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In Silico Analysis of the NPC1L1 Inhibitor of Catechins from Green Tea

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Abstract

The main contributor to cardiovascular disease is atherosclerosis. The Liver X Receptor is one of the unexplored signaling pathways in atherosclerosis that contributes to cholesterol efflux and inhibitory inflammation (LXR). Catechin, as an LXR agonist, influences the expression of the NPC1L1 protein transporter, which inhibits cholesterol absorption. The objective of this study is to predict the NPC1L1 inhibitor of Catechins from Green Tea. The role of NPC1L1 inhibitors is to prevent atherogenesis. Molecular docking is the research method used. Pyrx's Open Babel was used for analysis. Autodock vina in Pyrx was employed for docking, and Chimera v1.8 was administered for visualization. The result of molecular interaction was assigned. Pose view was used in this study. Catechins have the potential to be an NPC1L1 inhibitor, according to the findings. The main parameters used to predict the biological effect were energy bonds, hydrogen bonds, and hydrophobic interactions of molecules with NPC1L1. All Catechins isolates had low affinity energy and a strong affinity for NPC1L1. Epigallocatechin gallate (EGCG) is the most effective inhibitor because it has the lowest binding energy and the most active sites, including Gln 200, Tyr 192, Trp 202, Cys 189, Gly 207, Asp 217, Gly 190, Phe 205, Asp 208. There are hydrogen bonds at Thr 219, Ile 218, Asn 204, Asn 211, Arg 201, and Asn 204. The interaction energy between NPC1L1 and EGCG is -7.5 kCal/mol. Based on the results of the in-silico analysis, the researchers concluded that Catechins have the potential to be an NPC1L1 inhibitor. Further research into molecular dynamic simulation and in vivo analysis is required to demonstrate the synergistic effect of Catechins as an inhibitor of atherogenesis.

Keywords: In silico, Catechins, NPC1L1, Atherogenesis.

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1. INTRODUCTION

The development of natural products as drugs continues, particularly for degenerative diseases. Because of the high prevalence of cardiovascular disease as a degenerative disease, complementary therapy is required to supplement the main drug. The in-silico methods are one of the research approaches used to demonstrate the activity of natural substances in preventing cardiovascular disease (Suryadi et al., 2021).

The in-silico method is useful for determining the activity of natural substances with pharmacological properties such as analgesic (Suryadi et al., 2021), anticancer (Ervina et al., 2021a; Ervina et al., 2021b; Sulistyowaty et al., 2021), antidiabetic, and recently antivirus (Rendi et al., 2021; Hidayat et al., 2021; Asdaq et al., 2021). On a computational basis, this method predicts drug activity based on drug interactions with receptors. Traditional methods for investigating the activity of natural substances are prohibitively expensive (Brooijmans & Kuntz, 2003; Mukesh & Rakesh, 2011). The in-silico method is now being used more extensively in the search for new compounds for medicinal or nutraceutical ingredients. Molecular docking is commonly used in drug development. Docking is based on the formation of complex proteins and ligands. The docking principle can be used to determine ligand binding to target proteins as well as predict the properties of complex molecules (Gohlke, & Klebe, 2002). The ultimate goal of in silico is to find bioactive compounds with biological effects for drug development. In this docking method, candidate molecules are placed at various positions and suitability on the desired target protein. The pose done assessment is used to determine the exact position (scoring). A high score indicates that the molecule is the best candidate for binding to the target (Halperin et al., 2002; Meng, et al., 2011).

Catechins, as LXR agonists, can reduce the expression of NPC1L1, also known as the Niemann-Pick C1-Like 1, a protein transporter on the apical of enterocytes (Altmann, et al., 2004). NPC1L1 is involved in intestinal cholesterol absorption. Cholesterol from the diet and cholesterol synthesized by HMG CoA are transported through cell membranes by Niemann Pick C1 Like 1 (NPC1L1), and ApoB48 phospholipids, along with triglycerides, are synthesized into triglyceride-rich cylomicrons by Mycrosomal Triglyceride Transport Protein (MTP). With the help of ABCG5/G8, some of the absorbed cholesterol is excreted in the gut.

Preliminary studies prove Catechins increase efflux cholesterol macrophage, increase eNOS expression, p 110 PI3K, decrease MMP9 expression, and p 38 MAPK (Susanti, 2021; Susanti, 2012; Susanti, 2015; Susanti 2019). All these factors contribute to the inhibition of atherogenesis. Another mechanism is that NPC1L1 expression parameters inhibit intestinal cholesterol absorption. Catechins' activity as an inhibitor of NPC1L1 must be demonstrated in silico as a predictable activity before in vivo studies are conducted. The objective of this study is to predict the NPC1L1 inhibitor of Catechins from Green Tea. In silico data is required in the early stages of drug discovery to select active compounds. Natural substances as NPC1L1 inhibitors have received little attention. As a result, we will conduct research to identify interactions between NPC1L1 inhibitors and natural substances for the prevention of degenerative diseases.

2. RESEARCH METHOD

This research aims to predict the interaction NPC1L1 with Catechins through in silico analysis. A literature review and in silico analysis were performed to ensure the active compounds of Catechins. The Bioinformatic Laboratory Department Biology Universitas Brawijaya Malang assisted in the collection of research data.

a. Homology modelling.

Homology modeling to generate Molecular NPC1L1 employed a web server Swiss Model (Grosdidier, Zoete, & Michielin., 2007; Guex & Peitsch, 1997). This study administered web server PROCHECK to validate 3D structures (Fradera et al., 2010). The active compounds of Catechins from PubChem Compound ZINC database in a file (sdf) (http://pubchem.ncbi.nlm.nih.gov).

