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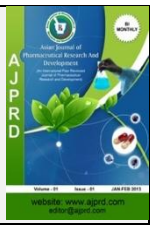

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Research Article

In-silico study of compounds of Curcuma xanthorriza against enzyme tyrosinase sac and α-MSH

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A B S T R A C T

Previous report determined that curcumin, demetoxycurcumin and xanthorrhizol are an active compounds contained in *Curcuma xanthorrhiza* which have activity in inhibiting the tyrosinase enzyme and α-melanocyte stimulating hormone (α-MSH) in vitro. This study aimed to determine and visualize the interaction of the three compounds with the tyrosinase enzyme and α-MSH in order to find their possibility for skin whitening. The experiment carried out using Auto Dock Vina program .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, AutoDock Vina, skin whitening agent

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INTRODUCTION

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.

The enzyme tyrosinase is an enzyme involved in the biosynthesis of melanin pigment (melanogenesis) wherein the substrate is a precursor amino acid tyrosine [2-4]. 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 *C.xanthorrhiza*has activity in inhibiting the enzyme tyrosinase with IC50 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 DAN METHOD

Tools: Hardware used for calculations, molecular modeling, and docking molecule includes a personal computer (personal computer) equipped with Intel Core Duo processor T6500 2.1 GHz, 800MHz FSB, operating system Windows Vista TM Home Basic, a hard disk capacity of 320 GB, and RAM memory 2 GB.

The software used in this study was Swiss PDBViewerv.4.01 which was downloaded from http://www.expasy.org. Chem Office 2004 software (by Cambridge Soft Corporation 2003 downloaded from www.cambridgesoft.com). Portable Hyper Chem 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.4.0.1ArgusLab downloaded from http://www.arguslab.com and Auto Dock Tools 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 Chem Office Chem Draw Ultra2004 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 Swiss PDB Viewer 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.

RESULT AND DISCUSSION

Ligand Preparation: 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.

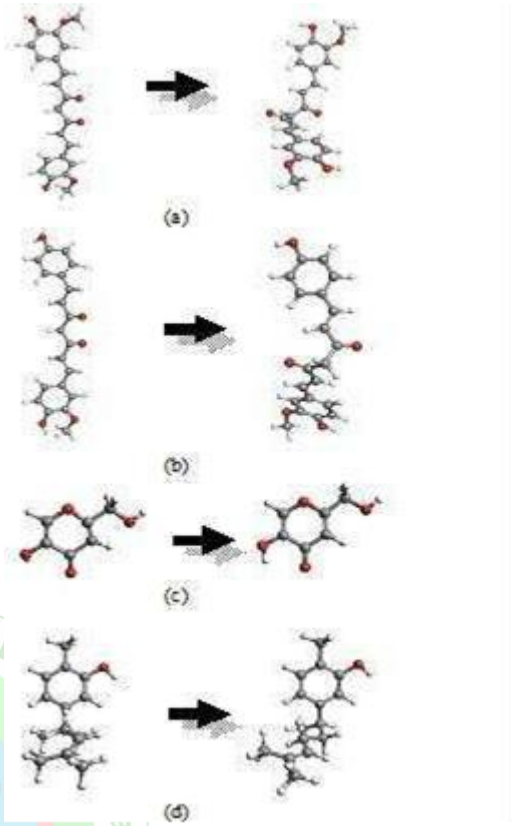

Figure 1:Three-dimensionalstructurebeforeandafteroptimization

(a)curcumin,(b)demethoxycurcumin,(c)kojikacidand(d)xanthorrizol

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 Hyper Chem Release 8.0.7 Portable. cLogP showed lipophilicity of a compound. Partition coefficient of 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 cLogPxanthorrizol 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.

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 Auto Dock Tools 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 µ m. 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 µ m Ki kojik acid. Ki kojik worth0.0 M acid, this might due to AutoDockTools v3.05 software used was not able to detect large Ki values. (see Table 2 and Table 3 showed results Top Score Against Enzyme Docking Results tyrosinase and Top Score Docking Results Against α-MSH, respectively). 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. Demethoxycurcumininteract 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 Waalsbonding 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]

Table 1: Top Score Against Enzyme Docking Results tyrosinase

	Parameter				
Compounds	EIa	Kib	Hydrogen bond	Hydrogen bond	Amino acid
				distancec	residues
Curcumin	-6.45	1520	O-KMN->H	1.886	Phe197,Gly200,
			Val218		Pro201,Asn205,
					Val217,Val218
					Arg209,Gly216,
Demethoxy-	-6.34	5650	-	-	Gly200, Pro201
curcumin					Phe197,Met184,
					Asn205,Arg209
					Gly216, Val217
Xanthorrizol	-6.63	1040	H-XNT->O	1.899	Glu158, Gly19
			Asn205		Phe197, Gly200,
					Asn205, Arg209
					Arg209
Kojik acid	-3.93	0.0	H-KJK O->	1.780	Glu158, Gly196,
			Glu158		Phe197, Gly200
			H-KJK O->	2.036	Pro201, Asn205
			Asn205		Arg209
Note :aInteraction energy (kcal/mole) obtained from the results of three times					
the value of the best repetition docking, bInhibition constants (μ M) and cHydrogen bond					

Table 2: Top Score Against α-MSH

	Parameter				
Compounds	**EI[a]**	**Ki[b]**	**Hydrogen bond**	**Hydrogen bond**	**Amino acid**
				distance[c]	**residues**
Curcumin	-5.49	10000	O-KMN->H-Cys1	1.986	Cis1,Glu2
			O-KMN->H-His3	1.733	His3,DPN4
			O-KMN->H-Arg5	2.204	Arg5, Cis7
Demethoxy-				2.074	Cis1,Glu2
curcumin				2.008	His3,DPN4
					Arg5, Trp6
					Cis7
Xanthorrizol	-536	7080	H-XNT->H	1.730	Cis1,Glu2
			Trp6		His3,DPN4
			H-XNT->H	1.780	Arg5, Cis7
			Cis7		
Kojik acid	-4.06	0.0	O-KJK-> H	1.884	Cis1, Dpn4
			Arg5		
			O-KJK-> H	2.018	Arg5, Tpn6
			Trp6		
			O-KJK-> H	2.211	Cys7,Lys8
			Cis7		
			O-KJK-> H	1.865	
			Lis8		
Note :[a]Interaction energy (kcal/mole) obtained from the results of three times					
the value of the best repetition docking, [b]Inhibition constants (μ M) and [c]Hydrogen bond					

CONCLUSION

Based on the research it can be concluded that curcumin, demethoxycurcumin, and xanthorrizolspontaneously 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).

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