewer in the Protein Data Bank (www.pdb.org). Next, preparation of ligand curcumin, demethoxycurcumin, xanthorrizol and Kojik acid with 3D ChemBio package v.12.0.2 Free Trial and HyperChem v8.0. Professional Edition. The preparation was done by making the structure of 2D and 3D using ChemBio program package 3Ddengan v.12.0.2 Free Trial, optimization of geometry with HyperChem software v8.0. Professional Edition and analysing of molecular properties with QSAR Properties on the program HyperChem v8.0. Professional Edition.

To make sure that the AutoDockVina program used was valid, the model structure was overlayed with a ligand that had been crystallized using program HyperChem v8.0. Professional Edition, redocking ligands had been crystallized into ERα binding pockets using the program AutoDock Vina and analysing of validation data.

Further, docking of curcumin, demethoxy curcumin, xanthorrizol and Kojik acid, enzyme tyrosinase, and α-MSH using AutoDock Vina program and last, interpretation of obtained data.

RESULTS AND DISCUSSION

Results Preparation of Ligand Curcumin, Demethoxycurcumin, Xanthorrizol and Acid kojik with software ChemOffice 2004 and Portable HyperChem Release 8.0.7: The first step in the preparation of the ligand was making a two-dimensional structure which was then converted into three-dimensional structures with ChemDraw Ultra program Chem3D Ultra v8.0.3 and v8.0.3 in Chemoffice program package 2004. Three-dimensional structure then optimized geometry to obtain the most stable conformation. Conformational changes before and after optimized shown in Fig. 1.

The next step was the analysis of the characteristic of the ligand using Portable HyperChem Release 8.0.7 software. Ligand analysis results shown in Table 1.

Principle Lipinski's Rule of Five [10] was used as a reference to determine the effectiveness of the theoretical and bioavaibilitas of an oral drug that was used. Principle Lipinski's Rule of Five is the first compound mass not more than 500 dalton. Mass owned by curcumin, demethoxycurcumin, xanthorrizol and kojik acid had met the criteria as oral medications which could produce an effect. Requirements Lipinski's Rule of Five others were reviewing the physical properties of a compound was the value of the partition coefficient (cLogP). Partition coefficient is the ratio of fat solubility concentration values with the concentration in the water. cLogP was the calculation of the partition coefficients obtained by using the software HyperChem Release 8.0.7 Portable. cLogP showed lipophilicity of a compound. Partition coefficient of both curcumin, demethoxycurcumin, xanthorrizol and kojik acid oral drug met the criteria according to Lipinski's Rule of Five, which was under five and less than minus two. Negative sign on the coefficient of curcumin and kojik acid showed that the two compounds were hydrophilic. This means that these compounds would easily be dissolved in water or body fluids so that the process of distribution to achieve the longer the cell

membranes. Unlike the cLogP xanthorrizol which had close to 5. This suggested that xanthorrizol had the ability to penetrate the cell membrane was higher than the other compounds because it was more lipophilic which xanthorrizol more soluble in fat than in water or body fluids.

All four compounds have the volume varies. If viewed from the volume sac active enzyme tyrosinase (320 Å ³) and α-MSH (43 Å ³), the four compounds would be difficult to get into the bag off in full because the volume was greater than the volume of the bag. Thus, all four of these compounds into the coffers of active only in part.
Moreover, the principle of Lipinski's Rule of Five can also be used as a reference to assess the feasibility of the chemical properties of drug compounds with the oral. Chemical properties are taken into account by this principle is the number of donors and acceptors of hydrogen bonds. Based on the principle of Lipinski's Rule of Five, a compound can be medication by mouth if it has a maximum of five donors and a maximum of 10 acceptors of hydrogen bonds. Curcumin has two donors and six acceptor hydrogen bonds.