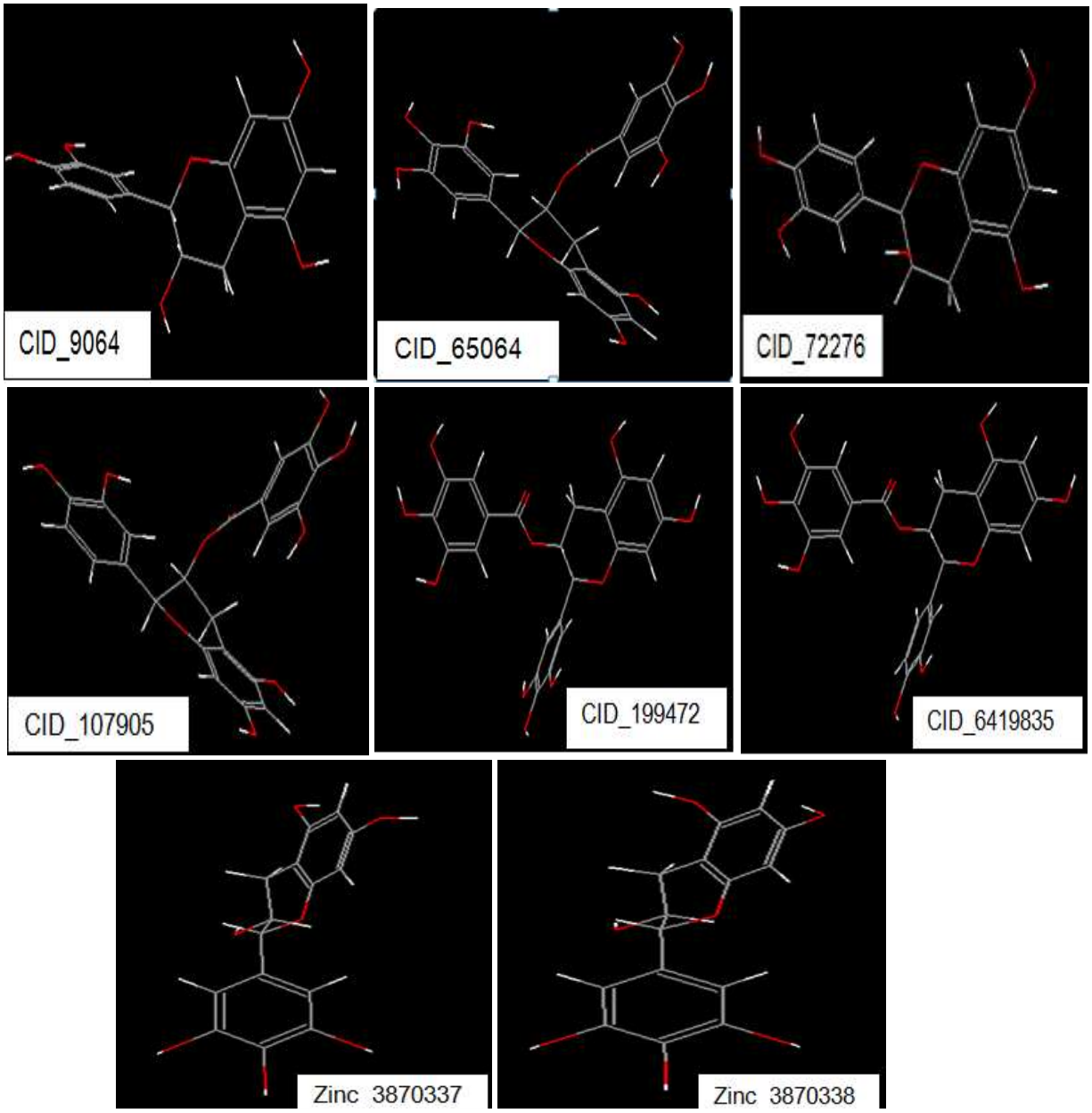

Figure 1. Structure of Catechins in three dimensions; Zinc_3870337: Gallocatechin, Zinc_3870328: Epigallocatechin, CID_9064: -(-) Catechin, CID_199472: Gallocatechin gallate, CID_65064: EGCG, CID_6419835: Catechin gallate, CID_72276: Epicathecin, CID_107905: Epicathecin gallate

b. The 3D structure of NPC1L1

The Swiss model was used in this study to generate a 3D structure of NPC1L1 (http://swissmodel.expasy.org). The 3D structures were visualized by Python Molecular Viewer (PyMOL) 3. Figure 2 demonstrates the 3D Structure of LXR.

