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Analysis of betanin from beetroot (*Beta vulgaris* L.) as an alternative prevention of Diabetes Mellitus Type 2: In Silico Study

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Abstract

Type 2 diabetes mellitus (T2DM) is one of the global health issues that is expected to continue increasing in prevalence. Various risk factors for T2DM can cause oxidative stress and chronic inflammation, which contribute to insulin resistance and β-cell damage. These conditions are closely related to dysfunction of the Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ). One alternative strategy for T2DM is utilizing bioactive compounds found in plants, such as betanin from beetroot (*Beta vulgaris* L.). Therefore, this article analyzes the potential of betanin from beetroot as an alternative for preventing T2DM through an in-silico approach, focusing on its interaction with the PPAR-γ receptor and its toxicity profile. The docking application used was PYRX, with visualization in BIOVIA Discovery Studio. Toxicity prediction tests were conducted using Swiss ADME, PK-CSM, and Pro-Tox tools. The results show that betanin can bind stably and strongly to the active site of the PPAR-γ receptor with a binding affinity value of -8.9 kcal/mol. Betanin also has an LD50 ranging from 305 mg/kg, categorizing it as toxicity class 4.

Keywords: Beetroot; Betanin; Insulin Resistance; PPAR-γ; Type 2 Diabetes Mellitus

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common global health problems, especially in developing countries, including Indonesia. T2DM is defined as a chronic metabolic disorder characterized by insulin resistance and progressive pancreatic β-cell dysfunction, leading to persistent hyperglycemia (1,2). T2DM falls into the category of non-communicable diseases, with a vulnerable group consisting of patients over 40 years old. According to the International Diabetes Federation in 2024, 1 in 9 people have diabetes, with a total of 589 million cases in the 20-79 age range (3). Meanwhile, the prevalence of diabetes cases in Indonesia is estimated to continue increasing from 9.19% in 2020 (18.69 million cases), to 11.3% (20.4 million cases) in 2024, and projected to reach 16.09% in 2045 (40.7 million cases) (3,4).

There are several risk factors that cause type 2 diabetes mellitus (DM), such as obesity, smoking, unhealthy eating patterns, lack of physical activity, hypertension, gut dysbiosis, epigenetics, and genetic predisposition (1,4). These factors lead to oxidative stress and chronic inflammation, which contribute to insulin resistance and β-cell damage (1,5). These conditions are closely related to dysfunction of the Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ), which plays an important role in regulating glucose homeostasis, lipid metabolism, adipocyte differentiation, and increasing insulin sensitivity. Reduced activity or dysregulation of PPAR-γ can disrupt insulin signaling pathways, thereby worsening insulin resistance and accelerating the progression of type 2 DM (6,7). Activation of PPAR-γ is also known to suppress the production of pro-inflammatory cytokines and oxidative stress through regulation of metabolic and antioxidant gene expression, making this receptor a potential therapeutic target in managing type 2 DM (8,9).

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Therefore, the use of bioactive compounds with strong antioxidant and anti-inflammatory properties is considered a potential therapeutic agent. One strategy that can be employed is utilizing bioactive compounds found in plants.

Beet (*Beta vulgaris* L.) belongs to the Chenopodiaceae family and has earned the nickname "functional food" due to its ability to prevent various diseases, such as diabetes, hypertension, dementia, heart disease, and anemia (10–12). Beets are known to contain the bioactive compound betanin, which is a natural pigment belonging to the betalain group (13,14). A 100-gram serving of beet contains 1,287 mg of betanin (15). Several experimental studies have shown that consuming beets can help control diabetes and insulin homeostasis, and betanin exhibits various biological activities, including antioxidant, anti-inflammatory, and antidiabetic effects (16–18).

Betanin is known to have antidiabetic effects through various mechanisms, including scavenging reactive oxygen species (ROS), increasing insulin sensitivity, and modulating key metabolic pathways involved in glucose homeostasis (16,19). However, the molecular interactions underlying these effects, particularly at the level of target proteins related to glucose metabolism, have not yet been adequately explored. In the era of technological advancements in computing, in silico studies have become an important initial approach before conducting in vitro or in vivo research. Using these studies allows for the evaluation of potential interactions between active compounds like betanin and PPAR-γ, providing an initial understanding of the mechanism of action of the compound in the context of preventing type 2 DM. This offers insight into its potential mechanism at the molecular level (20) ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis can also provide an overview of the safety of candidate compounds before further experimental testing (21).

Therefore, researchers are interested in analyzing the potential of betanin from beetroot (*Beta vulgaris* L.) as an alternative for preventing type 2 DM through in silico approaches, focusing on molecular interactions and toxicity profiles. By evaluating its interactions with receptors involved in glucose regulation, this study aims to provide a scientific basis for developing betanin as a natural compound with antidiabetic potential. These findings are expected to contribute to the growing body of evidence supporting the use of plant-based bioactive compounds in the prevention of metabolic diseases.