Demethoxycurcumin has two donors and five acceptor hydrogen bonds. Xanthorrizol have one donor who also serves as an acceptor of hydrogen bonds, while the acid kojik have two donors and four acceptors of hydrogen. The fourth of these compounds meet the criteria according to the principles Lipinski's Rule of Five. Number of donor and acceptor bonding is one of the capital of the interaction between the five compounds with amino acids making up the bag off the enzyme tyrosinase and α-MSH. These atoms provide the ability for four to interact with sac active through hydrogen bonding. Hydrogen bond is a bond between the H atom that has a partial positive charge with other atoms that are electronegative and has a lone pair with octets complete, such as O, N, and F [11]. Overall, the four compounds is compliant with the rules of Lipinski's Rule of Five, so it can be said that all four of these compounds have bioavaibilitas good to be preparation orally.
In addition, curcumin and demethoxycurcumin have 2 ring aromatics, xanthorrizol only have one ring aromatic while the kojik acid have no ring aromatics. Ring aromatic donating properties of non-polar to a compound. In addition, the ring aromatics can donate interaction van der Waals with the amino acids making up the bag off the enzyme tyrosinase enzyme and α-MSH.

Results of Tyrosinase Enzyme Preparation (PDB code: 3NQ1) and alpha-melanocyte stimulating hormone (α-MSH) (PDB code: 1B0Q) were obtained from the Protein Data Bank: Data enzymes that have been downloaded from the Protein Data Bank was reduced to a single chain. Chain used was a chain of the enzyme tyrosinase. Ligand Explorer Viewer Tool was used to analyze the amino acids making up the active enzyme tyrosinase bag and α-MSH. Analysis done by record amino acids located around kojik acid and Rhenium atom during crystallization. On the enzyme tyrosinase, there weree 6 amino acids were around kojik acid, namely Gly196, Phe197, Gly200, Pro201, Asn205, Arg209, whereas in α-MSH contained four amino acids, namely Cys1, Arg5, Trp6, and Cys7.

To see more precisely the active enclave on a receptor, used Q Site Finder application.
Active enzyme tyrosinase bag on light blue area (site 1) with a volume of 320 Å where all the constituent amino acids in the enzyme tyrosinase active sac contained on exploler ligand for Q Site Finder (Table 2).

Similarly, the enzyme tyrosinase, active sac of α-melanocyte stimulating hormone (α-MSH) were in light blue area (site 1) with a volume of 43 Å ³. All the amino acids making up the active α-MSH sac contained on exploler ligand for Q Site Finder (Table 3).

In addition, through the Site Q finder can be known coordinates (x, y, z) of a bag activated receptor. On the enzyme tyrosinase, the minimum coordinates were (-19, -28, -3) and maximum coordinates (-1, -5, 14). Minimum coordinates (-6, -7, -3) and maximum coordinates (7, 4, 9) for the active α-MSH bag.

On the enzyme tyrosinase, there is no hydrogen bonding between the kojik acid and the enzyme tyrosinase but there is a bond with hydrophobic amino acids Phe197, Pro201, and Asn205. Similarly, the enzyme tyrosinase, bonding pouch α-melanocyte stimulating hormone (α-MSH) do not form hydrogen bonds with rhenium atom. In addition, the alpha-MSH α there is a hydrophobic bond. This is because an atom of rhenium is not a compound that has a hydrogen bond donor or acceptor which allows for better interaction with the receptor through hydrogen bonding and hydrophobic bonding.

Results Validation Program v3.05 AutoDockTools in MGLTools Program Package v1.5.2 with Re-Docking Inhibitor crystallized to enzyme Tyrosinase Pouch: For the validation process initially made two-and three-dimensional kojik acid structure. The three-dimensional

structure was then optimized geometry with AM1 and PM3 methods. The AM1 buildup result better than PM3 method (AM1 method produces 0.07 Å RMSD whereas the PM3 method gave 0.8 Å). These results indicate that the AM1 method is better used than PM3. RMSD values showed similarities with the model structure crystalliser. Stacking the AM1 method produces models kojik acid structure similar to the crystal structure kojik acid. That is, kojik acid can be used to validate the model program AutoDockTools v3.05.

