Evaluation of Pharmacokinetic Properties, Toxicity, and Bioactive Cytotoxic Activity of Black Rice (Oryza sativa L.) as Candidates for Diabetes Mellitus Drugs by in silico

Fadjar Kurnia Hartati 1,*, Arlin Besari Djaahari 1, Viol Dhea K 2

1 Department of Food Technology, Dr Soetomo University, Surabaya, Indonesia
2 Laboratory of Computational Biology and Bioinformatics, Biology Departement, Faculty of Mathematics and Natural Science, Brawijaya University, Malang, East Java, Indonesia

* Correspondence: fadjar.kurnia@unitomo.ac.id; Scopus Author ID 57194443683

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Abstract: The in silico method is widely used to search for drug candidates, namely by predicting physicochemical properties, pharmacokinetics (ADME), toxicity, know the description of drug interactions with target proteins, Rerank Score (RS), and then carried out the Molecular Docking (MD) process. This study intends to predict the physicochemical, pharmacokinetics (ADME) properties, toxicity, and cytotoxic activity of 15 bioactive compounds in black rice (Oryza sativa L.) drug candidates for diabetes mellitus using the GLP-1 target protein. The In silico test results showed that campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin found in black rice have physicochemical, pharmacokinetic (ADME), toxicity, and better Rerank Score (RS) properties than omarigliptin (drug). Black rice can also be safely recommended to be consumed by people with diabetes mellitus.

Keywords: black rice; GLP-1; in silico; Campesterol ferulate; Cycloartenol ferulate; lutein; β-carotene; zeaxanthin.

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1. Introduction

Diabetes mellitus (DM) is a disease that has a high risk of causing fatal health complications [1]. On the other hand, the number of DM sufferers increases and cannot be cured [2]. One of the efforts to minimize the risk of these health complications is to adjust the lifestyle and diet [3]. Diet is related to the type of food consumed, so it is necessary to find ingredients safe for consumption by people with DM and potentially reduce blood sugar. One of the ingredients is black rice (Oryza sativa L.). Phytochemicals in black rice are cyanidin-3-O-arabinoside, cyanidin-3,5-diglucoside, and pelargonidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside, campesterol ferulate, cycloartenol ferulate, 24-methylenecycloartenol ferulate, quercetin-3-O-glucoside,isorhamnetin-3-O-glucoside, lycopene, zeaxanthin, lutein, β-carotene, quercetin -3-O-Rutinoside [4]. Black rice extract can inhibit pro-inflammatory cytokines produced by immunocompetent cells in DM model mice [5]. Pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), gamma interferon (IFN-γ), and alpha tumor necrosis factor (TNF-α) could inhibit insulin secretion that stimulates glucose [6].

The method widely developed in the search for drug candidates is the in silico method using molecular modeling methods to predict pharmacokinetics (ADME), toxicity, and the
description of drug interactions with target proteins [7]. Furthermore, Molecular Docking (MD) was carried out between the selected target cells and the molecules whose activity would be predicted [8],[9]. This method is believed to explore two molecules’ interactions, such as the interaction between a drug candidate and a binding target protein. Docking programs can identify target bioactive molecule/ligand-protein complexes’ interactions and their bond affinities evaluated using free energy simulations [10]. The in-silico test results are in the form of a bond energy value or Rerank Score (RS), which indicates the energy’s amount needed to form bonds between ligands and receptors. The smaller the bond energy, the more stable the bond. Thus, the more stable the ligand bond with the receptor, it is predictable that the activity will also be more significant [11].

Especially in the current COVID-19 pandemic condition, almost all laboratories are closed, making it difficult to conduct research. This study aimed to reduce the cost and time needed to convert active ingredients into drugs that can be publicly available [12]. Research in the United States’ pharmaceutical industry shows that in 2001, the cost of making a drug from active ingredients to a drug was valued at around $ 880 million with a production time of around 12 years. In addition to cost and duration, several other factors can cause the drug manufacturing process to fail, such as the insignificant effects of illegal drugs, toxic effects, and obstacles in the marketing process [13].

