The cyanidin-3-O-glucoside of Black Rice inhibits the interaction of HMG-CoA and HMG-CoA Reductase: three- and two-dimension structure

Fatchiyah Fatchiyah¹²*, Hazna Noor Meidinna¹², Eko Suyanto¹²
¹Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia
²Research Center of Smart Molecule of Natural Genetics Resource, Brawijaya University, Indonesia

*Corresponding author email: fatchiya@ub.ac.id

Abstract. The hypercholesterolemia condition is one metabolic disorder has affected to onset and prevalence of the dyslipidemia and cardiovascular disease. The cholesterol synthesized from HMG-CoA by co-enzyme HMG-CoA reductase. The research focus was to determine the effect of Cyanidin-O-glucose of black rice to prevent the HMG-CoA and HMG-CoA reductase interaction. The anthocyanins retrieved from the database of PubChem, is cyanidin-3-O-glucoside (CID: 12303221). We obtained HMG-CoA canonical SMILES (CID: 445127) from PubChem and reconstructed it using VEGAZZ software. RCSB Protein Data Bank (PDB) was used as a database to get the 3D structures of HMGR (PDB ID: 1DQ9). The water molecules and or previously existing ligands incorporated in their 3D structures were removed using Discovery Studio 2019. The docking results were visualized and analyzed by Discovery Studio, and Hex 8.0.0 software. This result determined that cyaniding-O-glucose of black rice prevented the interaction between HMG-CoA into HMG-CoA reductase respectively. This result indicates that the cyaniding-O-glucose of black rice has potential function as lowering cholesterol biosynthesis.

1. Introduction
Dyslipidemia is a condition plasma lipid abnormality that may play a major role in the pathogenesis of atherosclerosis in the walls of blood vessels, which is the cause of coronary heart disease (CHD) and stroke [1]. According to Global Health Observatory (GHO) data from the World Health Organization (WHO), which shows the prevalence of dyslipidemia in 2008 was 37% in the male population and 40% in the female population and was thought to be responsible for 2.6 million deaths and causes. The data results of Indonesia national basic health research reported that 15.9% of the population aged ≥15 years have a very high proportion of LDL (≥190mg/dl), 22.9% HDL levels less than 40 mg / dl, and 11.9% with very high triglyceride levels (≥500mg/dl) [2, 3].

Hypercholesterolemia is common disorder status with overabundance the amount of cholesterol in the blood. There are three types of cholesterol in the blood, namely high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL), and triglycerides. HDL is good cholesterol that can prevent clogging of blood vessels, while high LDL and triglycerides can cause blockage of blood vessels. The
cholesterol can build up and narrow blood vessels and as a result with high-risk for cardiovascular disease (CVD). The 3-hydroxymethyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase is one enzyme that play potential role of biosynthesis cholesterol from 3-hydroxymethyl-3-methylglutaryl coenzyme A (HMG-CoA) through Mevalonate pathway. Many studies reported that the bioactive compounds succeed to reduce cholesterol levels through the inhibition of the HMG-CoA-Reductase induced the HMG-CoA in mammals and other eukaryote [4, 5].

Research of food compounds ingredient to improve the cholesterol biosynthesis related with dyslipidemia and cardiovascular diseases becomes important. The ingredient of red yeast rice has similar function of statin to inhibit the biosynthesis of cholesterol in mammal and other organisms [5, 6]. The bioactive food compounds can be explored from pigmented rice. The pigmented rice ingredient is the rich of fiber, anthocyanin, phenol, macro and micro nutrition, including the bioactive compound for body health [7]. Moreover, Fatchiyah reported that the pigmented rice from Java Island has macronutrient contents involved carbohydrates, proteins, and fat, which is the black rice from West Java has the lowest carbohydrates and the highest protein composition. Living organism needed not only the macronutrients but also micronutrients in their dietary nutrients. The compositions of amino acid residues in black rice are more abundant than red and white rice. The black rice from West Java and then East Java displayed rich of amino acids residues and total anthocyanin, which are the advantage as anti-inflammatory, anti-apoptosis, and anti-oxidative [7, 8, 9].

The influence of Cyanidin-O-glucose as one of black rice anthocyanin to prevent cholesterol biosynthesis from HMG-CoA reductase through Mevalonate pathway is not yet observed. Therefore, this in silico research analyzed the potential effect of Cyanidin-O-glucose on inhibition of the HMG-CoA and HMG-CoA reductase interaction.