Value of RMSD (Root Mean Square Deviation) re-docking kojik acid on tyrosinase worth 1:30 Å. Because the value of the results of re-docking RMSD less than 2 Å, then AutoDock v.3.05 software used in this study has been proven valid.

Docking Simulation results Curcumin, Demethoxycurcumin, Xanthorrizol and Acid kojik Using v3.05 AutoDockTools Program and Data Interpretation Results: In the docking simulation, run used 10 times run with grid points at 40 x 40 x 40. Formation intended to limit the grid box, so that other atoms or other parts of the receptor are not necessary, do not interfere with the process of docking calculations. Docking simulations on the enzyme tyrosinase using the same grid coordinates with re-docking grid box which wass placed in the middle position of the ligand, which was at position -12 584, -24 146, 8417 (x, y, z) in Cartesian coordinates.

Interaction energy or free energy is the energy required for a ligand can enter into binding pockets and interact with the receptor. The negative sign indicates that the three compounds curcumin and others can interact spontaneously with the enzyme tyrosinase. Curcumin can interact with the enzyme tyrosinase through the hydrogen bonds and van der Waals bonding. The hydrogen bonding between the O atom in curcumin with H atoms in the amino acid Val218 (1.886 Å). Hydrogen bond is a bond between the H atom has a partial positive charge with another atom that is electronegative and has a lone pair with complete octets, such as O, N, and F [11]. According Siswandono and Soekardjo [12], an amino acid which is about 4 - 6A will form van der Waals interactions. Curcumin forming van der Waals bonds with several amino acids on tyrosinase namely Val217 (4,393 Å), Pro201 (4,908 Å), and Gly216 (5,528 Å). Curcumin had the inhibition constants (Ki) of 1520 µm. This value was smaller than the value of Ki kojik acid. This indicated that the strength of the bond between the curcumin with the tyrosinase enzyme was stronger than kojik acid. This is evident from the value of 0.0 µm Ki kojik acid. Ki kojik worth 0.0 M acid, this might due to AutoDockTools v3.05 software used was not able to detect large Ki values.

In contrast to curcumin, demethoxycurcumin interact with the enzyme tyrosinase only through van der Waals bonding. The absence of hydrogen bonds in this interaction suggests that the ability to bind to demethoxycurcumin for tyrosinase smaller than curcumin. It can be seen from demethoxycurcumin interaction energy greater than curcumin. Demethoxycurcumin forming van der Waals bonds with several amino acids on tyrosinase which Met184 (5,383 Å), Gly200 (4,014 Å), Pro201 (4,442 Å), Asn205 (4,957 Å), Gly216 (5,396 Å), Val217 (4,527 Å). Demethoxycurcumin has a Ki value of 5650 µm. This value is greater than Ki curcumin. Showed inhibition constants of affinity ligand-receptor binding. Low Ki value indicates that the ligand has a higher strength than the Ki values were higher (weaker bond strength). A good values range of Ki is 10^{-6}-10^{-12} M. When ligands bound to the receptor too powerful, the ligand will be difficult excreted from the body and the ability to cause an activity is inhibited, but when the ligand binds weakly to the receptors, the ligands will be easily removed before generating an activity.

Similarly, curcumin, xanthorrizol interact with the enzyme tyrosinase through the hydrogen bonds and van der waals interactions. Hydrogen bonds occur between atoms H on xanthorrizol with O atoms in the amino acids Asn205 (1.899Å). Xanthorrizol bonding van der Waals with some amino acids on tyrosinase which Glu158 (5,757 Å), Phe197 (4,303 Å), Gly200 (4313 Å), Pro201 (5,315 Å), and Arg209 (4,931 Å). Xanthorrizol has a Ki of 1040 lm. This value is smaller than the kojik acid. Means of interaction xanthorrizol with tyrosinase stronger than the acid kojik. Research of Batubara *et al*., (2010) showed that the extract of turmeric (Curcuma xanthorrhiza) has activity in inhibiting the enzyme tyrosinase with IC_{50} of 267.3 ± 6.1 mg / mL. This value indicates that the concentration needed to inhibit the enzyme tyrosinase is quite large. With the value of Ki owned curcumin, demethoxycurcumin and xanthorrizol showed that doses in vivo are needed to inhibit the enzyme tyrosinase larger but smaller than the kojik acid.