The target protein used for various chronic therapies for inflammatory-related diseases, including diabetes mellitus types 1 and 2, in vitro and in vivo is GLP-1 [14]. GLP-1 is an incretin hormone that can stimulate insulin secretion [15,16]. GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4). Inhibition of proteolytic enzymes increases the biological half-life [17]. GLP-1 has many beneficial effects on controlling blood glucose levels, including stimulation of insulin secretion and inhibition of glucagon secretion; expansion of beta-cell mass through the stimulation of beta-cell proliferation and differentiation and inhibiting beta-cell apoptosis; gastric delay, emptying; and reducing food intake [18][19]. Therefore, GLP-1 has been widely studied as an alternative treatment for type 2 diabetes [20,21].

Based on these facts, this study was conducted to determine the potential of bioactive compounds in black rice (Oryza sativa L.) as a drug candidate for diabetes mellitus in silico, with GLP-1 as the target protein and omarigliptin as a comparison compound. Omarigliptin (46209133) has been shown to increase insulin secretion by up to 97% in type 2 DM patients without other drugs [22]. Omarigliptin is a DPP-4 inhibitor group that is widely used as an oral drug for DM sufferers [23].

2. Materials and Methods

The potential of black rice active compounds and the binding affinity of active compounds to the GLP-1 receptor protein can be known computationally with the Descriptive Analytical research design. Black rice bioactive compounds were obtained from the PubChem server (https://pubchem.ncbi.nlm.nih.gov) with the recorded CID. Whereas, the GLP-1 receptor is obtained from a data server (http://www.rcsb.org/pdb/home/home.do) with ID 3iol. Reference to GLP-1 protein activity is reviewed at http://www.string-db.org and prediction of its pathway in KEGG through GLP1R (ID 04911) [24].

The physicochemical properties studied include molecular weight / BM, the logarithm of the octanol/water partition coefficient (log P), the bonds’ numbers between rotating atoms (Torsion), Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), and Polar Surface Activity (PSA). Meanwhile, the pharmacokinetic properties that were monitored were
ADME (absorption, distribution, metabolism, and excretion). Toxicity of all black rice bioactive compounds and comparison compounds obtained by using the pkCSM online tool (http://biosig.unimelb.edu.au/pkcsmprediction) [25]. Toxicity test (LD50) by mouth on rodents and classification of compound toxicity based on the globally harmonized system (GSH) was carried out using the Protox online tool (http://tox.charite.de/tox/).

Then, the docking process was done using the PyRx program (Autodock vina) [26]. The docking process was carried out specifically with ‘bioactive compounds’ as a reference for ligands and receptors (ID 04911). The best complex conformation selection was based on the binding energy that has the smallest or the most negative value. The docking results were then stored and visualized using Ligandscout and PyMOL ligands [27].

Visualization of docking results by LigPlot indicated the interaction between two molecules (GLP-1-ligand compounds) [28]. LigPlot results showed hydrophobic bonds and hydrogen bonds that occurred in the complex. Its potential activity can be observed from the active site’s amino acids, bound by active compounds. Omarigliptin acts as a comparison compound (46209133) [29,30].

3. Results and Discussion

3.1. Analysis of physicochemical properties, pharmacokinetics, and toxicity of bioactive compounds.

Based on in silico analysis, obtained values of physicochemical properties of black rice bioactive compounds and comparative compounds can be seen in Table 1.

| Comp. No. | Compound                  | BM   | LogP | Torsion | HBA | HBD | PSA (Å²) |
|-----------|---------------------------|------|------|---------|-----|-----|----------|
| 1.        | Campesterol ferulate      | 576.85 | 5.61 | 10      | 15  | 5   | 145.76   |
| 2.        | Cycloartenol ferulate     | 602.89 | 6.36 | 9       | 15  | 6   | 135.76   |
| 3.        | Lutein                    | 568.87 | 5.75 | 10      | 13  | 6   | 131.46   |
| 4.        | β-carotene                | 536.87 | 5.96 | 10      | 17  | 6   | 136.16   |
| 5.        | Zeaxanthin                | 568.87 | 5.96 | 10      | 11  | 6   | 140.46   |
| 6.        | Omarigliptin comparison   | 598.43 | 5.96 | 8       | 13  | 6   | 136.83   |
| 7.        | Cyanidin-3-O-arabinoside  | 454.81 | -1.00 | 3     | 10  | 7   | 173.21   |
| 8.        | Cyanidin-3,5-diglucoside  | 709.58 | -3.73 | 1      | 19  | 10  | 304.96   |
| 9.        | Pelargonidin-3-O-glucoside| 433.39 | -1.27 | 4     | 10  | 7   | 173.21   |
| 10.       | Quercetin-3-O-rutinoside  | 610.52 | -3.89 | 6      | 16  | 10  | 269.43   |
| 11.       | Cyanidin-3-O-glucoside    | 449.38 | -1.76 | 4      | 11  | 8   | 193.44   |
| 12.       | Peonidin-3-O-glucoside    | 463.41 | -1.54 | 5      | 11  | 7   | 182.44   |
| 13.       | Isorhamnetin-3-O-glucoside| 478.40 | -2.37 | 5      | 12  | 7   | 199.51   |
| 14.       | Lycopene                  | 536.87 | 4.21 | 16      | 0   | 0   | 0        |
| 15.       | Quercetin-3-O-glucoside   | 464.38 | -2.59 | 4      | 12  | 8   | 210.51   |
| 16.       | 24-Methylenecycloartenol ferulate | 314.33 | 2.30 | 7      | 5   | 2   | 75.99   |

Table 1. Results of in silico analysis, the values of the physicochemical parameters of black rice bioactive compounds and comparison compounds.

| Comp. No. | Compound                  | BM   | LogP | Torsion | HBA | HBD | PSA (Å²) |
|-----------|---------------------------|------|------|---------|-----|-----|----------|
| 1.        | Campesterol ferulate      | 576.85 | 5.61 | 10      | 15  | 5   | 145.76   |
| 2.        | Cycloartenol ferulate     | 602.89 | 6.36 | 9       | 15  | 6   | 135.76   |
| 3.        | Lutein                    | 568.87 | 5.75 | 10      | 13  | 6   | 131.46   |
| 4.        | β-carotene                | 536.87 | 5.96 | 10      | 17  | 6   | 136.16   |
| 5.        | Zeaxanthin                | 568.87 | 5.96 | 10      | 11  | 6   | 140.46   |
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| 13.       | Isorhamnetin-3-O-glucoside| 478.40 | -2.37 | 5      | 12  | 7   | 199.51   |
| 14.       | Lycopene                  | 536.87 | 4.21 | 16      | 0   | 0   | 0        |
| 15.       | Quercetin-3-O-glucoside   | 464.38 | -2.59 | 4      | 12  | 8   | 210.51   |
| 16.       | 24-Methylenecycloartenol ferulate | 314.33 | 2.30 | 7      | 5   | 2   | 75.99   |

Table 1. Results of in silico analysis, the values of the physicochemical parameters of black rice bioactive compounds and comparison compounds.

1Table legend: BM = molecular weight; LogP = logarithm of octanol / water partition coefficient; Torsion = bond between rotating atoms; HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors; PSA = Polar Surface Activity.

The World Drugs Index database has analyzed 2,245 drugs and concluded that these compounds will be difficult to absorb. The permeability will be low if the compound had a higher molecular weight of 500, had a log value of octanol partition coefficient/water (log P) is more significant than +5, has a donor H-bond (HBD) expressed by the number of OH and
NH groups, greater than 5, and has an H-acceptor (HBA) bond expressed by the number of O and N atoms, greater 10. Based on the statement, the bioactive compounds of black rice and comparison compounds that met Lipinski criteria were the comparison compounds, namely campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin [31].

Table 2. *In silico* analysis of pharmacokinetic properties (ADME), toxicity, and cytotoxic activity (Rerank Score) of black rice bioactive compounds and comparison compounds.