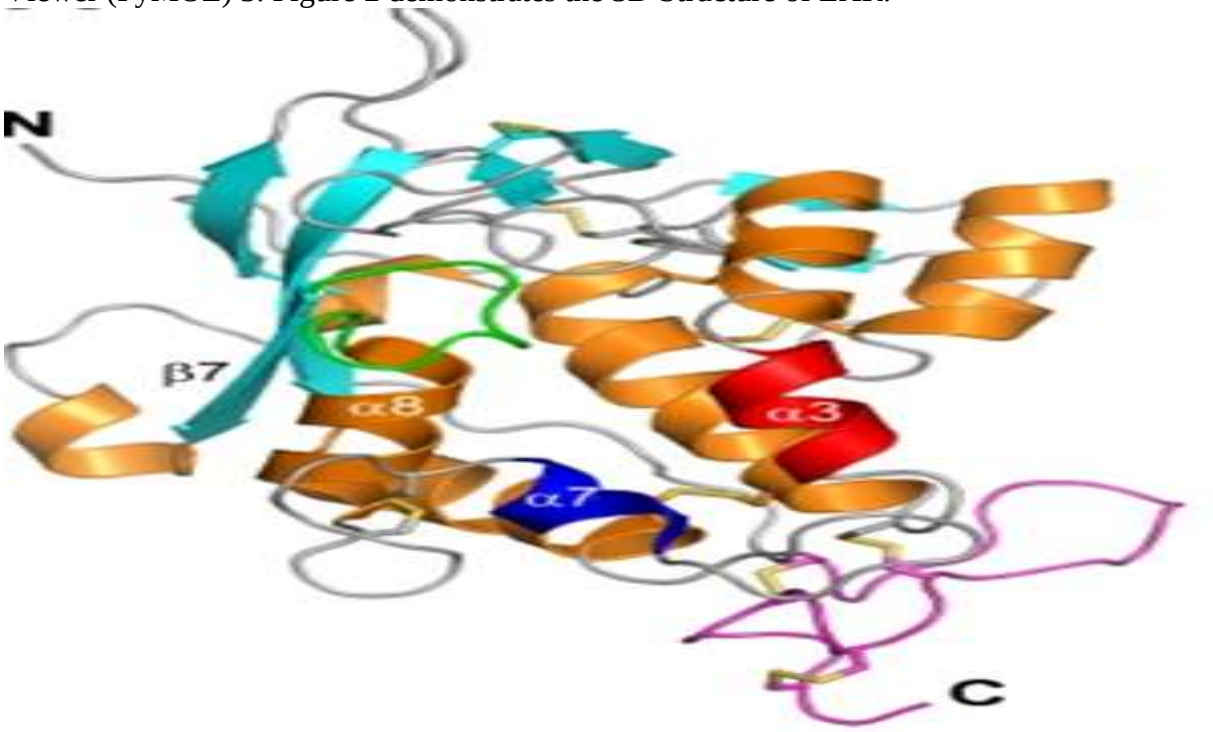

Figure 2. Three-dimension structure of NPC1L1.

c. Molecular docking study.

The bond energy strength, the number of hydrogen bonds, and the number of hydrophobic interactions is the three main parameters of in silico analysis. To demonstrate catechins as NPC1L1 inhibitors with the lowest binding energy and the greatest number of hydrophobic interactions and hydrogen bonding. Open Babel in Pyrx was employed for in silico analysis, and Autodock 4.0 in Pyrx was administered for docking. Docking results are determined using Pose view. To see both sides of the interaction, Chimera v 1.8 is required.

3. RESULTS AND DISCUSSION

Table 1. Binding energy (Kcal/mol) of Catechins with NPC1L1.

Catechins (Ligan)	**NPC1L1 (Receptor)**
Epigallocatechin gallate (EGCG)	-7.5
(-)-Catechin gallate (CG)	-7.4
(-)-Gallocatechin Gallate (GCG)	-7.3
-(-) Catechin (C)	-6.7
Epigallocatechin (EGC)	-6.6
Epicathechin (EC)	-6.6
Gallocatechin (GC)	-6.5
Ezetimibe (control)	-6.1

Table 1 illustrates binding energy of Catechins with NPC1L1.The binding affinity of Catechins with NPC1L1 had been evaluated.

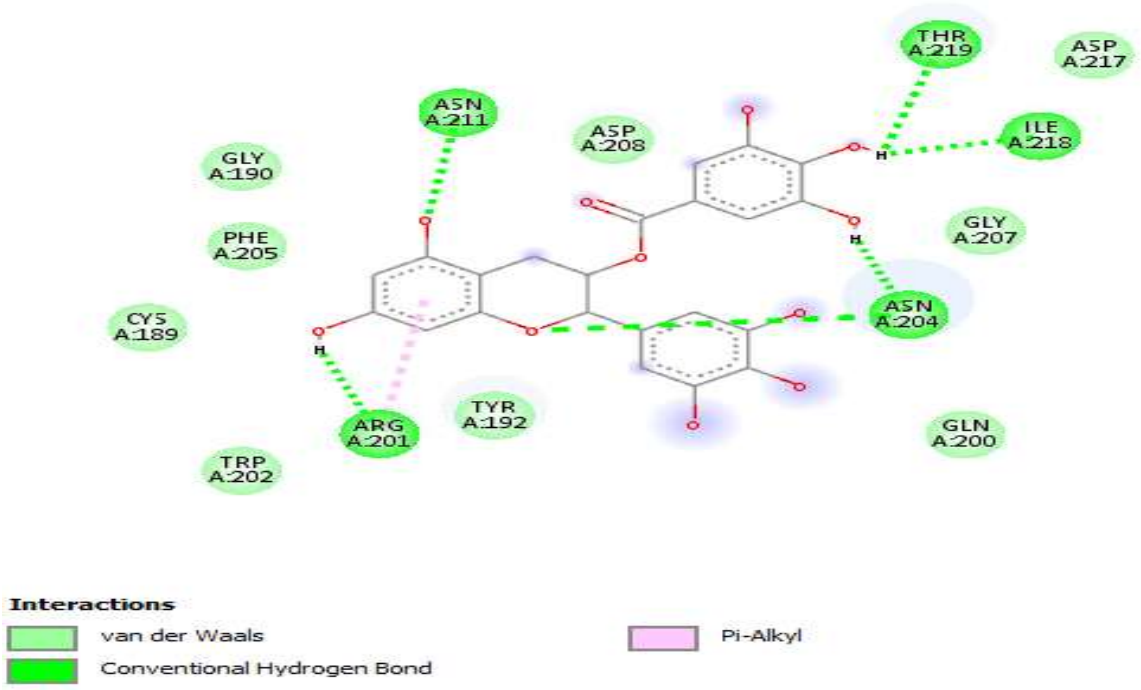

Figure 3. The hydrogen bonding and hydrophobic interaction of NPC1L1 with EGCG.

The binding energy of EGCG with NPC1L1 is -7,5 kcal/mol. The hydrogen bonding was at Thr 219, Ile 218, Asn 204, Asn 211, Arg 201, and hydrophobic interactions were at Gln 200, Tyr 192, Trp 202, Cys 189, Gly 207, Asp 217, Gly 190, Phe 205, and Asp 208.

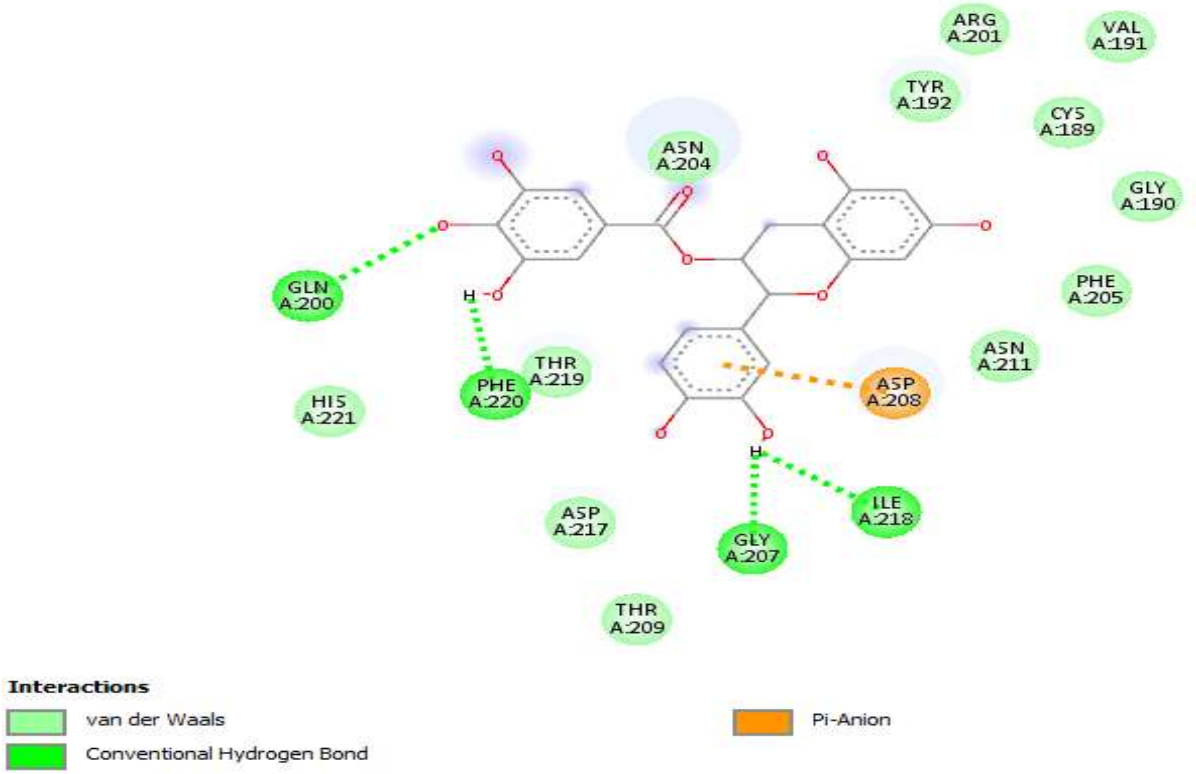

Figure 4. The hydrogen bonding and hydrophobic interaction of NPC1L1 with Catechin gallatte.

The binding energy of Catechin gallate with NPC1L1 is -7.4 kcal/mol.The hydrogen bond at Gln 200, Phe 220, Gly 207, Ile 218, and hydrophobic interactions at Arg 201, Val 191, Gly 190, Tyr 192, Cys 189, Phe 201, Asn 211, Asn 204, Thr 219, Asp 217, Thr 209, and His 221.

