International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.3, pp 340-346, 2015

Molecular Docking of Catechins with Lxrα and Lxrβ as Potensial Inhibitor Aterogenesis

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Abstract: Catechins from Green tea clones GMB4 is the natural substance that have the potential to be developed as inhibitor of atherogenesis through LXR signaling. LXR is one of the signaling pathways that contribute to the inhibition of atherogenesis through mechanisms *reverse cholesterol transport* (RCT) and decreased LDL. There are two LXR isoforms in mammals, namely LXRα (NR1H3) and LXRβ (NR1H2). The results of molecular docking LXRα and LXRβ to Catechins showed the potential of Catechins as LXR agonists. The results of the analysis bioinformatics showed that bioactive compounds of Catechins have a great potential in the inhibition of atherogenesis. This analysis process was done using OpenBabel in Pyrx and docking was performed using Autodock vina in Pyrx. Visualization using PyMOL. To determine the sides of the interaction of molecular docking results using LigPlot +. All of isolates from Catechins have negative affinity energy, this shows all of the isolates have a strong affinity to LXR. The most potent as agonist LXR of Catechins showed that Epicathecin gallate (CID_107905) binds to LXR at many active sites including: Phe315, Leu260, Leu316, Ser228, Val263, Ile268, Glu301, Met298, Thr302. Further analysis revealed that these binding sites are maintained by hydrogen bonds with Ser264, Arg305, Asn225, Glu267. The interaction energy between LXR and Epicathecin gallate (CID_107905) is -9.86 Kcal/mol.
Key words: LXR agonist, Catechins, aterogenesis.

Introduction

One of the signaling pathways that contribute to the inhibition of atherogenesis through mechanisms reverse cholesterol transport (RCT) and decreased LDL is the liver X receptor (LXR). LXR acts as a cholesterol sensor that play to lower cholesterol levels through increased expression of the target gene associated with RCTs, the conversion of cholesterol into bile acids and intestinal cholesterol transport. RCT is the transport of cholesterol from peripheral tissues to the liver to be excreted into the bile duct in the form of cholesterol that has not changed or after converted to bile acids. RCT begins with the removal of cholesterol from cells into HDL or lipid-free apolipoprotein such as Apo-A1 or Apo E[1].

Liver X receptors play an important role in the maintenance of lipid homeostasis by functioning as a transcription factor that regulates genes that control the transport, catabolism and excretion of cholesterol. LXR is one of the signaling pathways that play a role in the process of cholesterol efflux subsequently play a role in

atherosclerosis. There are two LXR isoforms in mammals, namely LXRα (NR1H3) and LXRβ (NR1H2). LXRα is expressed in large amounts in the liver, intestine, kidney, spleen, and adipose tissue, while LXRβ is expressed in various places in small quantities. Both isoforms nearly 80% identical amino acid sequences[2] .

To prove the activity of Catechins as inhibitors of atherosclerosis can be explored activity on LXR signaling. With molecular docking to predict binding site position and strength of the bonds between the complex of Catechins as LXR ligands with LXR so that it can be seen whether the tested compounds have the ability as a candidate LXR agonists. The results of the analysis bioinformatics showed that bioactive compounds of Catechins have a great potential in the inhibition of atherogenesis.

Materials and Methods

Ligand preparation

We downloaded the major compound structures of *Catechins* from NCBI PubChem and Zinc.docking.org. The ID of Catechins : includes CID_9064 : -(-) catechin; CID_199472 : gallocatechin gallat; CID_65064 : EGCG; CID_6419835 : catechin gallate; CID_72276 : epicathecin; Zinc_3870337 : gallo catechin; CID_107905 : Epicathecin gallate; Zinc_3870328 : epi gallo catechin. The 3D structure of Catechins are in Figure 1.