| Compound No. | Intestinal absorption (human) (%) | Skin Permeability (log Kp, cm/h) | VDss (human) (log L/kg) | BBB Permeability (logBB) | CYP2D6 Substrate | CYP2D6 Inhibitor | Total Clearance (log ml/min/kg) | Renal OCT2 Substrate | Ames Toxicity | LD50 (mg/kg) | Rerank Score |
|--------------|---------------------------------|-------------------------------|------------------------|-------------------------|------------------|-----------------|-------------------------|----------------------|--------------|--------------|--------------|
| 1.           | 92.46                           | -3.06                         | -0.71                  | 0.26                    | No               | No              | -0.22                   | No                   | Yes          | 2885         | -123.26±0.36 |
| 2.           | 91.73                           | -3.29                         | -0.69                  | 0.25                    | No               | No              | -0.48                   | No                   | Yes          | 2885         | -133.91±0.3  |
| 3.           | 90.90                           | -3.25                         | -0.46                  | 0.30                    | No               | No              | -0.52                   | No                   | Yes          | 2865         | -122.82±0.35 |
| 4.           | 90.48                           | -2.79                         | -0.51                  | 0.23                    | No               | No              | -0.38                   | No                   | Yes          | 2860         | 147.24±1.20  |
| 5.           | 92.04                           | -3.00                         | -0.64                  | 0.43                    | No               | No              | -0.41                   | No                   | Yes          | 2850         | 124.53±2.10  |
| 6.           | 91.63                           | -2.96                         | -0.48                  | 0.31                    | No               | No              | -0.20                   | No                   | Yes          | 2850         | 116.18±0.24  |
| 7.           | 67.45                           | -2.16                         | -0.10                  | -0.04                   | No               | No              | -0.33                   | No                   | No           | 2850         | 112.91±0.19  |
| 8.           | 72.19                           | -0.06                         | -0.14                  | -0.01                   | No               | No              | -0.56                   | No                   | No           | 2850         | 100.82±2.11  |
| 9.           | 57.72                           | -1.02                         | -0.23                  | 0.21                    | No               | No              | -0.43                   | No                   | No           | 2860         | -99.83±0.67  |
| 10.          | 65.85                           | -0.70                         | -0.11                  | 0.12                    | No               | No              | -0.66                   | No                   | No           | 2665         | 112.61±2.8   |
| 11.          | 74.28                           | -0.08                         | -0.08                  | -0.45                   | No               | No              | -0.45                   | No                   | No           | 2880         | -98.82±1.37  |
| 12.          | 81.08                           | -1.98                         | -0.19                  | -0.65                   | No               | No              | -0.48                   | No                   | Yes          | 1850         | 121.82±4.26  |
| 13.          | 89.78                           | -2.01                         | -0.21                  | -0.05                   | No               | No              | -0.51                   | No                   | No           | 1000         | 114.61±0.19  |
| 14.          | 86.98                           | -1.46                         | -0.09                  | -0.18                   | No               | No              | -0.23                   | No                   | No           | 1850         | 113.28±0.39  |
| 15.          | 68.24                           | -1.86                         | -0.14                  | 0.23                    | No               | No              | -0.50                   | No                   | No           | 2850         | -98.75±3.21  |
| 16.          | 74.85                           | -2.03                         | -0.22                  | 0.14                    | No               | No              | -0.64                   | No                   | Yes          | 1000         | 113.45±1.94  |

Clinically approved drugs have the following ranges of physicochemical properties: Molecular Weight (130-725), HBD (0-6), HBA (2-20), Log P (-2 to 6.5), and the number of RB (0-15). In conclusion, the bioactive compounds and comparative compounds of the black rice that fulfill these criteria were comparison compounds, campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin [32].

The central place of absorption of drugs given orally is in the intestine. Compounds are said to have proper absorption if the value is more than 80% and must not be less than 30% [33]. Based on this case, bioactive compounds and comparative compounds that met these criteria were compounds number 1, 2, 3, 4, 5, 6, 12, 13,14, and 15. A compound is said to have relatively low skin permeability if it has a log value of Kp > -0.25. The results of this study indicated that the test compounds having good skin permeability were 1, 2, 3, 4, 5, and 6.

Volume distribution (VDSS) is the total dose of the drug that needs to be evenly distributed to give the same concentration in blood plasma. The higher the VD value, the more drugs are distributed in the tissue than in plasma. A compound has a low VD if the log VD

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value < -0.15; and high if > 0.45 [33]. Based on this, the test and comparison compounds 1, 2, 3, 4, 5, and 6 could be distributed evenly in blood plasma in this study.