2. Methods

2.1 The 3D Structure Preparation of Anthocyanins, HMG-CoA, and HMGR

The anthocyanins retrieved from the database of PubChem, is cyanidin-3-O-glucoside (CID: 12303221), we minimized their energy and converted their SDF format into PDB format by using PyRx software. We obtained HMG-CoA canonical SMILES (CID: 445127) from PubChem and reconstructed it using VEGAZZ software. RCSB Protein Data Bank (PDB) was used as a database to get the 3D structures of HMGR (PDB ID: 1DQ9). The water molecules and or previously existing ligands incorporated in their 3D structures were removed using Discovery Studio 2019.

2.2 Molecular Docking

We would like to investigate whether each of the anthocyanins could interfere with the interaction of HMG-CoA and HMGR in silico. Firstly, we predicted the interactions between HMG-CoA and HMGR. Each of anthocyanins was docked to HMG-CoA. Then, we established the anthocyanin-HMG-CoA-HMGR complex. Using the default parameter of Hex 8.0.0 software did docking. All molecular interactions were further visualized in the Discovery Studio 2019.

3. Result and Discussions

The in silico analysis showed the three-dimension structure of cyaniding-O-glucose of black rice anthocyanin blocked the HMG-CoA protein interacted with HMG-CoA reductase (Fig. 1). The differential interaction energy are displayed the cyaniding-O-glucose and HMG-CoA reductase interaction is -334cal/mol, cyaniding-O-glucose and HMG-CoA reductase into HMG-CoA interaction is -539.9 kJ/mol, cyaniding-O-glucose and HMG-CoA interaction is -218 kJ/mol, and cyaniding-O-glucose and HMG-CoA into HMG-CoA reductase interaction is -501.6 kJ/mol. The highest energy is between cyaniding-O-glucose and HMG-CoA interaction. In both interaction among cyaniding-O-glucose and HMG-CoA reductase into HMG-CoA or cyaniding-O-glucose and HMG-CoA into HMG-CoA reductase is tight blocking with low energy as respectively. This result demonstrated that cyaniding-O-glucose of black rice prevented the interaction between HMG-CoA into HMG-CoA reductase. Similar with this result, recently our study reported that bioactive peptides of goat milk alpha
casein-S2 protein completely prevented the interaction of HMG-CoA reductase into HMG-CoA with binding energy 498.8 \text{kJ/mol}, and \text{–} 1010.4 \text{kJ/mol} [10]. The energy docking determined the atoms of compounds would be easy interaction to prevent or enhance the biological function approach [11].

The two-dimension structure of cyanidin-O-glucose (yellow line in Figure 2) and HMG-CoA into HMG-CoA reductase exhibited the differential distance and bonding type. Fig. 2. The interaction distance are the lowest was 1.11608 (:LIG1-A:MET655) and the highest was 5.28192(A:GLY808:HN-LIG1:H). The bond type is six of hydrogen bonds, one of electrostatic, one of other, four of hydrophobic and two of unfavorable. The amino acid residue of HMG-CoA reductase (blue line in Figure 2) are connected in both of cyaniding-O-glucose and HMG-Co that the hydrogen bonds were formed in ASN658, LYS691, GLY807, ASP767, and GLN766 bon. The GLUT559 residue was bond by electrostatic, MET657 bond by other type and GLY765 and GLY808 bond by unfavorable
interactions. The other of amino acid such as MET655, MET657 and ALA654 of HMG-CoA reductase only interacted with HMG-CoA (red line in Figure 2). Hydrophobic bonds in these complexes can up-regulated the affinity of HMG-CoA against co-enzyme of HMG-CoA reductase, which promoted by some hydrogen bonds and other interactions type [12, 13]. Recently, study showed that HMG-CoA reductase blocked by methoxyl pectin of banana peel to interact with ligands that may reducing the cholesterol level [14]. This result indicated that hydrogen bonds, hydrophobic binding, electricity and unfavorable interactions performed the inhibition of cyanidin-O-glucose to HMG-CoA against HMG-CoA reductase, respectively.