At the protein α-MSH, curcumin interacts through 3 hydrogen bonds and Van der Waals interactions. The hydrogen bonding between the O atoms contained in curcumin with H atoms contained in amino acids Cys1 (1,986 Å), His3 (1.733 Å), and Arg5 (2,204 Å). Curcumin makes three hydrogen bonds have a high enough affinity to bind to α-

MSH. It can be seen from the interaction energy of -5.49 kcal / mol. Number of hydrogen bonds formed in the ligand-receptor interaction depends on the presence of atomic H, O, N, or S ligands which are located around the H atom acts as a hydrogen bond donor, while O atoms, N, and S acts as a hydrogen bond acceptor.

According to Bohm and Schneider [13], the range of hydrogen bond distances are good docking simulation results are 1.72-2.85 Å. The four compounds had bond distances in the range, in other words that the four compounds had a hydrogen bond distance corresponding requirements so that they could interact with either the tyrosinase enzyme through hydrogen bonding. Curcumin formed van der Waals bond with the amino acids in α-MSH with DPN4 (4,645 Å) and Cys7 (4,571 Å). Van der Waals interaction is the force of attraction between molecules or atoms are not charged and is located adjacent to the bond strength of 0.5-1 kcal / mol [11]. According Siswandono and Soekardjo [12], amino acids that are about 4-6A will form van der Waals interactions. Although the van der Waals bonds are weak, but the sum of van der Waals bonding is a substantial binding factor, especially for compounds with high molecular weight. Van der Waals interactions also give effect to the lipid solubility of the ligand. The more the van der Waals interaction that happens it will be easier ligand is soluble in lipid ligands that can penetrate the cell membrane to be able to bind to the receptor. Curcumin has a Ki of 10000 lm. This value is smaller than the Ki kojik acid. It means that the bond between the curcumin-enzyme tyrosinase is stronger than kojik acid.

Demethoxycurcumin have a smaller interaction energy compared with curcumin, xanthorrizol and kojik acid. This suggests that demethoxycurcumin has a higher affinity than the other three compounds, so demethoxycurcumin more easily interact with amino acids in the active α-MSH sac. Demethoxycurcumin interact with α-MSH via 2 hydrogen bonds and van der Waals interactions. Hydrogen bonds are formed from the O atom to atom H demethoxycurcumin on His3 amino acids (2,074 Å) and Arg5 (2.008 Å). Demethoxycurcumin forming van der Waals bonds with several amino acids that Cys1 (4.413Å), Glu2 (5,084 Å), DPN4 (4.781Å), and Cys7 (4.400 Å). Xanthorrizol can interact with α-MSH via 2 hydrogen bonds and van der Waals interactions. Hydrogen bonds are formed between atoms O on xanthorrizol with H atoms in the amino acid Trp6 (1.730 Å) and Cys7 (1.780 Å) and van der Waals bonding with some amino acids are Cys1, Glu2, His3, DPN4, and Arg5 which has a distance between 4-6 Å. Unlike the other compounds, 4 kojik acid has hydrogen bonding, but still has a lower affinity compared with the three compounds. Kojik acid forming van der Waals interactions with amino acids Cys1 (4,208 Å) and DPN4 (5,572 Å). This suggests that the weak acid bound kojik with α-MSH. It can be seen from the Ki value and the value of the bond energy is greater than the other three compounds.

Curcumin, demethoxycurcumin, and xanthorrizol have a smaller interaction energy than kojik acid which is a compound synthesis. So it can be said that all three of these compounds have a high affinity to bind with the amino acids making up the active enzyme tyrosinase bag and α-MSH. All three of these compounds are able to interact as inhibitors that can later suppress the production of melanin. This indicates that these three compounds may provide pharmacological effects as skin whitening agent as an alternative to the use of acid kojik [14], who have carcinogenic effects.

