Potential of Purple Corn Anthocyanin Extract as A Hypolipidemic Agent: An In-Silico Analysis

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Abstract. Hyperlipidemia remains an important risk factor for cardiovascular diseases, including myocardial infarction and stroke. Some standard hypolipidemic drugs available in the market still do not meet expectations in lowering lipid levels. Investigation of alternative hypolipidemic agents continues to be carried out, including using purple corn plants with high-anthocyanin content. Plant anthocyanins have been shown to play an important role in lowering blood lipid levels. Here, we explored the potential of anthocyanin compounds in purple corn as a lipid-lowering agent through various mechanisms of action using an in-silico analysis. A total of fourteen proteins known to play a role in modulating blood lipid levels were set as target in molecular docking. Three active compounds from purple corn anthocyanin extract namely cyanidin-3-glucoside, cyanidin chloride, and peonidin-3-glucoside were used as ligands. The docking methods used the Protein-Ligand ANT System (PLANTS) to determine the binding affinities of those ligands to all target proteins and visualization of docking results displayed using Discovery Studio. Molecular docking of the three ligands with target proteins showed that purple corn anthocyanin extracts bound effectively to the active site of AMPK (adenosine monophosphate-activated protein kinase), ATP (adenosine triphosphate) citrate lyase, FABP4 (fatty acid-binding protein), glucokinase, and proprotein convertase subtilisin/kexin type 9 (PCSK9). Testing ligands c3g and p3g showed a better potency than cya-ch in lowering lipid serum level. In-silico analysis showed that purple corn anthocyanin extract has potential as a hypolipidemic agent, and it should be confirmed by a wet lab setup.

Keywords: Purple Corn Anthocyanin Extract · Hypolipidemic Agent · Mechanism Of Action · In-Silico Study

1 Introduction

The prevalence of cardiovascular disease keeps on increasing throughout the year, which atherosclerosis as one of the main causes. An important modifiable factor in atherosclerosis is dyslipidemia, thus controlling serum lipid levels become a target in reducing atherosclerosis and cardiovascular disease[1]. Although reducing LDL cholesterol levels using statins reduces the risk of cardiovascular disease by 15–37% [2], but various efforts are ongoing in developing new hypolipidemic agents, including from plants [3].

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For many years, plants have become part of herbal medicine for the management of many disorders, including dyslipidemia. The International Lipid Expert Panel (ILEP) states that nutritional therapy containing part of plants, either as herbal monomers or their derivatives, is proven to be safe and effective [4]. In the context of drug discovery, testing the activity and mechanism of action of drug candidates through an in-silico study, has become an important and inseparable part. In-silico techniques are commonly used in the early phase of drug discovery process, and are able to predict the binding activity of drug candidates to certain receptors, also optimizing their pharmacokinetic profile [5]. One of the methods in the in-silico study is molecular docking, as a part of the structure-based drug design. This method allows identification of the predicted binding activity between a drug candidate (ligand) and its receptor (can be an enzyme, protein, hormone, or other biological structures) [6]. Thus, we are capable to predict whether a drug candidate will act on which receptors.

Some receptors are known to play a role in controlling serum lipid levels. Among them are β-hydroxy β-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), peroxisome proliferator-activated receptor (PPAR), PPAR, PPAR, squalene synthase (SqS), AMP-activated protein kinase (AMPK), ATP citrate lyase, diacylglycerol O-acyltransferase (DGAT), fatty acid binding protein-4 (FABP4), glucokinase, 11-beta-hydroxysteroid dehydrogenase (11--HSD), farnesoid X receptor (FXR), proprotein convertase subtilisin/kexin type 9 (PCSK9), dan liver X receptor (LXR).

A previous study showed that corn kernels have various pharmacological activities, including preventing cardiovascular disease, improving type 2 diabetes mellitus, overcoming overweight and obesity, and improving various digestive tract disorders [7]. However, the effect of purple corn anthocyanin extract containing cyanidin-3-glucoside (c3g), peonidin-3-glucoside (p3g), and cyanidin chloride (cya-ch) in improving dyslipidemia has not been widely studied. Therefore, this study was conducted to explore the potential of purple corn anthocyanin extract as a hypolipidemic agent, through an in-silico study.

2 Method

2.1 Study Design

This was a non-laboratory experimental study, using computational methods. All computational program were run on a computer with specifications: Processor Intel® Pentium® CPU 2117U @ 1.80GHz, RAM 4.00 GB, Harddisk drive 500 GB and operating system Windows 10.