Another critical parameter is the drug’s ability to penetrate the blood barrier (Blood-Brain Barrier), an important parameter that needs to be considered to reduce the side effects and toxicity or increase drugs' efficacy pharmacological activity is in the brain. The permeability of brain-blood is measure in vivo in animal models as Log BB, the logarithmic ratio of concentration in the brain to plasma. The compound can penetrate the blood-brain barrier well when it has a Log BB value > 0.3, and cannot be distributed well if Log BB < -1. Based on this, the bioactive compounds of black rice and comparative compounds that we're able to penetrate the blood barrier in this study were compound no. 1, 2, 3, 4, 5, 6, 9, 10, 15, and 16.

It is generally known that most metabolic reactions will involve oxidation processes. Cytochrome P450 is an important detoxification enzyme in the body found in the liver. This enzyme works by oxidizing foreign compounds, including drugs, and facilitating the excretion of these compounds. Inhibitors of these compounds can affect drugs' metabolism, so they are contraindicated against the cytochrome P450 enzyme. Therefore, it is essential to assess the ability of compounds that can inhibit cytochrome P450, which is represented by the cytochrome P2D6 isoform (CYP2D6). The analysis results showed that all the bioactive compounds of black rice and the comparative compounds did not affect or inhibit the CYP2D6 enzyme, so it can be said that the P450 enzyme could metabolize the compound.

Total Clearance (CLTOT) and Renal Organic Cation Transporter 2 (OCT2) are parameters to determine a compound's excretion. CLTOT is a mix of hepatic (metabolism in the liver and bile) and renal clearance (excretion through the kidneys). This is related to bioavailability, and it is crucial to determine the dose level in achieving steady-state concentration. The analysis showed that the CLTOT value of the test compounds ranged from -0.20 to -0.66. From these values, it can be seen the speed of excretion of compounds.

Organic Cation Transporter 2 is a transporter in the kidney which plays a vital role in the disposition and drugs’ clearance and endogenous compounds. OCT2 substrate also has the potential to cause side interactions when terminated jointly with OCT2 inhibitors [33]. The analysis showed that all test compounds did not affect the OCT2 substrate, so it can be concluded that all tests were not OCT2 substrates.

The Ames toxicity test is a widely used method for assessing mutagenic compounds’ potential using bacteria [34]. The analysis results showed that the comparative compound was able to cause mutagenic effects and the compound no. 1, 2, 3, 4, 5, 12, and 16.

LD50 (oral toxicity of rodents) is used to complete the observation of a compound's toxicity and compound toxicity classification based on the Globally Harmonized System (GSH). LD50 is the number of stopped compounds that can cause the death of 50% of experimental animals [35]. Based on the results of the analysis showed that LD50 values ranged between 1000 - 2885 mg/kg, and were included in the 5 GSH toxicity class, which means the compound has a low acute toxicity effect.

The smaller the RS value of a compound, the higher the cytotoxic activity of the Sirtuin enzyme 1. The RS value of the comparative compound (no. 6) is -116.18 kcal/mol. The black rice bioactive compounds with lower RS value than the comparison compound are the compound no. 1, 2, 3, 4, 5, and 12, which means that these compounds have high cytotoxic activity [35].
Based on the values of the parameters of physicochemical, pharmacokinetic (ADME), toxicity, and cytotoxic activity (Rerank Score, black rice bioactive compounds that have potential as drug candidates are campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin.

3.2. Interaction analysis between ligand (black rice bioactive compounds) and GLP-1 protein target with in silico method.

The results of the interaction between black rice bioactive compounds as ligands and the target protein GLP-1 are shown by the binding affinity value (Table 3). According to [36], binding affinity shows the binding strength between the target protein and the ligand. The smaller the value, the stronger the bond.