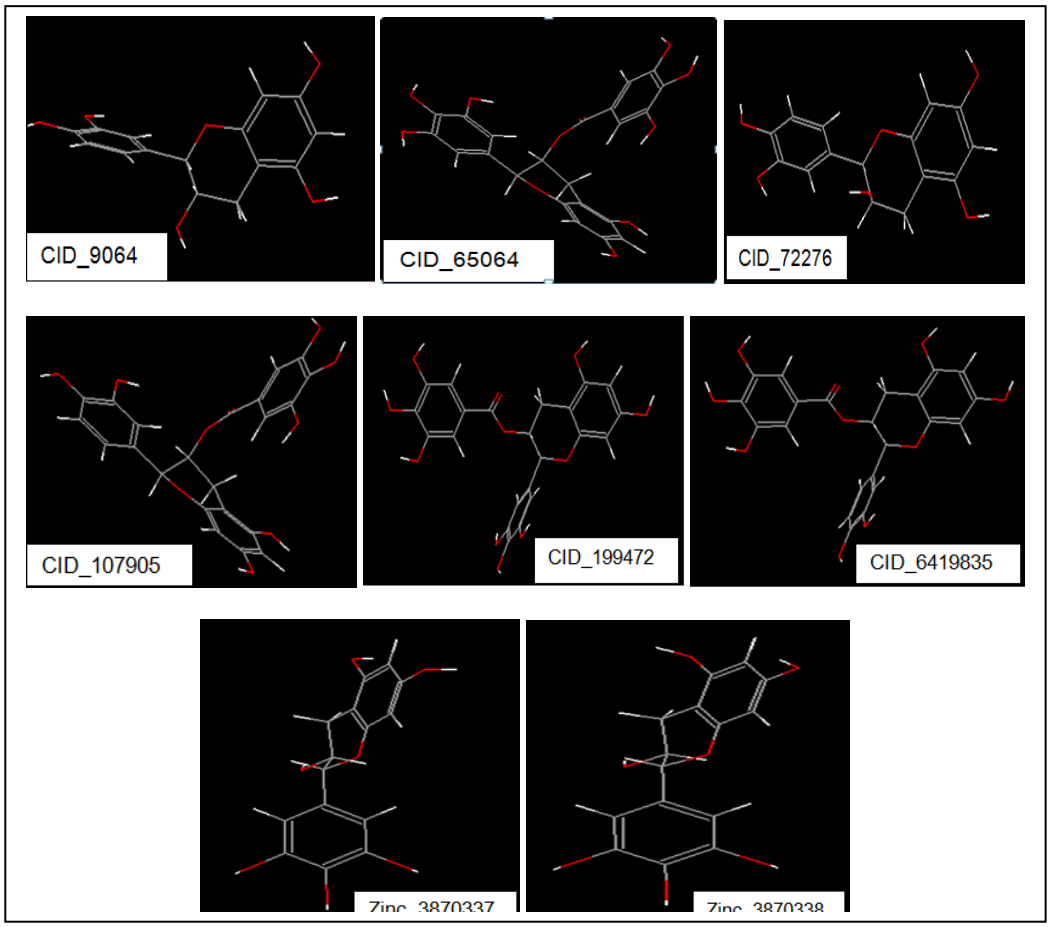


Figure 1. Three dimension Stucture of *Catechins*

CID_9064: -(-) Catechin; CID_199472: Gallocatechin gallat; CID_65064: EGCG; CID_6419835: Catechin gallate; CID_72276: Epicathecin; Zinc_3870337: Gallo catechin; CID_107905: Epicathecin gallate; Zinc_3870328 : Epigallo catechin.

The 3D Structure of LXR

Modeling the 3D structure of LXR used swiss model (http://swissmodel.expasy.org). For visualization of the 3D structures used Python Molecular Viewer (PyMOL)[3]. The 3D Structure of LXR is as in Figure 2.

Based on the images of 3D structures LXR composed of structures α helix and β-sheet while the amino acid sequence shown by notation alphabet based on IUPAC naming.