2.2 Protein and Ligan Preparations

Proteins as target receptors in molecular docking study were listed in the Introduction section. The three-dimensional (3D) structure of proteins and their native ligands were downloaded form the Research Collaboratory for Structural Bioinformatics (RCSB) (www.rcsb.org). Separation of native ligands from protein molecular complexes was carried out using YASARA software (www.yasara.org). The preparation of the receptor

molecule for the docking simulation was done by removing residues that were non needed in the docking program, removing water molecules, and adding hydrogen atoms to the remaining residues. Ligand preparation was carried out using Marvinsketch software series 16.5.2 (https://chemaxon.com/products/marvin), by protonating native ligands, generating conformations, and storing them as copy ligand [8].

The active compounds in purple corn anthocyanin extract used as testing ligands, namely cyanidin-3-glucoside (c3g), peonidin-3-glucoside (p3g), and cyanidin chloride (cya-ch). The two-dimensional (2D) structures of the three testing ligands were downloaded from https://pubchem.ncbi.nlm.nih.gov. The testing ligands preparation was carried out in the same procedure as the previous copy ligands.

2.3 Simulation and Validation of Docking Process

The docking process was performed according to the method as described previously [8]. Briefly, the docking simulation carried out using PLANTS software, with the binding site set at a distance of 5Å from the coordinate location of the ligand binding site on the receptor. The results of docking copy ligands were validated using YASARA. Receptors that meet the requirements for molecular docking are those with the root mean square deviation (RMSD) value of < 3.0Å, when ligand copy redocked against its receptor (self-docked). The RMSD value < 2.0Å indicates that the position of the copy ligand to its native ligand is very close and appropriate (good); a value between > 2.0Å and < 3.0Å indicates that the copy ligand position is deviated compared to its native ligand, but is still in the same orientation (acceptable); whereas if > 3.0Å it means that the copy ligand position is very different from its native ligand, so it does not meet the requirement as a docking target (poor) [9].

The docking simulation using PLANTS results a scoring function, which is the minimum binding energy between the ligand and its receptor. The active binding site was visualized using the Discovery Studio series 21.1.0.20298 (https://discover.3ds.com/discovery-studio-visualizer-download), to display the presence of various types of bonds between ligands and amino acid residues on the target receptor.

2.4 Analysis

The results of docking simulation were analyzed descriptively, by comparing the binding energies between native ligand and testing ligands (c3g, p3g, and cya-ch). The hydrogen bonds position of testing ligands and amino acid residues of receptors also compared with its positions in native ligand.

3 Result and Discussion

The interaction between the ligands and their target receptors was indicated by the magnitude of the binding energy generated from the docking simulation. The binding energies of the testing ligands were compared with the native ligands to see the interaction ability. Of the fourteen proteins as hypolipidemic target, the three testing ligands contained in

Table 1. Ligand-receptor bond energies between native ligand and testing ligands against proteins

No.	Receptors	PDB ID	Binding energies (kcal/mol)						
			Native ligand	c3g		p3g		cya-ch	
1.	HMG-CoA reductase	1HWI	-103.53	-90.19	87%	-86.36	83%	-72.47	70%
2.	PPAR α	3G8I	-119.49	-98.9	83%	-101	85%	-76.31	64%
3.	PPAR δ	2ZNQ	-123.04	-81.04	66%	-81.79	66%	-79	64%
4.	PPAR γ	4EMA	-100.71	-86.83	86%	-88.12	87%	-76.26	76%
5.	SqS	3VJC	-176.38	-100.8	57%	-95.44	54%	-79.54	45%
6.	AMPK	4CFF	-85.68	-83.72	98%	-82.59	96%	-73.45	86%
7.	ATP citrate lyase	6O0H	-106.52	-99.77	94%	-90.05	85%	-85.1	80%
8.	DGAT	4CK0	-140.85	-96.86	69%	-98.54	70%	-79.82	57%
9.	FABP4	4NNS	-80.29	-88.94	111%	-88.37	110%	-71.15	89%
10.	Glucokinase	4DHY	-86.6	-91.22	105%	-85.91	99%	-80.87	93%
11.	11β-HSD	3HFG	-111.2	-86.69	78%	-85.91	77%	-76.16	68%
12.	FXR	7D42	-118.22	-83.34	70%	-79.93	68%	-80.54	68%
13.	PCSK9	6U38	-80.68	-105	130%	-106.5	132%	-84.1	104%
14.	LXR	5KYJ	-112.44	-83.59	74%	-74.62	66%	-82	73%

the purple corn anthocyanin extract provided binding energies with their receptors as listed in Table 1.