Table 3. Analysis of the potential of black rice bioactive compounds that can increase insulin in silico.

| No | Compound                  | CID       | Target Protein | Binding Affinity (kcal/mol) |
|----|--------------------------|-----------|----------------|----------------------------|
| 1  | Campesterol ferulate     | 173183    | GLP1           | -8.5                       |
| 2  | Cycloartenol ferulate    | 56840602  | GLP1           | -8.3                       |
| 3  | Lutein                   | 5281243   | GLP1           | -8.2                       |
| 4  | β-carotene               | 5280489   | GLP1           | -7.8                       |
| 5  | Zeaxanthin               | 5280899   | GLP1           | -7.6                       |
| 6  | Omarigliptin (comparison compound) | 46209133 | GLP1 | -7.5 |
| 7  | Cyanidin-3-O-arabidoside | 91810602  | GLP1           | -7.3                       |
| 8  | Cyanidin-3,5-diglucoside | 44256812  | GLP1           | -6.8                       |
| 9  | Pelargonidin-3-O-glucoside | 443648 | GLP1 | -6.7 |
| 10 | Quercetin-3-O-rutinoside | 5280805   | GLP1           | -6.6                       |
| 11 | Cyanidin-3-O-glucoside   | 441667    | GLP1           | -6.5                       |
| 12 | Peonidin-3-O-glucoside   | 14311151  | GLP1           | -6.5                       |
| 13 | Isorhamnetin-3-O-glucoside | 5318645 | GLP1 | -6.4 |
| 14 | Lycopene                 | 446925    | GLP1           | -6.4                       |
| 15 | Quercetin-3-O-glucoside  | 5748594   | GLP1           | -6.4                       |
| 16 | 24-Methylenecycloartenol ferulate | 637308 | GLP1 | -5.7 |

Table 3 shows that the bioactive compounds campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin in black rice have less binding energy than the comparison compounds. Thus, these bioactive compounds have the potential to increase insulin secretion. The binding energy of the comparison compound and GLP-1 protein is -7.5. The free binding energy amount (ΔG) is a binding indicator of the active compound to the target protein. In a spontaneous process, protein-ligand binding occurs only when the alteration in Gibbs free energy (ΔG) of the system is negative when the system reaches equilibrium at constant pressure and temperature. Since the magnitude of negative ΔG determines the degree of protein-ligand association, ΔG can be considered on determining the stability of the present protein-ligand complex or the ligand’s affinity bond to a particular acceptor [37]. Based on this statement, the bioactive compounds campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin in black rice predict spontaneous bonding to the active site of the GLP-1 receptor protein to form a stable protein-ligand complex.

The results show that GLP-1 based therapy regulates inflammatory mediators’ expression and influences immunocytes, and protects against immunocyte attacks, contributing to pancreatic maintenance. Increased production of pro-inflammatory cytokines and chemokines in adipose tissue is a significant contributor to insulin resistance in type 2 diabetes.
and blocks inflammatory signaling pathways or infiltration of immune cells in adipose tissue and increasing insulin sensitivity [38]. Giving recombinant adenovirus that produces GLP-1 (4 × 109 PFU/rat) in mice reduces the population of macrophages and the production of TNF-α, MCP-1, and IL-6 in adipose tissue through activation of nuclear factor-Kappa B (NF-kB) inhibition, ERK1/2 phosphorylation and c-Jun N-terminal kinase. Sitagliptin (4 g/kg) also showed the same effect and reduced mRNA expression for inflammatory cytokine genes and macrophage infiltration in adipose tissue in high-fat fat (HFD-) mice that induced obese mice [39]. In patients with type 2 diabetes, sitagliptin therapy (100 mg/day) significantly reduces plasma C-reactive protein (CRP) levels, IL-6, IL-18, which secrete phospholipase-A2, dissolved intracellular adhesion molecules (ICAM-)1, and E-selectin compared to placebo. Inflammatory values and the homeostatic model assessment index for insulin resistance were significantly reduced in patients with type 2 diabetes treated with sitagliptin [40]. Therefore, suppressing inflammatory mediators in adipose tissue by GLP-1 based therapy can contribute to increased insulin sensitivity.

GLP-1-based therapy for diabetes contributes to reducing inflammation and has additional beneficial effects such as the preservation of ‘islets’ and increased insulin sensitivity in addition to the effects of glucose reduction. Based on the above statement, it can be concluded that the bioactive compound cholesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin contained in black rice, can interact with the active side of GLP1 protein spontaneously. The interaction can increase insulin secretion.

### 3.3. Analysis of the position of hydrogen and hydrophobic bonds between ligands (black rice bioactive compounds) and the target protein GLP-1 using in silico method.