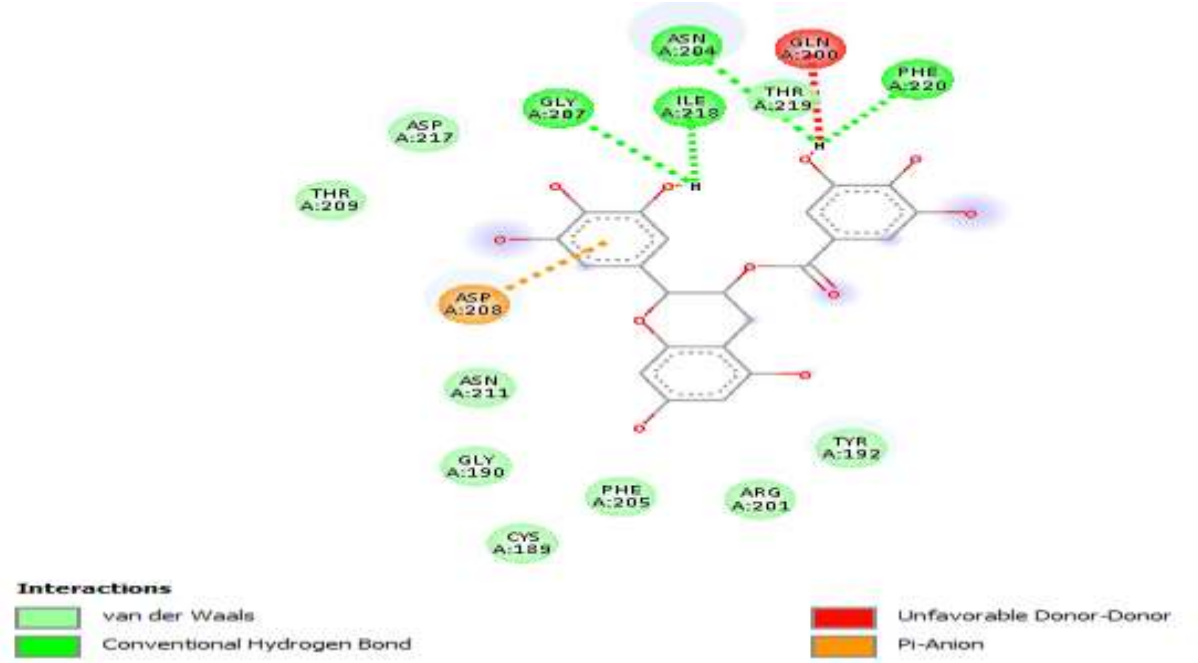

Figure 5. The hydrogen bonding and hydrophobic interaction of NPC1L1 with Gallocatechin gallate.

The binding energy of Gallocatechin gallate with NPC1L1 is - 7.5 kcal/mol. Meanwhile, the type of bond that is generated is a hydrogen bond at Asn 204, Ile 218, Gly 207, Phe 220, and hydrophobic interactions at Thr 219, Asp 217, Thr 209, Asn 211, Gly 190, Phe 205, Arg 201, Tyr 192, and Cys 189.

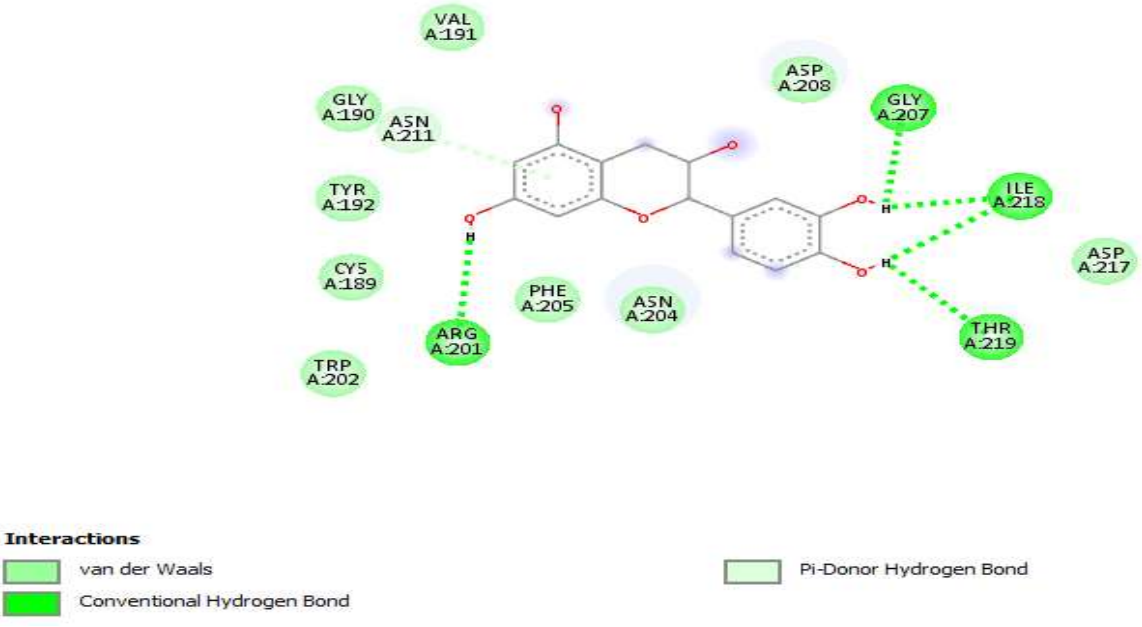

Figure 6. The hydrogen bonding and hydrophobic interaction of NPC1L1 with Catechin.

NPC1L1 has a binding energy of -6,7 kcal/mol with Catechin. Hydrophobic interactions at Asp 217, Asp 208, Val 191, Gly 190, Asn 211, Tyr 192, Cys 189, Trp 192, Cys 189, Trp 202, Phe 205, and Asn 204, as well as hydrogen bonds at Arg 201, Thr 219, Ile 218, Gly 207.

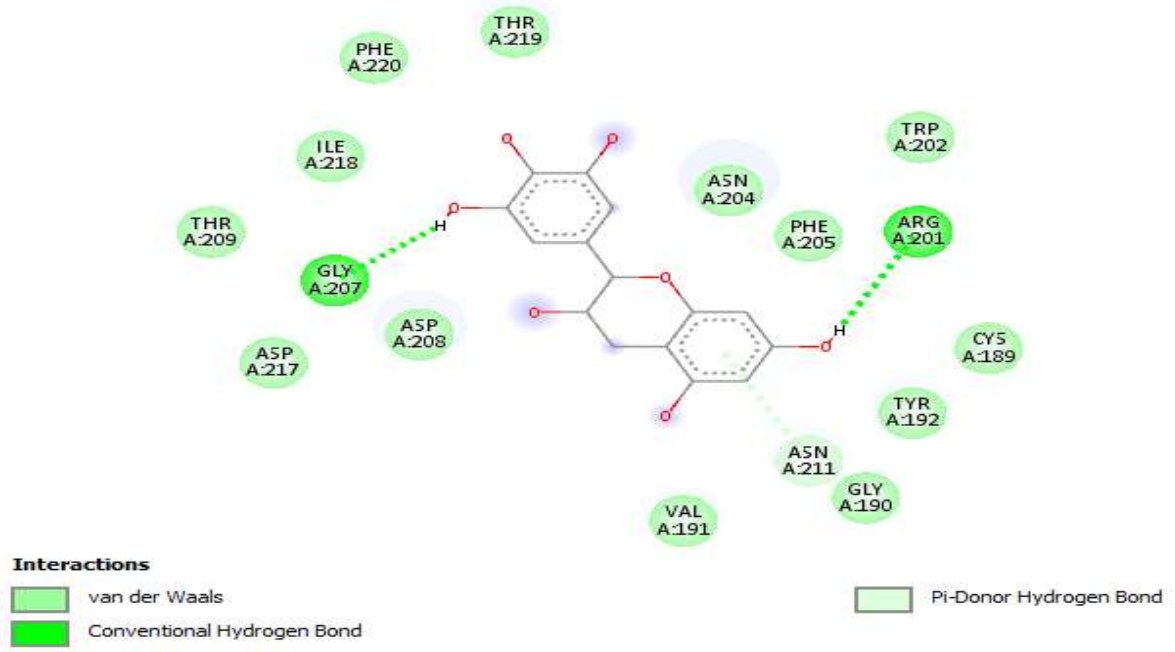

Figure 7. The hydrogen bonding and hydrophobic interaction of NPC1L1 with Epigallocatechin.

The binding energy of NPC1L1 with Epigallocatechin is -6,6 kcal/mol. The hydrogen bond is at Gly 207, Arg 201, and hydrophobic interactions at Thr 219, Phe 220, Ile 218, Cys 189, Tyr 192, Val 191, Thr 209, Asn 204, Phe 205, Asn 211, Gly 190, Trp 202, Asp 217, and Asp 208.

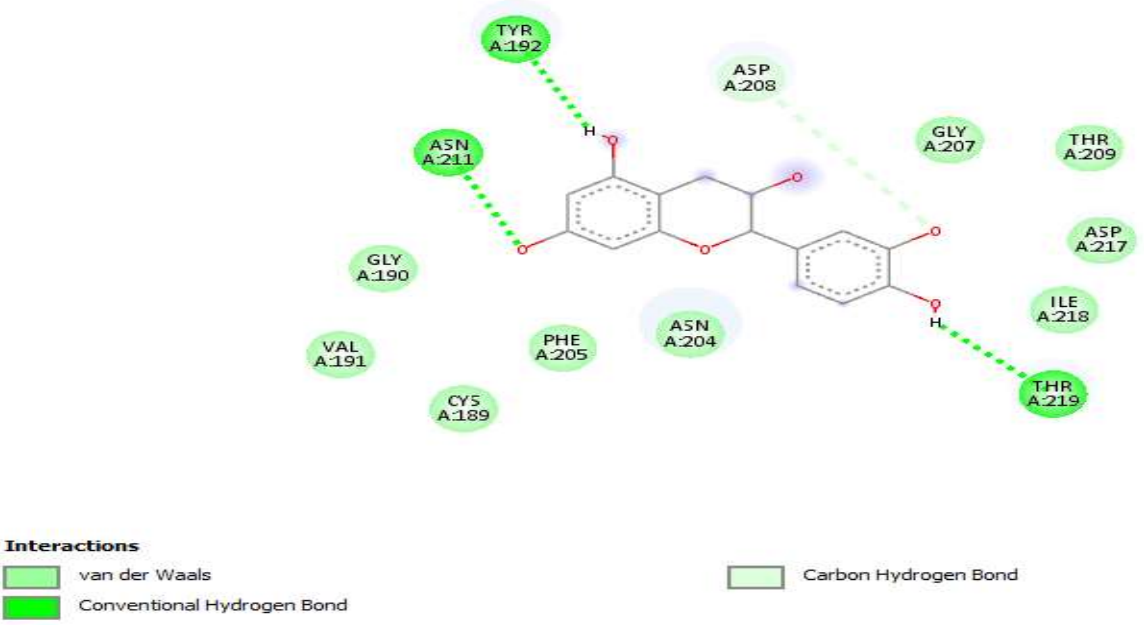

Figure 8. The hydrogen bonding and hydrophobic interaction of NPC1L1 with Epicatechin.

NPC1L1 has a binding energy of -6,6 kcal/mol with Epicatechin. Hydrophobic interactions are at Asp 208, Gly 207, Thr 209, Asp 217, Val 191, Ile 218, Asn 204, Phe 205, Cys 189, and Gly 190.