Table 1 showed that the best binding energies between the ligand-receptor of the testing ligands achieved by c3g and p3g, while cya-ch had the lowest binding energy compared to the native ligand. The good performance of docking results showed by five proteins, namely 4CFF (AMPK), 6O0H (ATP citrate lyase), 4NNS (FABP4), 4DHY (glucokinase), and 6U38 (PCSK9), as displayed by small differences in binding energy between native ligand and testing ligands. These findings suggested possible mechanism of action of the active compounds in purple corn anthocyanin extract as a hypolipidemic agent. Visualization of various bonds between native ligand and testing ligands to the AMPK receptor (PDB ID: 4CFF) using the Discovery Studio software displayed in Fig. 1.

The native ligand of AMPK protein receptor was located on the active site surrounded by amino acid residues as shown in Fig. 2 (a), while position of the testing ligands showed in Fig. 2 (b), (c), and (d). With the similar procedures in the visualization of the AMPK receptor, position of native and testing ligands on the active site of ATP citrate lyase (PDB ID: 6O0H), FABP4 (PDB ID: 4NNS), glucokinase (PDB ID: 4DHY), dan PCSK9 (PDB ID: 6U38) were carried out (docking visualization are not displayed). The amino acid residues on the active site of each protein and the number of hydrogen bonds were displayed in Table 2 and Table 3.

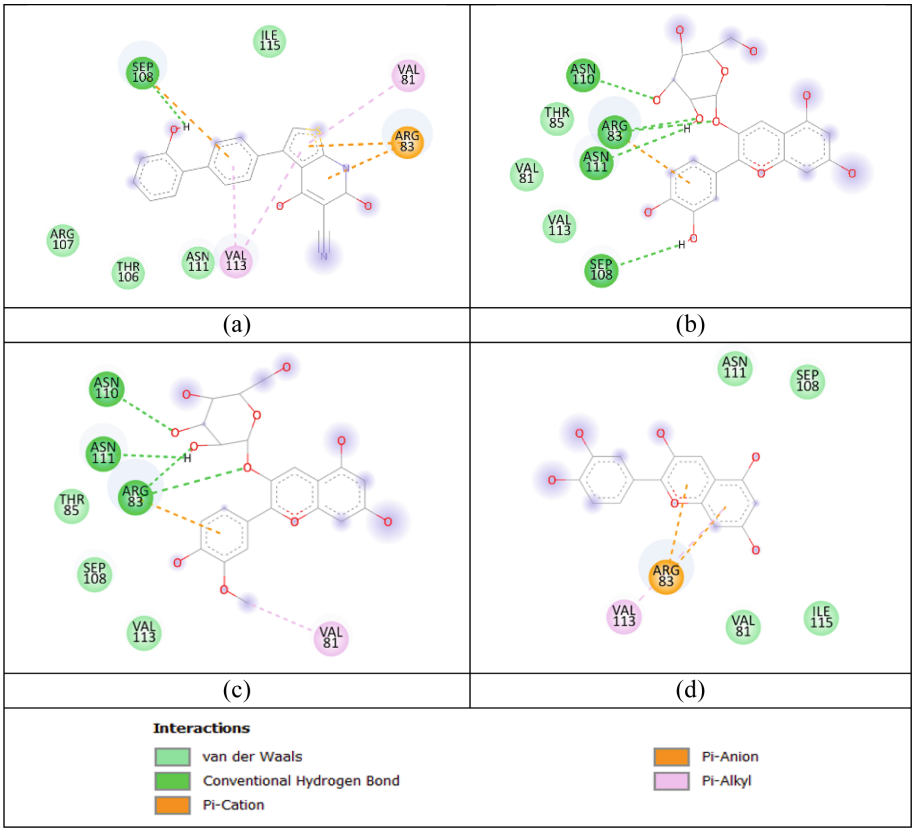

Fig. 1. Visualization of docking results at AMPK receptor (PDB ID: 4CFF).

Table 2. Amino acid residues on the active site of each receptor

No.	Receptor	PDB ID	Amino acid residues
1.	AMPK	4CFF	Sep108 (serin phosphorylated108), Ile115, Val81, Arg83, Val113, Asn111, Thr106, dan Arg107
2.	ATP citrate lyase	6O0H	Arg379, Arg378, Gly342, Gly380, Thr353, Phe347, Phe354, Val 350, Pro382, Ile357, Thr316, Met278, Tyr307, Ala280, Ile340, Gly341, dan Val377
3.	FABP4	4NNS	Phe16, Phe57, Ala36, Lys58, Arg126, Ser53, Val115, Cys117, Ile104, Arg106, Tyr19, Val25, Met20, Arg78, Asp76, Ala33, dan Pro38
4.	Glucokinase	4DHY	Ala456, Val452, Thr65, Tyr214, Met210, Met235, Ile211, Tyr215, Val91, Ser69, Leu451, Ala454, Arg63, Tyr61, Val62, Val 455, dan Ile159
5.	PCSK9	6U38	Ala330, Val333, Ile334, Thr335, Ala328, Cys358, Arg357, Pro331, Trp441, Arg476, Val460, Ala478, Arg458, Arg412, dan Asp360

Table 3. The number of hydrogen bonds formed in each receptor

No.	Receptor	PDB ID	The number of hydrogen bonds			
			Native ligand	c3g	p3g	cya-ch
1.	AMPK	4CFF	1	5	4	0
2.	ATP citrate lyase	6O0H	3	5	4	4
3.	FABP4	4NNS	0	6	5	2
4.	Glucokinase	4DHY	2	6	5	5
5.	PCSK9	6U38	5	11	7	4

4 Discussion

This in-silico study investigated the potential of purple corn anthocyanin extract as a hypolipidemic agent. The three active compounds in purple corn anthocyanin extract, namely c3g, p3g, and cya-ch, showed potential hypolipidemic activity against several types of proteins involved in lipid metabolism. These were indicated by a less negative binding energy than their native ligands. The smaller binding energy value between testing ligands and proteins, the better the binding affinity and the more stable the interaction between complexes [10]. The position of testing ligands is indicated by the amino acid residues surrounding the ligands, while the ligand affinity to its receptor can be seen from the interaction between the ligands (native and testing ligands) with the amino acid residues that are responsible for the catalytic side of each protein [11].

Of the fourteen receptors studied, it appeared that the binding energy of testing ligands closest to the native ligand of five receptors, namely AMPK, ATP citrate lyase, FABP4, glucokinase, and PCSK9. This suggested that, computationally, testing ligands potential as a hypolipidemic agent through those mechanism of actions. The closer value of testing ligands to native ligand indicating that testing ligands require a lower energy to bind to the receptor, also describing the magnitude of the ligand's affinity for the receptor [12]. The more similar the position of amino acid residues between the native ligands and testing ligands, the more likely the mechanism of action of a ligand [13].

Against the AMPK receptor, the three testing ligands positioned in the same active site as its native ligand. The active compound c3g and p3g even provided more hydrogen bonds to their target than the native ligand. The active site of AMPK protein was surrounded by Arg83, Val81, Val113, and Asn111 residues. This finding was in line with a previous study, as indicated that salvianolic acid that is known as an AMPK activator, formed hydrogen bonds at Arg83, Val81, Val113, and Asn111. Salvianolic acid also formed electrostatic bonds at Lys29, Lys31, and Arg83 residues [14]. This showed the potential of c3g and p3g to act as AMPK activators, as is salvianolic acid. AMPK functions as an energy sensor and a major mediator in lipid metabolism in skeletal muscle. AMPK concentration is inversely proportional to lipid content in skeletal muscle thus, AMPK is a negative regulator of lipid accumulation in skeletal muscle. This has implications for AMPK inhibition which will cause an increase in lipid levels, while AMPK activation will decrease muscle lipid content [15].

The docking results of native ligand of ATP citrate lyase (PDB ID: 6O0H) indicated that the active site of ATP citrate lyase receptor was characterized by amino acid residues Gly341 and Gly342, also surrounded by amino acid residues from Ser 343 to Thr-348 [16]. The three testing ligands formed hydrogen bonds in several amino acid residues including Asn346, Thr348, and Asn349. These three amino acid residues are important in ATP citrate lyase inhibitory activity in lipid metabolism [17]. ATP citrate lyase is a key enzyme in fatty acid and cholesterol synthesis because it produces acetyl CoA, thus it is often a target for reducing serum lipid levels [18]. Mitochondrial citrate is converted to acetyl CoA and oxaloacetate in the cytosol. Acetyl CoA is the main ingredient for the synthesis of fatty acids, cholesterol, and non-histone protein acetylation [19].