2. Materials and methods

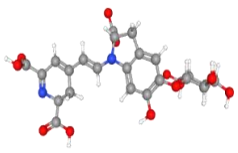
2.1. In Silico Tools

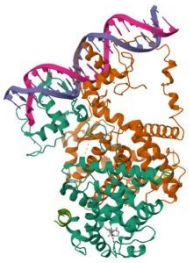
The equipment used in this research includes a set of LENOVO Ideapad 1 AMD Ryzen 3 64-bit laptops with 8GB RAM, 477GB SSD, and Windows 11 operating system. The applications used are PyRx and BIOVIA Discovery Studio. The results include binding affinity and Root-Mean-Square Deviation (RMSD) values.

2.2. Preparation of Ligand and Protein

The structure of phytochemicals from *B. vulgaris*, namely betanin, was prepared through the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Meanwhile, the Peroxisome proliferation-activated receptor gamma (PPAR-γ) receptor structure was obtained from the RCSB Protein Data Bank (PDB) website (https://www.rcsb.org/).

Table 1 Proteins and Ligands Used

	Name	**Structure**
Ligand	Betanin (C24H26N2O13) (https://pubchem.ncbi.nlm.nih.gov/compound/6540685)	

Protein	Receptor of PPAR-γ (3DYZ) (https://doi.org/10.2210/pdb3DZY/pdb)	

2.3. Prediction Test of Compound Toxicity

2.3.1. Swiss ADME

Tools Swiss ADME are used as an initial step to predict the toxicity of compounds through the website http://www.swissadme.ch/ by inputting the SMILES of betanin. The subsequent output includes predictions of lipophilicity, water solubility, pharmacokinetics, and drug-likeness.

2.3.2. PK-CSM

Prediction of compound toxicity using pK-CSM Tools through the website http://biosig.unimelb.edu.au/pkcsm/prediction is performed by inputting SMILES to analyze ADMET (absorption, distribution, metabolism, excretion, and toxicity) more specifically. The purpose of this testing is to observe initial indications of potential toxicity and systemic safety.

The SMILE from betanin is as follows:
C1[C@H](N=C(C=C1/C=C/N2[C@@H](CC3=CC(=C(C=C32)O)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)C(=O)O)C(=O)O)C(=O)O

2.3.3. Pro-Tox

Prediction of compound toxicity using Pro-Tox 3.0 Tools through the website http://https://tox.charite.de/protox3/, is carried out by entering the PubChem name and Conical SMILES of betanin. The output predictions from this tool include toxicity class, LD50, average similarity, prediction accuracy, and several target toxicity models. The purpose of this stage is to confirm and demonstrate detailed toxicity classification according to the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals, as well as to identify potential molecular mechanisms.

3. Results and discussion

3.1. Docking Analysis

Our study focuses on the binding interaction of betanin as a potential antidiabetic agent through its interaction with the PPAR-γ receptor, which plays a crucial role in regulating insulin sensitivity in type 2 diabetes mellitus. The docking results show that betanin can bind stably to the active site of the PPAR-γ receptor with a binding affinity value of -8.9 kcal/mol. This value indicates a potential for stable and strong interactions between the ligand and the target protein, suggesting a promising biological activity (22). A negative bond affinity value also indicates that the bonding process occurs spontaneously thermodynamically (22,23). In addition, the best bond conformation (poses) is selected based on the lowest bond energy, and an RMSD value of 0.0 Å indicates that the conformation position resulting from docking is identical to the reference conformation or the initial predicted position, which suggests that the result is a pose with high conformational stability (23,24).

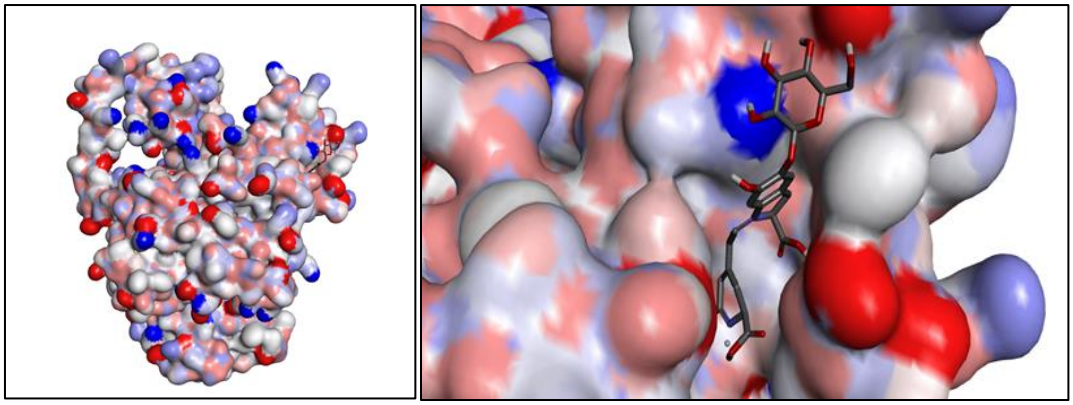


Figure 1 Docking results of betanin with PPAR-γ receptor

The interaction between betanin and the PPAR-γ receptor is dominated by the formation of conventional hydrogen bonds. