Research

Anti-Atherosclerotic Activity of *Eleutherine americana* Merr. as the Peroxisome Proliferated-Activated Receptor γ Agonist: In Silico Study

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ABSTRACT:

Peroxisome proliferated-activated receptor γ (PPARγ) has central role in Atherosclerosis process. PPARγ that is activated by ligand could inhibit atherosclerosis process through some mechanisms such as the decrease of inflammation, the increase of cholesterol efflux and stabilisation of atheroma plague. There were many research about the use of PPARγ agonist to solve the effect of and the complication of atherosclerosis especially about how to find an effective PPARγ with minimum side effect. One of the PPARγ agonists is *Eleutherine americana* Merr. which is a natural substance. The purpose of the study is to examine the anti-atherosclerotic activity of *Eleutherine americana* Merr. active substance as PPARγ agonist through in silico approach. There were 18 active substances being examined of their anti-atherosclerotic activity. Examination prediction of the anti-atherosclerotic activity was conducted through Way2Drug PASS Online. Specific docking by using Autodock Vina, 3D visualized by PyMOL(TM) 2.3.1 and the visualization of ligand-receptor was done with LigPlot+ V.2.1. The results showed that there were 4 highly potential anti-atherosclerotic substances namely eleutherinoside A, β-Sitosterol, eleuthoside B and eleutherinoside B. From the four substances, eleutherinoside A has the most negative binding affinity towards PPARγ which was -8 kcal/mol, with 8 bonds of hydrogen and was hydrophobic similar to the control substance. In conclusion, through in silico study *Eleutherine americana* Merr. has anti-atherosclerotic activity through the mechanism of PPARγ agonist.

KEYWORDS: Anti-Atherosclerosis, PPARγ Agonist, *Eleutherine americana* Merr., in silico.

INTRODUCTION:

Atherosclerosis is one of the manifestations of metabolic complication which caused cardiovascular disease[1,2]. The occurrence of atherosclerosis is a very complex process, initiated by endothelial dysfunction, monocytes recruitment, maturation and monocytes activation which becomes macrophage, the formation of foam cells, proliferation and smooth muscle cell migration, the formation of atheroma plague as well as the process of inflammation that often occurs during the development of atherogenesis[3-6]. Various strategies have been set in order to inhibit in the atherosclerosis as well as to decrease the cardiovascular complication that it causes, but the strategies did not promise a satisfying result[7,8].

Numerous studies showed that PPARγ hold important role in inhibiting the development of atherosclerosis in all above-mentioned cell[6]. PPARγ is one of the subfamily from the PPARs, the nuclear transcription factors which activated by ligand either endogenous or exogenous, that has anti atherosclerotic activity[9,10]. The increase of ligand that has agonist properties towards PPARγ will stimulate the formation of dimer structure together with retinoid X receptor (RXR), followed by the change of conformation of the receptor forming a complex transcription that are activate the target gen[9,11]. PPARγ activation could inhibit the development of atherosclerosis through many mechanisms such as on endothelium cell which hold the expression of the adhesive molecule and chemokine, on macrophage inhibit the expression of cytokine pro-inflammation and increase reverse cholesterol transport, on smooth muscle cell inhibit the proliferation and cell migration and on extra cellular matrix inhibit the accumulation of MMPs and TIMPs[1]. On macrophage, PPARγ agonist could also slow down the formation of foam cell through the increase of cholesterol efflux by increasing the expression of protein transport i.e. ATP-binding cassette A1 (ABCA1) and ATP-binding cassette G1 (ABCG1) and delay the inflammation process[12-14]. Cholesterol efflux is responsible on 70% of the free cholesterol transport process from cell to the cell membrane actively[15-18].

As the role of PPARγ is very important to inhibit the atherosclerosis, many studies have been conducted in the past few years, especially in the area of the study about the discovery of new medicine for cardiovascular [11,19]. The use of synthetic PPARγ agonist as anti-atherosclerosis such as thiazolidinedione (TZD) has not been clinically satisfying[10], although the results were significant in in-vitro test and on testing animals[9]. This has led researchers to conduct expansive study to get an alternative medicine. It is a common knowledge that one of the reasons why TZD is not very effective as PPARγ agonist is because the TZD agonist is very strong towards PPARγ, so that it causes side effects[20]. Therefore, it triggers other studies to find out other partial PPARγ agonist as anti-atherosclerosis, and one of the ways is through studying natural substance from medicinal plants[5,11,21-23].

Eleutherine americana Merr. also called as *Bawang Dayak* in Indonesian language is very well-known for traditional medicine in East Kalimantan, Indonesia. Some of the medicinal properties that *Eleutherine americana* Merr. could offer is that it could be antipyretics, coping with intestinal disorder, anti-diabetic as well as cardiovascular disorder therapy[23,24]. At the moment *Eleutherine americana* Merr. has been identified containing active substances with the properties as anti-oxidant[25-27], anti-inflammation[23,24], anti-bacteria[28,29], anti-diabetic[30] and anti-cancer[31,32]. There has not any single study examining the potential of *Eleutherine americana* Merr. as anti-atherosclerosis. The aim of the study was to examine the potential of anti-atherosclerotic activity from *Eleutherine americana* Merr. active substances as PPARγ agonist through in silico approach.

MATERIALS AND METHODS:

Materials:

The materials which were used in the examination process were 18 active substances of *Eleutherine americana* Merr. they were eleutherol, eleutherin, isoeleutherin, elecanacin, isoeleutherol, eleutheriol,1,5- dihydroxy-3-methyl anthraquinone, dihydroeleutherinol, eleuthoside A, eleuthoside B, eleucanarol, eleutherinoside A, eleutherinoside B, 1,3,6-trihydroxy-8-methyl-anthraquinone, β-sitosterol, kadsuric acid, 2-acetyl-3,6,8-trihydroxy-1-methyl Anthraquinone and eleuthoside C.

Instruments:

All computation work were performed using Acer Vivabook A442U with Windows 7, Intel Core i5, ip tp 3,4 GHz, 64 bit operation system, 1TB HDD, and 4,00 GB RAM. The software were used to analize are PyMol (TM) 2.3.1, PyRx V.9.5 and LigPlot+ V.2.1. Software.

Methods:
The method used for the examination of the anti-atherosclerotic activity of *Eleutherine Americana* Merr. active substances was by using Way2Drug PASS Online (http://www.pharmaexpert.ru/passonline). From the abovementioned active substances, the canonical SMILE was examined through a website at https://pubchem.ncbi.nlm.nih.gov/. Active substances can only be examined in the Way2Drug PASS Online software if the substances have canonical SMILE. After getting the results of prediction of the active substances activity, shown by Pa (probability to be active) value, the average value of Pa was then calculated for their anti-atherosclerotic activity by analysing the activities that are related to the mechanism of anti-atherosclerosis. If the Pa value is > 0.7 it means that the substance has high potential both in computation and in laboratory test. If the Pa value is >0.3 but <0.7 it means that the substance may have the potential in computation but may not have good results in laboratory test. Then, the results that showed the anti-atherosclerosis examination through PASS online were examined by using molecular docking.

Ligands preparation:
The ligands were chosen based on the results from Way2Drug PASS Online of *Eleutherine americana* Merr. There were 4 ligands namely eleuthoside B (Pubchem ID 95224384), eleutherinoside A (Pubchem ID 101855662), eleutherinoside B (Pubchem ID 101855663), β-sitosterol (Pubchem ID 222284) and PPARγ with co-agonist (2S)-3-(1-{[2-(2-chlorophenyl)-5-methyl-1,3-oxazol-4-yl]methyl}-1h-indol-5-Yl)-2-ethoxypropanoic acid (PDB ID 2GTK chain A) as the control[33]. PPARγ agonists used as the control were the PPARα/γ co-agonist, they were modified tesaglitazar PPARα/γ ligand agonist with amino acid residual with the purpose in order to get the agonist properties that is better than tesaglitazar (Kuhn et al, 2006). The 3D view of ligands was saved with ".sdf" format.

Protein preparation:
The 3D structure of PPARγ nuclear receptor was downloaded from the Protein Data Bank (PDB) through http://www.rscb.org. Pymol was used to clean the unnecessary molecules.

Docking:
Docking was specifically conducted by using Autodock Vina program of PyRx 9.5. The visualization result of the docking was created by using PyMOL(TM) 2.3.1[34-36] and the interaction between ligand and receptor was visualized by using LigPlot+ V.2.1. Software.

RESULTS:
By using Way2Drug PASS Online software, the examination of the anti-atherosclerosis related activity was conducted. The results showed anti-atherosclerotic activity of *Eleutherine americana* Merr. as presented in the following figure.

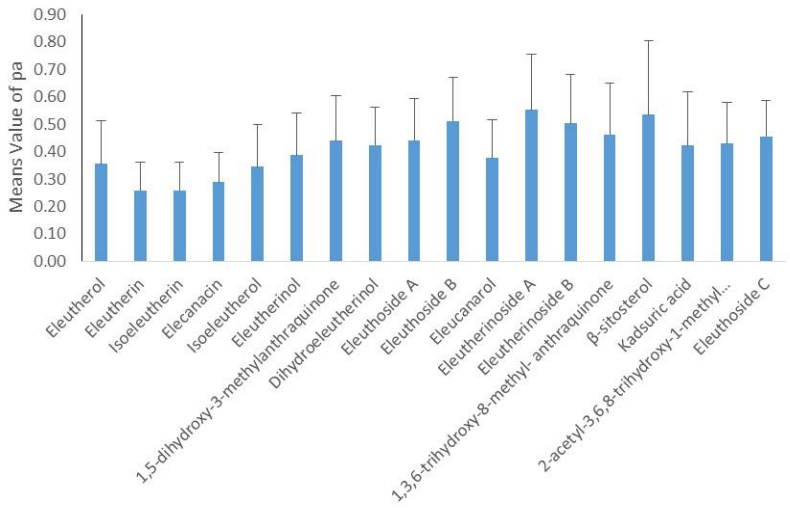


Fig. 1: The prediction of *Eleutherine americana* Merr. anti-atherosclerotic active substances activity by using PASS Online.

Table 1: The results of anti-atherosclerosis analysis from Eleutherine *americana* Merr. active substances.

Compounds	CID	SMILE	ACTIFITY	AVERAGE of PA (PASS Online)
Eleutherol	120697	CC1C2=C(C=C3C=CC=C(C3=C2O)OC)C(=O)O1	Antioxidant, Free radical scavenger, Superoxide dismutase inhibitor, Peroxidase inhibitor, Glutathione peroxidase inhibitor, Lipoprotein lipase inhibitor, TNF expression inhibitor, Antiinflammatory, Atherosclerosis treatment, Cholesterol antagonist, Antihypercholesterolemic, APOA1 expression enhancer, Cholesterol synthesis inhibitor, ICAM1 expression inhibitor, Lipid metabolism regulator, Lipid peroxidase inhibitor, Macrophage colony stimulating factor agonist, MMP9 expression inhibitor, NADPH peroxidase inhibitor, Oxygen scavenger, VCAM1 expression inhibitor	0.36
Eleutherin	10166	CC1CC2=C(C(O1)C)C(=O)C3=C(C2=O)C=CC=C3OC		0.26
Isoeleutherin	10445924	CC1CC2=C(C(O1)C)C(=O)C3=C(C2=O)C=CC=C3OC		0.26
Elecanacin	5491405	CC1CC23C(CC2O1)C(=O)C4=C(C3=O)C=CC=C4OC		0.29
Isoeleutherol	10800314	CC1C2=C(C=C3C=CC=C(C3=C2O)OC)C(=O)O1		0.34
Eleutherinol	136623083	CC1=CC2=CC(=O)C=C(C2=C3C1=C(C=C(O3)C)O)O		0.39
1,5-dihydroxy-3-methylanthraquinone	5316800	CC1=CC(=C2C(=C1)C(=O)C3=C(C2=O)C=CC=C3O)O		0.44
Dihydroeleutherinol	102473740	CC1CC(=O)C2=C(C=C3C=C(C=C(C3=C2O1)O)O)C		0.42
Eleuthoside A	101709341	CC1C2=C(C=C3C=CC=C(C3=C2OC4C(C(C(C(O4)CO)O)O)O)OC)C(=O)O1		0.44
Eleuthoside B	95224384	COC1=CC(=CC(=C1OC2C(C(C(C(O2)CO)O)O)O)OC)C=CCO		0.51
Eleucanarol	120697	CC1C2=C(C=C3C=CC=C(C3=C2O)OC)C(=O)O1		0.38
Eleutherinoside A	101855662	CC1=CC(=O)C2=C(C=C3C=C(C=C(C3=C2O1)O)OC4C(C(C(C(O4)CO)O)O)O)C		0.55
Eleutherinoside B	101855663	CC1C2=C(C3=C(C=CC=C3OC)C(=C2C(=C(O1)C)O)OC4C(C(C(C(O4)CO)OC5C(C(C(C(O5)CO)O)O)O)O)O)O		0.50
1,3,6-trihydroxy-8-methyl-anthraquinone	12309204	CC1=C2C(=CC(=C1)O)C(=O)C3=CC(=CC(=C3C2=O)O)O		0.46
β-sitosterol	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C		0.54
Kadsuric acid	5384417	CC(CCC=C(C)C(=O)O)C1CCC2(C1(CC=C3C2CCC(C3(C)CCC(=O)O)C(=C)C)C)C		0.42
2-acetyl-3,6,8-trihydroxy-1-methyl Anthraquinone	86224715	CC1=C2C(=CC(=C1C(=O)C)O)C(=O)C3=CC(=CC(=C3C2=O)O)O		0.43
Eleuthoside C	10722258	CC1CC2=C(C3=C(C(=CC=C3)OC)C(=C2C(O1)C)O)OC4C(C(C(C(O4)COC5C(C(C(C(O5)CO)O)O)O)O)O)O		0.46

Table 1 and Figure 1 showed that the active substances from *Eleutherine americana* Merr. which have the potential as anti-atherosclerosis from the biggest to the smallest are Eleutherinoside A, β-Sitosterol, Eleuthoside B and Eleutherinoside B with Pa values are 0.55, 0.54, 0.51 dan 0.50 respectively. The value was within the range of $0.3 > Pa < 0.7$. This means that the active substances may have the activity on anti-atherosclerosis when computed but may not have the anti-atherosclerotic activity on the laboratory experiment.

Docking was conducted to investigate the binding affinity of the receptor protein towards the control agonist and top 4 anti-atherosclerotic substances of the *Eleutherine americana* Merr. based on PASS Online. Docking was specifically conducted by using Autodock Vina program on PyRx V.9.5 Software. Binding site which was utilized in the current study was imitating the placement of the control agonist. The more negative of the binding affinity value between the ligand and the receptor interaction is to be stronger.

Table 2 shows that the binding affinity from 3 out of 4 active substances which were examined had strong value although the value was still under the PPARγ control. These substances were β-sitosterol, Eleuthoside B and Eleutherinoside A, which the Eleutherinoside A binding affinity was highest (-8 kcal/mol). The visualization of the docking result by using PyMOL(TM) 2.3.1 and ligand interaction visualized by using LigPlot+ V.2.1 software (Fig. 2).

Table 2: The result of Binding Afinity analysis of 4 active substance of *Eleutherine americana* Merr. towards PPARγ

	Binding Affinity (kcal/mol)
β-sitosterol	-7.7
Eleutherinoside B	-5.7
Eleutherinoside A	-8
Eleuthoside B	-7.8
Control	-9.7

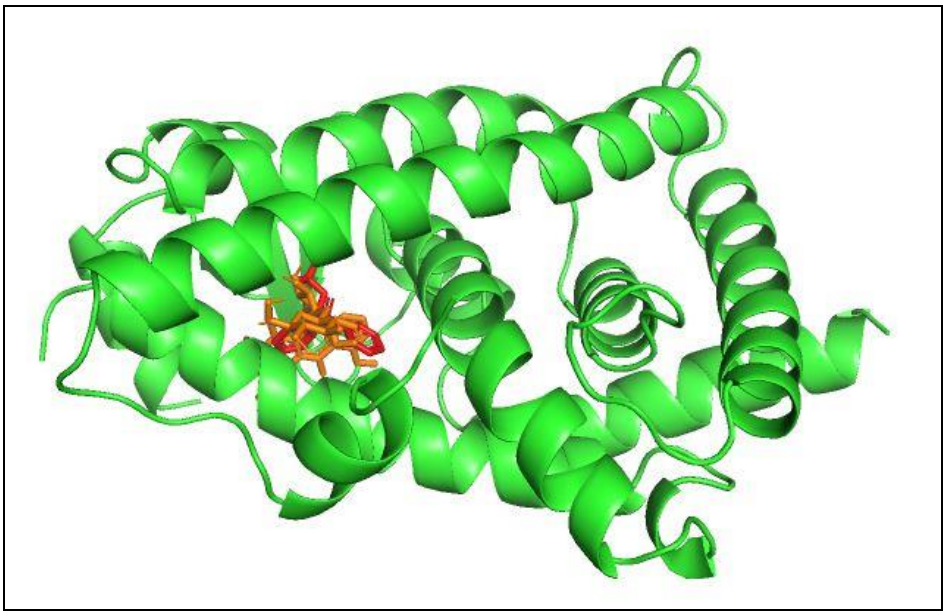
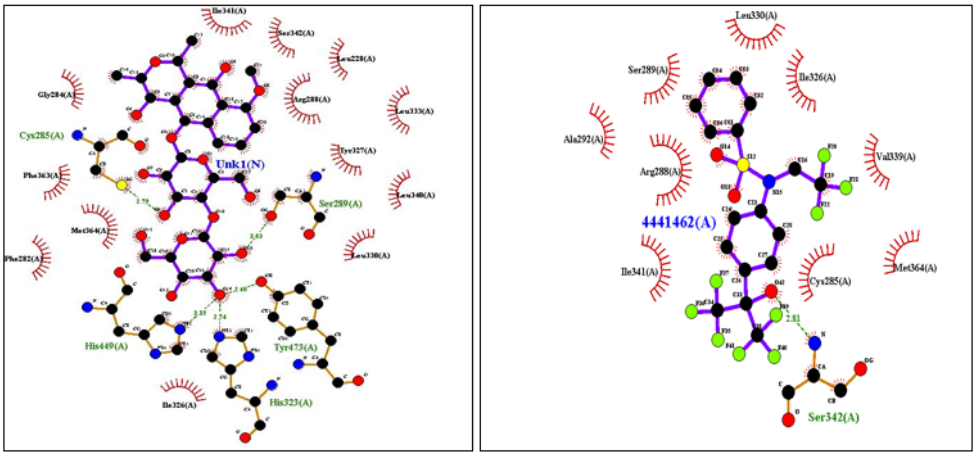