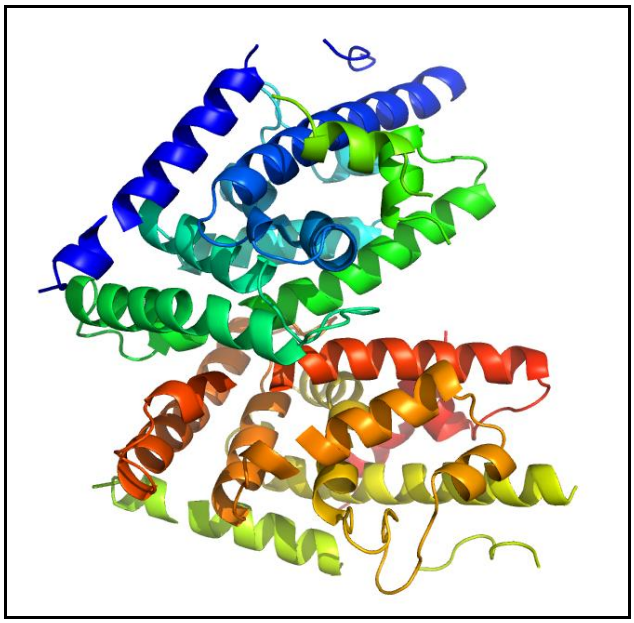


Figure 2. Three dimension Stucture of LXR

Docking Ligand- Protein

LXR docking process with compounds of *Catechins* performed with the following stages: 1) Molecular model of LXR generated through homology modeling using a web server Swiss Model[4-6]. Validation was performed using a 3D structure webserver PROCHECK[7]. While epicathechin molecules obtained from ZINC and PubChem Compound database file (sdf). To find out how much energy binding affinity between molecules LXR and molecular *Catechins* performed using rigid docking Pyrx by doing preparation C*atechins* molecules advance through energy minimization. This process is done using OpenBabel in Pyrx and docking was performed using Autodock vina in Pyrx. Visualization using PyMOL to determine the sides of the interaction of molecular docking results using LigPlot[8].

The Result of Docking LXRα-Catechins and LXRβ-Catechin

We evaluate the binding affinity of Catechins with LXRα and LXRβ. The result of docking are show in Table 1 (Supp. Data 2; Supp. Data 3).

Table 1. Binding energy (Kcal/mol) Isolate of Catechins

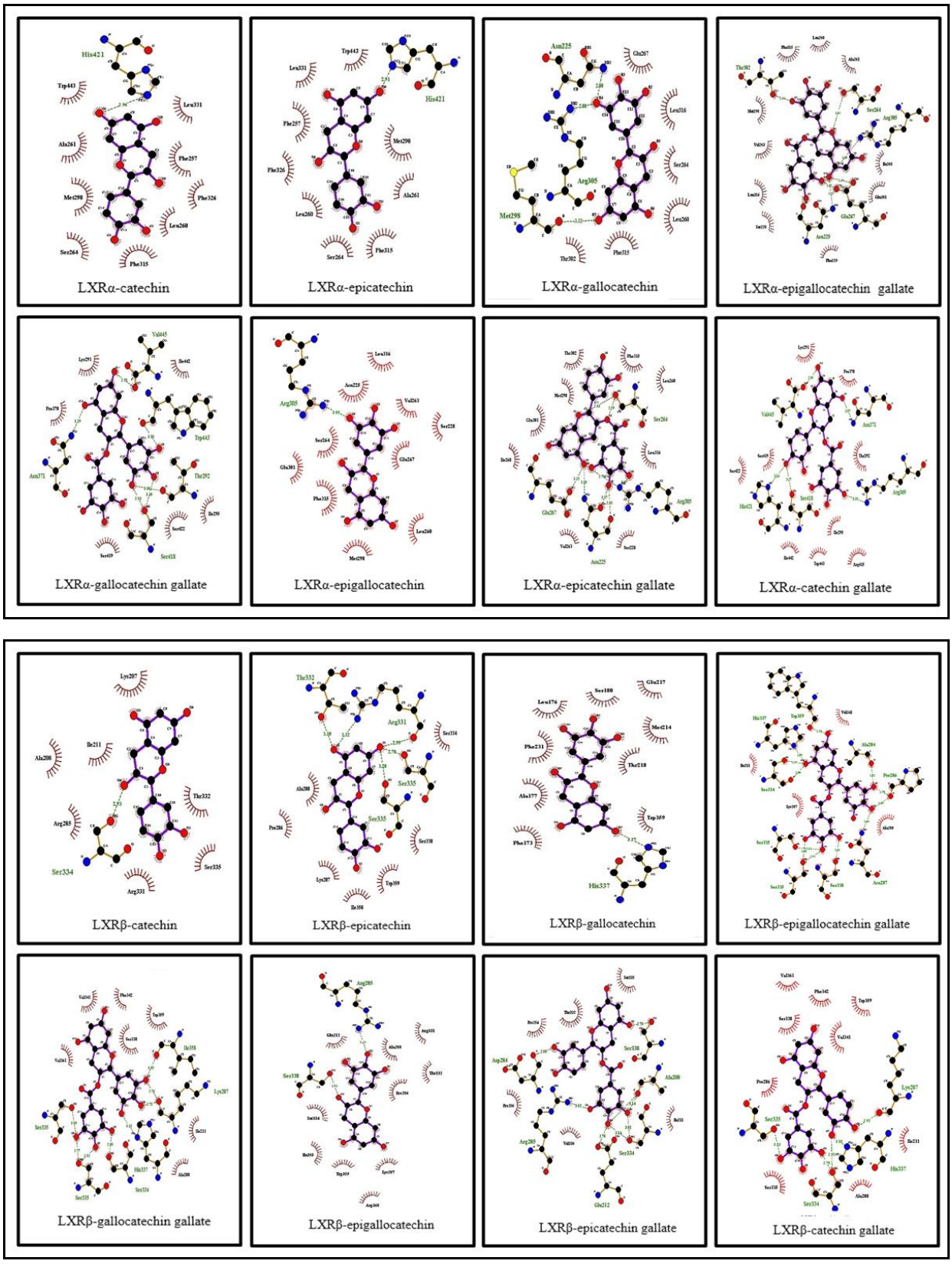
Cathecins	LXR_{α}	LXR_{β}
Epicathechin gallate (ECG)	-9,8	-9,6
Epogallocatechin gallate (EGCG)	-9,5	-8,8
Epigallocatechin	-8,7	-7,2
Catechin gallate (CG)	-8,3	-8,6
Gallocatechin (GC)	-8,3	-8,8
Catechin	-8,1	-7,1
Gallocatechin gallate	-7,9	-8,6