A previous study has shown that Phe19, Met20, Ala33, Pro38, Lys58, Phe57, Ala75, Glu72, Arg106, and Arg126 are important amino acid residues in the interaction process between FABP4 and its inhibitors [11]. The presence of mutations in Phe57 residue shows a decrease in the binding function of the FABP4 protein to fatty acids, indicating the important role of this residue in FABP4 function. Likewise, substitution of amino acid residues Ile104, Val115, and Cys117 also causes a decrease in the affinity of FABP4 protein for fatty acid [20]. FABP4 is a cytokine secreted by adipose tissue and plays a major role in the incidence of obesity. Serum FABP4 levels are positively correlated with body fat percentage, as well as with metabolic risk factors including obesity, dyslipidemia, and markers for heart failure, such as N-terminal pro Brain-Natriuretic Peptide (NT-pro BNP) [21].

The active sites of glucokinase enzyme consists of residues that are polar (Arg63, Thr65, Glu67 and Lys459) dan hydrophobic (Tyr61, Val62, Pro66, Trp99, Met210, Ile211, Tyr214, Tyr215, Val452, Val455 and Met462). Arg63 which forms hydrogen bonds with native ligand is an important feature in the docking interactions of various types of glucokinase activators [22]. The interaction of Arg63 with ligands is also a key in the induction of allosteric charge, which will trigger glucokinase activation and result in glucose phosphorylation in the catalytic site of this enzyme [23]. All the testing ligands formed hydrogen bonds with glucokinase, even in significantly higher amounts than their native ligands. This showed the potential of c3g, p3g, and cya-ch in binding to glucokinase, which plays a role in glucose and lipid metabolism. Hepatic glucokinase catalyses the phosphorylation of glucose-6-phosphate, which is an important step in hepatic glucose synthesis, as well as for the induction of glycolysis and lipogenic genes. Glucose metabolism via hepatic glucokinase is required for the transcriptional effect of sterol regulatory element binding protein 1c (SREBP1c), a protein that plays an important role in the expression of lipolytic genes [24].

The native ligand of PCSK9 protein was located in the active site of the enzyme, surrounded by amino acid residues Ala330, Val333, Ile334, Thr335, Ala328, Cys358, Arg357, Pro331, Trp441, Arg476, Val460, Ala478, Arg458, Arg412, and Asp360. Hydrogen bonds between native ligand and the receptor formed at the amino acid Pro331, Arg357, Cys358, and Arg458, while all testing ligands formed the much higher amount of hydrogen bonds in the same residues. This finding indicated a possible good interaction between testing ligands and the PSKC9 target protein. In the PSKC9 protein structure, the amino acid residues on the active site were Pro331, Arg357, Cys358, Asp360, Arg458, dan Arg476 [10]. Other study showed that Pro331, Glu332, Arg357, Asp360,

Arg412, Arg458, Trp461 residues were the active sites for the binding of atorvastatin to PSKC9 protein [25]. This raises the hypothesis, that the potential of hypolipidemic effect of purple corn anthocyanin extract may be similar to atorvastatin, but this needs to be elaborated further.

Overall, this study has demonstrated the potential of purple corn anthocyanin extract as a hypolipidemic agent, computationally. The proposed mechanism of action was probably through the interaction of the active compound in this extract (c3g, p3g, and cya-ch) with receptors of AMPK, ATP citrate lyase, FABP4, glucokinase, and PCKS9. However, the limitation of study was not predicting pharmacokinetic parameters including absorption, distribution, metabolism, and excretion, as a part of the initial step in testing drug candidates. In addition, further investigation should be performed involving laboratory testing.

5 Conclusion

The docking simulation of purple corn anthocyanin extract showed the potential of c3g, p3g, and cya-ch as hypolipidemic agents. Of the fourteen protein target tested, the lowest binding energy achieved in the docking of testing ligands to the receptors of AMPK, ATP citrate lyase, FABP4, glucokinase, and PCSK9. The visualization of docking results also placed testing ligands on the amino acid residues as active sites of each target receptors. These findings indicate that active compounds contained in purple corn anthocyanin extract probably act as hypolipidemic agents through those mechanisms.

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Author's Contribution. IM planned the study design, analyzed and interpreted the findings, wrote and organized the manuscript. SN contributed to the docking simulation, interpretation of findings, and the manuscript review.

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