| Anthocyanin type       | Point Interaction | Distance | Binding type  |
|------------------------|-------------------|----------|---------------|
| Cyanidin-3-O-glucoside | A:ASN658:HN - :LIG1:O | 2.55245  | Hydrogen Bond |
|                        | A:ASN658:HD21 - :LIG1:O | 3.05072  | Hydrogen Bond |
|                        | A:LYS691:HZ2 - :LIG1:O | 2.2681   | Hydrogen Bond |
|                        | A:GLY807:HN - :LIG1:O | 2.53656  | Hydrogen Bond |
|                        | :LIG1:H - A:ASP767:OD2 | 2.72275  | Hydrogen Bond |
|                        | A:GLN766:CA - :LIG1:O | 2.77907  | Hydrogen Bond |
|                        | B:GLU559:OE2 - :LIG1 | 4.00845  | Electrostatic |
|                        | A:MET657:SD - :LIG1 | 4.31476  | Other         |
|                        | :LIG1 - A:MET655 | 4.46377  | Hydrophobic   |
|                        | :LIG1 - A:MET657 | 4.26305  | Hydrophobic   |
|                        | :LIG1 - A:ALA654 | 4.56977  | Hydrophobic   |
|                        | :LIG1 - A:MET655 | 5.28192  | Hydrophobic   |
|                        | A:GLY765:O - :LIG1:O | 2.0703   | Unfavorable   |
|                        | A:GLY808:HN - :LIG1:H | 1.11608  | Unfavorable   |

**Figure 2.** The two-dimension Structure of Interaction Cyanidin-O-glucose of Black Rice with HMG-CoA and HMG-Co-A reductase. Yellow is Cyanidin-O-glucose, Red is HMG-CoA, and Blue is HMG-Co-A reductase, Bold is amino acid residue.

**4. Conclusion**
The Cyanidin-O-glucose of Black Rice demonstrated the play a role of inhibition HMG-CoA interacts into HMG-Co-A reductase. That result indicates Cyanidin-O-glucose of black rice has potential function for downgrade cholesterol biosynthesis.
Acknowledgement
This study is a part of research that supported by RISPRO-PRN-LPDP National Research Grant from the Ministry of Research & technology-BRIN and the Ministry of Finance, Republic of Indonesia, at 2020-2024.

References
[1] Linton MF, Yancey PG, Davies SS, Jerome G, Linton EF, Song WL, Doran AC, and Vickers KC. The Role of Lipids and Lipoproteins in Atherosclerosis. Endotex. 2019. https://www.ncbi.nlm.nih.gov/books/NBK343489/
[2] WHO. World health statistic 2018 monitoring health for SDGs. ISBN 978-92-4-156558-5.
[3] Riskesda. 2019. Laporan Nasional Riskesda 2018. Badan Penelitian dan Pengembangan Kesehatan. 674 pp.
[4] Burg, J. S. Espenshade, P. J. 2011. Regulation of HMG-CoA reductase in mammals and yeast. Progress in lipid research. 50(4): 403-410
[5] Bradshaw, R & P. Stahl. 2015. Encyclopedia of Cell Biology. Academic Press: USA
[6] Pharm AS, Maria-Corina Serban M-C, Głuba-Brzozka A, Mikhailidis DP, Arrigo F. Cicero AF, Rysz J, and Banach M. Nutrition 32 (2016) 1179–1192.
[7] Fatchiyah F, Sari DRT, Safitri A, and Cairns JRK. Phytochemical Compound and Nutritional Value in Black Rice from Java Island, Indonesia. Sys Rev Pharm 2020; 11(7):414-421
[8] Sari DRT, Cairns JRK, Safitri A, Fatchiyah F. Acta Informatica Medica. 2019. Vol. 27 (3), 152
[9] Sari DRT, Safitri A, Cairns JRK, Fatchiyah F. Journal Of Tropical Life Science. 2020. 10 (1) :15 – 25
[10] Ravi L, Jasmine SE, Krishnan K, and Khanna G, International Journal of Pharma and Bio Sciences 6(1), 1190-1195 (2015).
[11] R. Patil, S. Das, A. Stanley, L. Yadav, A. Sudhakar, A.K. Varma, PLoS ONE 5(8), e12029 (2010).
[12] T. Young, R. Abel, B. Kim, B.J. Berne, R.A. Friesner, PNAS 104(3), 808–813 (2007).
[13] Tapiory AA, Pertiwi KO, Fadilla K, Reyhanditya D, and Fatchiyah F. JSMARTECH 1 (2), 046-050, 2020.