Supplementary Data 1. Binding energy, hidrofobic interaction and hydrogen binding complex isolate of Catechins and LXRα

Isolate of Catechins	Binding Energy (kcal/mol)	Hidrofobic Interaction	Hydrogen binding
Catechin	-8.1	Trp443, Leu331, Ala261, Phe257, Ala261 ,Met298, Phe326, Leu260, Ser264, Phe315	Ligand-His421 Ligand-Thr302
Epicatechin	-8.2	Trp443, Leu331, Ala261, Phe257, Ala261, Met298, Phe326, Leu260, Ser264, Phe315.	Ligand-His421 Ligand-Thr302
Gallocatechin	-8.3	Glu267, Leu316, Ser264, Leu260, Phe315, Thr302.	Asn225, Arg305, Met298
Epigallocatechin gallate	-9.5	Phe315, Leu260, Ala261, Ile268, Glu301, Phe229, Ser228, Leu316, Val263, Met 298.	Ser264, Ser264, Arg305, Thr302, Glu267, Asn225
Gallocatechin gallate	-7,9	His421, Asn371, Ser422, Val445, Trp443, Thr292, Ser418, Asn371	Ile442, Ile295, Ser422, Ser419, Pro370, Lys291
Epigallocatechin	-8,7	Leu316, Val263, Asn225, Ser228, Glu267, Leu260, Met298, Phe315, Glu301, Ser264.	Thr302,Glu267, Asn225, Arg305
Epicatechin gallate	-9,8	Phe315, Leu260, Leu316, Ser228, Val263, Ile268, Glu301, Met298, Thr302.	Ser 264 (Donor) Ser 264,Arg305,Asn225, Glu267
Catechin gallate	-8,3	lys291, Phe370, Thr292, Ile295, Arg415, Trp443, Ile442, Ser422, Ser419.	Thr292,Arg369, His421, (donor)Asn371, Arg369, Ser418, His421, Val 445

Supplemantray Data 2. Binding energy, hidrofobic interaction and hydrogen binding complex isolate of Catechins and LXRβ

Isolate of Catechins	Binding Energy (kcal/mol)	Hidrofobic Interaction	Hydrogen binding
Catechin	-7.1	Ala208 , Thr332, Ile211, Ser335, Arg331, Arg285	(aceptor) Thr332, Ser335, Lys207, (Donor) Arg331, Thr332, Ser334
Epicatechin	-7.4	Ser334,Ser338, Trp359,Ile358, Lys207, Pro286, Ala203.	(aceptor) Ser335, Ser338, Arg285, Thr332 (donor) Arg331, Ile 358, Ser338, Trp359, Thr332
Gallocatechin	-8.8	-	(aceptor) His337, (donor) Thr218, Leu176.
Epigallocatechin gallate	-8.8	-	(aceptor) His337, Asn287, Ser338, Ser335 (donor) Trp 359, Ser334, Ala204, Pro286, Ser 338, Ser335, Ser334
Gallocatechin gallate	-8.6	-	(aceptor) Ser338, His337, Ser334, Ser335, (donor) Ser 338, Ile358, Lys207, Ser334, Ser335
Epigallocatechin	-7.2	-	(donor) Lys207, Ser335, Thr332, (aceptor) Arg331, Thr332, Ser334
Epicatechin gallate	-9,6	-	(aceptor) Ser335, Ser338, Arg285, (donor) Ser335, Ser338, Ser334, Ala208
Catechin gallate	-8,6	-	(aceptor) Ser338, His337, Ser335, Ser335, Ser334, (donor) Ser 338, Lys207, Ser335, Ser334