Our similar works on indication of pharmacological effects based on docking procedure has been published elsewhere [15, 16]

CONCLUSION AND RECOMMENDATION

Based on the research it can be concluded that curcumin, demethoxycurcumin, and xanthorrizol spontaneously interact with amino acids in the active enzyme tyrosinase sac and α-MSH. On the enzyme tyrosinase, the easiest interaction occurs in xanthorrizol because the low energy interactions. Xanthorrizol interact through via the formation of hydrogen bonds with the amino acids Asn205 and van der Waals interactions with amino acids Glu158, Phe197, Gly200, Pro201, and Arg209. On α-MSH, demethoxycurcumin interact easiest because the low energy interactions through the formation of two hydrogen bonds with the amino acid His3, and Arg5 and form van der Waals interactions with amino acids Cys1, Glu2, DPN4, and Cys7. This indicates that these compounds have a high potential to be used as a bleaching agent candidates (whitening agent) as an alternative to the use of kojik acid which have carcinogenic effects. It is suggested that further in-vivo research on curcumin, demethoxycurcumin and xanthorrizol to know the best compounds that have a lower dose with the highest activity as a skin whitening agent (skin whitening agent).

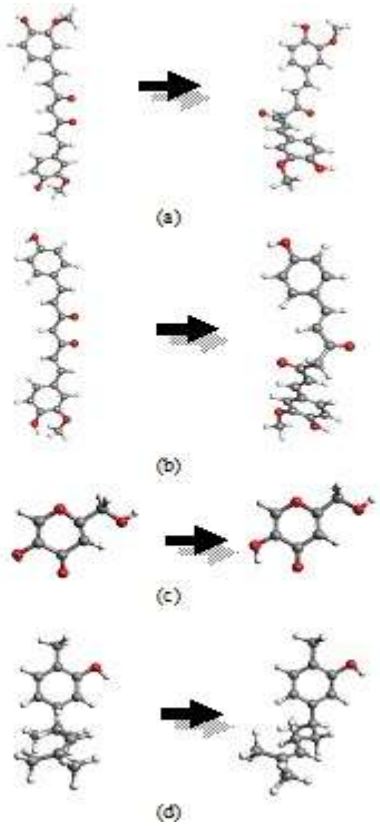
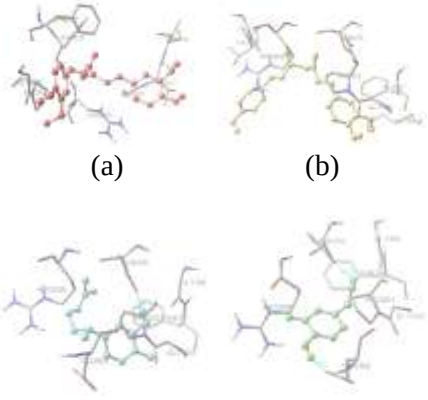


Fig. 1 Three-dimensional structure before and after optimization
(a) curcumin, (b) demethoxycurcumin, (c) kojik acid and (d) xanthorrizol

(a) (b)

Figure 2. Active enclave (a) the enzyme tyrosinase
and (b) α-MSH using Q Site Finder application



(a) (b)

(c) (d)

Fig.3 Docking results in active enzyme tyrosinase pocket
(a) curcumin, (b) demethoxycurcumin (c) xanthorrizol, and (d) kojik acid

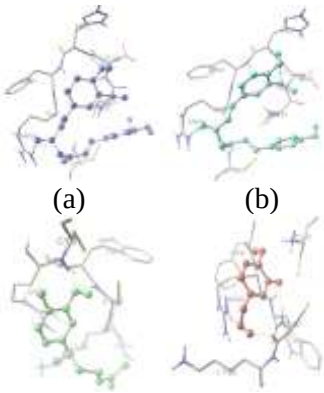


Fig. 4. Docking results of the α-MSH active pocket (a) curcumin, (b) demethoxycurcumin (c) xanthorrizol, and (d) kojik acid

Table 1. Results Analysis of Curcumin, demethoxycurcumin, xanthorrizol and kojik acid nature

Compounds	Energy (Kcal/mol^{-1})	cLogP	Volume (Å^3)	Mass (amu)
Curcumin	-5138.7127	-0.14	1082.26	368.39
Demethoxycurcumin	-4769.2845	0.86	1006.19	338.36
Xanthorrizol	-3819.6365	3.08	784.28	218.34
Kojik acid	-1708.0339	-1.66	423.10	142.11

Table 2. Amino acid constituent Active Enzyme Tyrosinase Pouch

No	Amino acids	*Ligan Exploler Viewer*	*Q Site Finder*
1	His42	-	√
2	His60	-	√
3	Met61	-	√
4	Phe65	-	√
5	His69	-	√
6	Glu158	-	√
7	Glu195	-	√
8	Gly196	√	√
9	Phe197	√	√
10	Gly200	√	√
11	Pro201	√	√
12	His204	-	√
13	Asn205	√	√
14	His208	-	√
15	Arg209	√	√
16	Met215	-	√
17	Gly216	-	√
18	Val217	-	√
19	Val218	-	√

Table 3. Amino acid constituent Pouch Active α-MSH

No	Amino acids	*Ligan Exsploler Viewer*	*Qsite Finder*
1	Cys1	√	√
2	Arg5	√	√
3	Trp6	√	√
4	Cys7	√	√
5	Lys8	-	√

Table 4. Top Score Against Enzyme Docking Results tyrosinase

Compound	Parameter				
	EI[a]	Ki[b]	Hydrogen bond	Hydrogen bond distance[c]	Amino acids residue
Curcumin	-6.45	1520	O-KMN→H-Val218	1.886	Phe197,Gly200, Pro201,Asn205, Arg209,Gly216, Val217,Val218
Demethoxy-curcumin	-6.34	5650	-	-	Phe197,Met184, Gly200, Pro201, Asn205,Arg209, Gly216, Val217
Xanthorrizol	-6.63	1040	H-XNT→O-Asn205	1.899	Glu158, Gly196, Phe197, Gly200, Pro201, Asn205, Arg209
Kojik acid	-3.93	0.0	H-KJK → O-Glu158 H-KJK → O-Asn205	1.780 2.036	Glu158, Gly196, Phe197, Gly200, Pro201, Asn205, Arg209

Note : [a] Interaction energy (kcal/mole) obtained from the results of three times the value of the best repetition docking, [b]Inhibition constants (μM) and [c]Hydrogen bond distance (Å)

Table 5. Top Score Docking Results Against α-MSH

Compound	Parameter				
	EI[a]	Ki[b]	Hydrogen bond	Hydrogen[c] bond distance	Amino acids residue
Curcumin	-5.49	10000	O-KMN→H-Cys1 O-KMN→H-His3 O-KMN→H-Arg5	1.986 1.733 2.204	Cys1, Glu2, His3,DPN4, Arg5, Cys7
Demethoxy-curcumin	-5.95	0.0	O-DKMN→H-His3 O-DKMN→H-Arg5	2.074 2.008	Cys1, Glu2, His3,DPN4, Arg5,Trp6, Cys7
Xanthorrizol	-5.36	7080	O-XNT→H-Trp6 O-XNT →H-Cys7	1.730 1.780	Cys1,Glu2, His3,DPN4,

					Arg5,Trp6, Cys7
Kojik acid	-4.06	0.0	O-KJK → H-Arg5 O-KJK → H-Trp6 O-KJK → H-Cys7 H-KJK → O-Lys8	1.884 2.018 2.211 1.865	Cys1,DPN4, Arg5, Trp6, Cys7, Lys8

Note : [a] Interaction energy (kcal/mol) obtained from the results of three times the value of the best repetition docking, [b]Inhibition constants (μM) and [c]Hydrogen bond distance (Å)

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