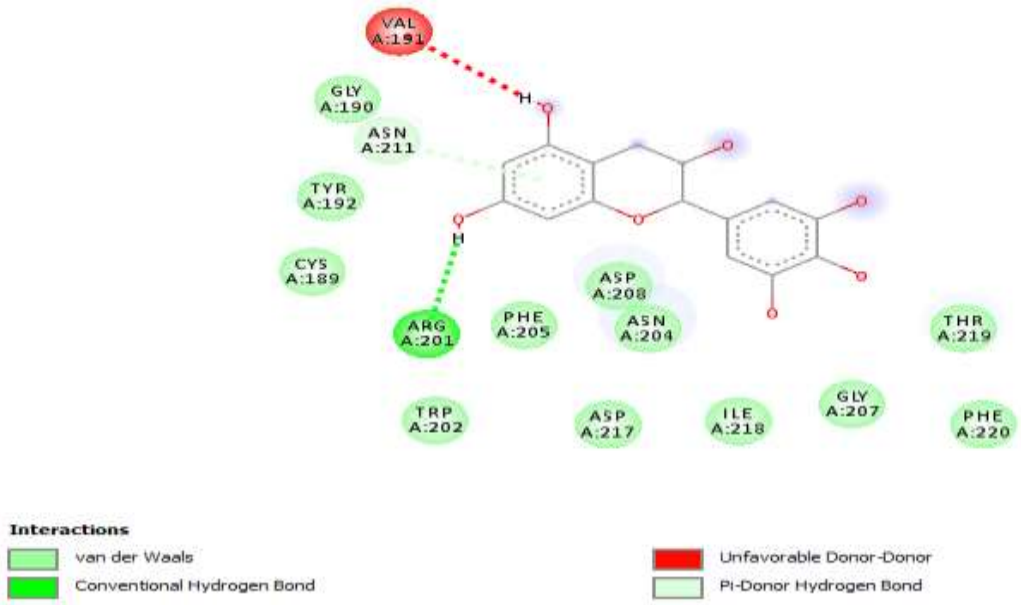

Figure 9. The hydrogen bonding and hydrophobic interaction of NPC1L1 with Gallocatechin.

The binding energy of NPC1L1 with Gallocatechin is -6,5 kcal/mol. The hydrogen bond is at Arg 201 and hydrophobic interactions at Gly 190, Phe 205, Asp 208, Asn 211, Asn 204, Tyr 192, Trp 202, Cys 189, Asp 217, Ile 218, Thr 219, Gly 207, Asp 217, Gly 207, and Phe 220.

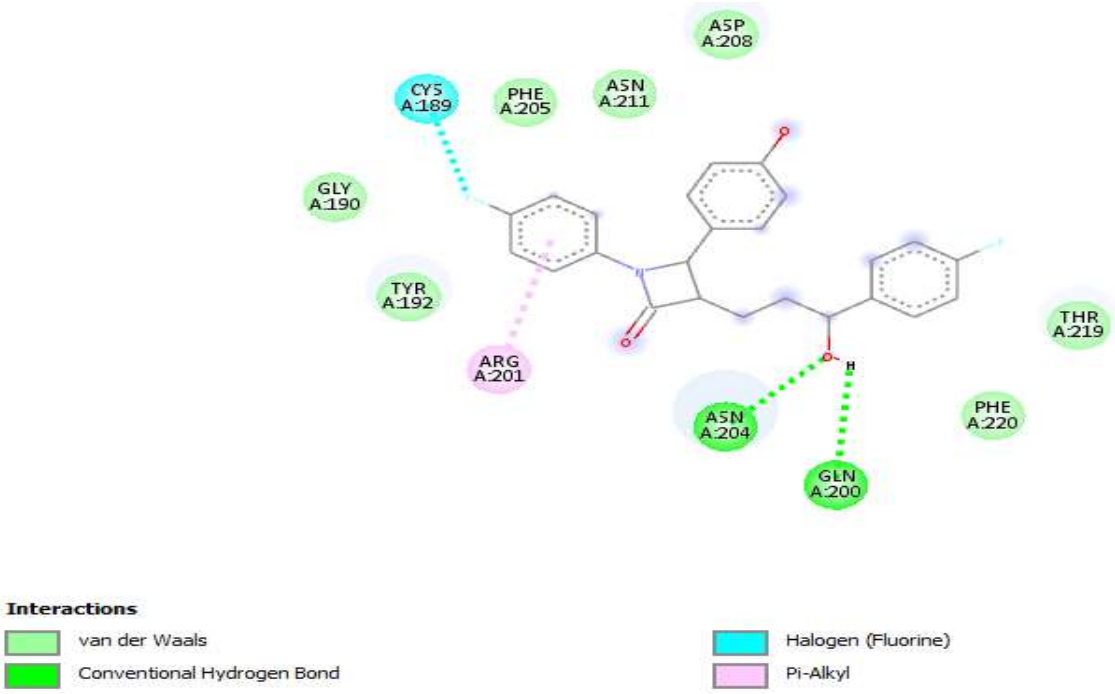

Figure 10. The hydrogen bonding and hydrophobic interaction of NPC1L1 with Ezetimibe.

NPC1L1 has a binding energy of -6,1 kcal/mol with Ezetimibe. The hydrogen bond is at Asn 204, Gln 200 and hydrophobic interactions at Asp 208, Gly 207, Thr 209, Asp 217, Val 191, Ile 218, Asn 204, Phe 205, Cys 189, Gly 190, Phe 320, Thr 220, Tir 192, Gly 190, Phe 205, Asn 211, and Asn 208.

Table 2. Binding energy, hydrogen binding, and hydrophobic interaction of complex Catechins and NPC1L1.

Catechins	Binding Energy (kcal/mol)	Hydrophobic Interaction van der walls	Hydrogen binding
Epigallocatechin Gallate	-7.5	Gln 200, Tyr 192, Trp 202, Cys 189, Gly 207, Asp 217, Gly 207, Asp 217, Gly 190, Phe 205, Asp 208	Thr 219, Ile 218, Asn 204, Asn 211, Arg 201
Catechin Gallate	-7.4	Arg 201, Val 191, Gly 190, Tyr 192, Cys 189, Phe 201, Asn 211, Asn 204, Thr 219, Asp 217, Thr 209, His 221	Gln 200, Phe 220, Gly 207, Ile 218
Gallocatechin Gallate	-7.3	Thr 219, Phe 220, Ile 218, Cys 189, Tyr 192, Val 191, Thr 209, Asn 204 Phe 205, Asn 211, Gly 190, Trp 202, Asp 217, Asp 208	Gly 207, Arg 201
Catechin	-6.7	Asp 217, Asp 208, Val 191, Gly 190, Asn 211, Tyr 192, Cys 189, Trp 192, Cys 189, Trp 202, Phe 205, Asn 204	Arg 201, Thr 219, Ile 218, Gly 207
Epigallocatechin	-6.5	Gly 190, Phe 205, Asp 208, Asn 211, Asn 204, Tyr192, Trp 202, Cys 189, Asp 217, Ile 218, Thr 219, Gly207, Asp 217, Gly 207, Phe 220	Arg 201
Epicatechin	-6.6	Asp 208, Gly 207, Thr 209, Asp 217, Val 191, Ile 218, Asn 204, Phe 205, Cys 189, Gly 190	Tyr 192, Asn 211, Thr 219
Gallocatechin	-6.5	Thr 219, Asp 217, Thr 209, Asn 211, Gly 190, Phe 205, Arg 201, Tyr 192, Cys 189	Asn 204, Ile 218, Gly 207, Phe 220
Ezetimibe	-6.1	Phe 320, Thr 220, Tir 192, Gly 190, Phe 205, Asn 211, Asn 208	Asn 204, Gln 200

The binding energy results of NPC1L1 docking with Catechins were obtained. The order of the binding energy is as follows: EGCG<CG<GCG<C<EGC<EC<GC< Ezetimibe as a positive control. The minimum interaction energy indicates the maximum receptor-ligand interactions (Yu et. al, 2005). Catechins' active compounds all have a low bond energy, indicating the strength of Catechins' binding to target receptors. Previous research suggests that EGCG has the potential to lower cholesterol by inhibiting the HMGCoA reductase enzyme and its binding to LDL receptors. Because EGCG has a low Gibbs free energy, the bond reactions that occur are more spontaneous and form more stable bonds (Adelina & Kurniarti, 2018).

Another parameter used to predict the activity of natural substances is hydrogen bonds. This binding is stronger than others. However, it is weaker than any other covalent or ionic bond. Hydrogen bonds are important in biological systems (Cuff et al., 2006). The molecular structure is one of many factors that influence hydrogen bonds. Catechins contain flavonoids with numerous hydroxyl groups (Kim et al., 2010). Catechins with hydrogen bonds similar to

Azetimibe are EGCG and GC. This interaction's binding site is located at Asn 204. Meanwhile, those with hydrophobic bonds similar to ezetimibe at Asn 211 are GC, EGC, C, GCG, CG. At Gly190 are GC, C, GCG, CG, EGCG. Nonpolar substances have a proclivity to organize hydrophobic interactions by forming aggregates. These aggregates can be dispersed to form micelles in aqueous solutions. A polar carboxyl group will surround the nonpolar. The greater the number of hydrophobic interactions between the ligand and its receptor, the stronger the interaction. Because EGCG from Catechins has the lowest binding energy, it may have the potential to inhibit NPC1L1.

The NPC1L1 sterol transporter is responsible for mediating intestinal cholesterol absorption. NPC1L1 is identified in enterocytes' apical membranes and hepatocytes' canalicular membranes. The other function is hepatobiliary cholesterol excretion that is counterbalanced (Alqahtani et al, 2015). Ezetimibe, a hypercholesterolemic medication, is an NPC1L1 inhibitor. NPC1L1 was discovered through a genomics and bioinformatics approach (Davies, Levy & Ioannou, 2000). The researchers discovered mutations in Niemann-Pick disease type C1, a genetic disorder characterized by lysosomal cholesterol and other lipid accumulation (Mukesh, & Rakesh, 2011). Previous research revealed that NPC1L1 knockout mice and ezetimibe-treated mice have similar reduced intestinal cholesterol absorption, indicating that NPC1L1 is involved in the ezetimibe-sensitive pathway (Janowski et al., 1999).

NPC1L1 inhibition may reduce cholesterol-dependent LXR activation and subsequent LXR-induced hepatic lipogenesis (Weinglass et al., 2008; Pirillo, Catapano & Norata., 2016). Intestinal cholesterol absorption may be necessary for the body to maintain basal LXR activity. According to the results of an in-silico analysis, Catechins have the potential to be an NPC1L1 inhibitor. However, molecular dynamic simulation and in vivo analysis are required to demonstrate a synergistic effect of Catechins as an inhibitor of atherogenesis.

The docking method has a limitation in drug design in that it can only predict protein-ligand interactions statically (Kitchen et al., 2004). More research on molecular dynamic methods is needed to understand how the structure of protein ligands changes over time.

4. CONCLUSION

The conclusion of these studies is Catechins of Green Tea are potential as NPC1L1 inhibitor be based energy bonds, hydrogen bonds and hydrophobic interactions of molecules. The GC>EC>EGC>C>GCG>CG>EGCG has the largest energy binding. Epigallocatechin Gallate (EGCG), the most potent NPC1L1 inhibitor of Catechins, binds to NPC1L1 at numerous active sites including Gln 200, Tyr 192, Trp 202, Cys 189, Gly 207, Asp 217, Gly 190, Phe 205, and Asp 208. EGCG is a potential NPC1L1 inhibitor candidate for preventing atherogenesis.

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