Supplementary Data 3. The result of docking LXRα-catechins and LXRβ-catechins

Discussion

Bond energy is the energy to break one mole of bonds of a molecule in the form of gas is expressed in units of Kj / mol or Kcal / mol. The minimum interaction energy indicates that the strength of the maximum receptor ligand interactions[9]. All of isolates from Catechins have little affinity energy, this shows all the isolates have a strong affinity to LXR.

Another parameter that can be used is to know that the receptor binding site with the formation of hydrogen bonds . Hydrogen bond is a bond that is formed due to attractive forces between the H atoms with other atoms are more electronegative (Nitrogen / N , Oxygen / O , Fluor / F) . The hydrogen bonds are the most powerful bonds with other molecular bonds but smaller than covalent bonds and ionic bonds . Secondary structure of the protein α - helix and both parallel and antiparallel β - sheets play a major role in the hydrogen bonding, which is one atomic interactions that play a role in biological and chemical systems. In addition to the structure of proteins and isolates from Catechins play a role in hydrogen bonding. These isolates are flavonoid compounds with polyphenols which have many hydroxyl groups[10]. Based on the results of docking with LXRα, isolates from Catechins that has the highest hydrogen bonds are Catechin Gallate (CG) Gallocatechin Gallate (GCG), Epigallocatechin Gallate(EGCG), Epicatechin gallate(ECG), Epigallocatechin (EGC), Gallocatechin(GC), Catechin (C) and Epicatechin (EC) (Supp. Data 3).

Isolates from Catechins that has the highest hydrophobic interaction are C, EC, EGCG, EGC, ECG, CG, GC and GCG. Hydrophobic interaction of nonpolar substance showed a tendency to form aggregates and can be dispersed in an aqueous solution to form micelles. The carboxyl group will be on the surface and the nonpolar substance will be inside. The more hydrophobic interactions indicates the stronger interaction between the ligand and its receptor.

Bond energy to isolate from Catechins with LXR β showed that ECG lowest binding energy subsequently ECG, EGCG, GC, GCG, CG, EC, EGC, C. The same tendency as shown by LXRα. Hydrogen bonds also occur in all of isolate from Catechins with LXRβ in which the order is based on the highest number of bonds is EGCG, CG, GCG, EC, ECG, C, GC. Whereas hydrophobic interactions are only shown Catechin and Epicatechin.

These results indicate that the isolates from Catechins show potential as LXR agonists both LXRα and LXRβ. LXRα and LXRβ structurally isoform which have the same molecular structure but differ only in the number of atoms so that the result of molecular docking is similar. Based on the expression, LXRα expression is widely expressed in the liver, spleen, kidney, adipose tissue and small intestine whereas LXRβ is expressed in a variety of places in small quantities[11]. The organ most responsible to inhibit aterogenesis are liver and small intestine subsequently to determine its potential is more focused on the results of docking with LXRα.

Catechins show potential effect to inhibit aterogenesis trough activating LXR signaling. There are accumulative effect isolate from Catechins that will enhance the ateroprotective effect.

Conclusion

All of isolates from Catechins show potential as LXR agonists both LXRα and LXRβ.The modeling analyses of isolate from Catechins suggest the maximum receptor ligand interaction showed by Epicathecin gallate (ECG) so Epicathecin gallate (CID_107905) binds to LXRα at many active sites including: Phe315, Leu260, Leu316, Ser228, Val263, Ile268, Glu301, Met298, Thr302. Further analysis revealed that these binding sites are maintained by hydrogen bonds with Ser264, Arg305, Asn225, Glu267. The interaction energy between LXRα and Epicathecin gallate (CID_107905) is -9.86 Kcal/mol. Thus, these theoretical data suggests all of isolate from Catechins as a potential inhibit aterogenesis trough activating LXR signaling. Required verification with in vivo and in vitro studies to proof the results of molecular docking Catechins with LXRα and LXRβ.

Acknowledment

The authors thank the Directorate of General Higher Education, Ministry of Education and Culture of Indonesia for the “BPPS” scholarship.

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