This analysis was carried out to determine the position of the hydrogen further and hydrophobic bonds between the selected compounds from the previous test (namely campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin) with the target protein GLP-1 and to determine the strength of the bonds between the active compound and target protein. An active compound is predicted to have a strong bond with the target protein if it can bind strongly by hydrogen bonding with the same amino acid residue compared to the control reference.

**Table 4.** Analysis of interactions between black rice bioactive compound ligands and GLP-1 protein by *in silico* method.

| Ligand ID                  | Interaction                  |
|----------------------------|------------------------------|
| Campesterol ferulate       | Hydrogen bond: Tyr88         |
|                           | Hydrophobic bond: Arg40, Trp91, Val36, Trp39, Thr35, Leu89, Tyr88, Glu127, Leu123, Cys126 |
| Cycloartenol ferulate      | Hydrogen bond: Tyr88         |
|                           | Hydrophobic bond: Arg121, Tyr69, Leu123, Glu127, Trp91, Val36, Trp39, Leu89, Tyr88, Pro90, Leu89, Thr35 |
| Lutein                    | Hydrogen bond: Glu68         |
|                           | Hydrophobic bond: Arg40, Trp39, Tyr88, Tyr69, Trp91, Trp33, Val36, Leu89, Glu127, Cys126 |
| β-carotene                | Hydrogen bond: Glu68         |
|                           | Hydrophobic bond: Glu127, Tyr69, Trp91, Val36, Leu123, Tyr88, Trp39, Arg40, Trp33 |
| Zeaxanthin                | Hydrogen bond: Glu68         |
|                           | Hydrophobic bond: Glu127, Tyr69, Leu123, Val36, Trp39, Tyr88, Arg43, Arg40, Trp33 |
| Omarigliptin (control compound) | Hydrogen bond: Glu68, Tyr88 |
|                           | Hydrophobic bond: Glu127, Cys126, Tyr69, Leu123, Leu89, Trp91, Val36, Trp39 |

Note: Bold shows the same protein-ligand complex as the protein-control complex.
The results of the analysis of the hydrogen and hydrophobic bond positions of selected black rice bioactive compounds with the GLP-1 target protein can be seen in Table 4 and Figure 1.

**Figure 1.** Visualization of interactions between target ligands-proteins. Figure Legends: Green cartoon structure indicates target protein; Sphere structures in red indicate active side area.

Table 4 shows that the reference compound is bound to the active site of the target protein through hydrogen bonds with the following amino acid types Glu68 and Tyr88 and bound through hydrophobic bonds with the following amino acids Glu127, Cys126, Tyr69, Leu123, Leu89, Trp91, Val36, and Trp39. The distance of hydrogen bonds between campesterol ferulate, cycloartenol ferulate, lutein, \(\beta\)-carotene, and zeaxanthin with amino acid residues, respectively 3.00 Å, 2.93 Å, 2.83 Å, 2.93 Å, and 2.91 Å, while the comparison compound is 3.002 Å. The results indicate that campesterol ferulate, cycloartenol fermentation, lutein, \(\beta\)-carotene, and zeaxanthin found in black rice have shorter hydrogen bonding distances with amino acid residues compared to similar compounds, so it is predicted to have the ability to synthesize insulin more strongly than control. The shorter the distance between the hydrogens, the stronger the bonds [41].

Bioactive compounds are predicted to have a strong bond with the target receptor if they can bind tightly through hydrogen bonds and bind with one amino acid residue from the active side than reference compounds or inhibitors [42]. Hydrogen bonds are electrostatic interactions between hydrogen atoms bound to electronegative atoms and other electronegative atoms. The strength of the hydrogen bond is below the covalent bond, but its presence is significant. Its presence contributes to the structure and characteristics of the molecule. In medicine, hydrogen bonds play a role in studying the design and interaction between drug molecules and metabolic systems in the body.
4. Conclusions

Of the fifteen types of bioactive compounds found in black rice, which have physicochemical, pharmacokinetic (ADME), toxicity, Rerank Score (RS), and better binding affinity than related compounds (drugs) are campesterol ferulate, cycloartenol ferulate, lutein, β-carotene, and zeaxanthin. These results indicate that black rice is one of the staple ingredients (rice types), which is safe to be consumed by people with diabetes.

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Conflicts of Interest

The authors declare no conflict of interest.

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