Fig. 2: The docking visualization Eleutherinoside A towards PPARγ (orange color is Eleutherinoside A and the red color is the control agonist).



A. Eleutherinoside A and PPARγ **B. Control and PPARγ**

Fig. 3: The interaction between ligands and PPARγ receptor. A) Eleutherinoside A- PPARγ, B) control- PPARγ. The green letter is the hydrogen bonds the black color is the hydrophobic bonds, and the blue is the name of the ligand.

Table 3 and fig. 3 showed that there were hydrogen bond and hydrophobic on some amino acid residual (bold) where Eleutherinoside A and PPARγ interacted similar to control.

Table 3: The comparison of amino acid where the interaction between Control - PPAR γ and Eleutherinoside A - PPAR γ occurs.

	Hydrogen bonds	**Hydrophobic bonds**
Control - PPAR γ	Ser342	Cys285,Met364, Val339, Ile326, Leu330, Ser289, Ala292, Arg288, Ile341
Eleutherinoside A - PPAR γ	**Ser289**, Tyr473, His323, His449, **Cys285**	**Ile341**, **Ser342**, Leu228, Leu333, **Arg288**, Tyr327, Leu340, **Leu330**, **Ile326**, Phe282, **Met364**, Phe363, Gly284

DISCUSSION:

The purpose of the research was to investigate the potential of anti-atherosclerosis activity of *Eleutherine americana* Merr. active substances through in silico approach. Out of 18 active substances that were investigated by using PASS Online for predicting anti-atherosclerosis activity, 15 active substances have the potential as anti-atherosclerosis with average value Pa >0.3. Four substances have the highest value with Pa >0.5; they are Eleutherinoside A, β-Sitosterol, Eleuthoside B and Eleutherinoside B (fig. 1). This finding can be used as the basis for investigating the effect of the substances in laboratory since they have bigger chances for laboratory experiment of anti-atherosclerosis.

If we take a look at the details of the Pa value of each biological activity from PASS Online results (data is not presented), the strongest anti-atherosclerosis activity of Eleutherinoside A was as free radical scavenger (Pa 0.9) and lipid peroxidase inhibitor (Pa 0.8). The laboratory study found that Eleutherinoside A could be an option as anti-diabetic since it has the property as α-glucosidase inhibitor in rats[29].

Molecular docking showed that there were 8 points of interaction of Eleutherinoside A which were similar to the points of interaction of the PPARγ control. They were hydrogen bonds at 2 amino acid ser289 and Cys285, and hydrophobic bonds at 6 amino acid that is Ile341, Ser342, Arg288, Leu330, Ile326 and Met364 (table 3). Many similarities of the amino acid residual between Eleutherinoside A and PPARγ control (8 out of 10 similar amino acids) that interacts on the active sites of PPARγ receptor, showed that the interaction between Eleutherinoside A and PPARγ was strong. It also indicates that there was similarity of the agonist properties between Eleutherinoside A and control towards PPARγ.

In this study, the control used was synthetic PPARγ agonist with modification at some clusters which was expected to generate more partial response of PPARγ agonist, different from TZD which has full agonist properties[20]. In addition, PPARγ from natural substance has more benefits as its property was weaker, similar to endogen ligand PPARγ[11]. Therefore this study can be a reference for investigating PPARγ, specifically for finding anti-atherosclerosis medicinal plants. A study on laboratory examination of *Eleutherine americana* Merr. anti-atherosclerosis was necessary to be conducted either with in vitro or in vivo approach.

CONCLUSION:

Based on the results of the study, it can be concluded that *Eleutherine americana* Merr. is proven to have anti-atherosclerosis activity, through Eleutherinoside A as PPARγ agonist.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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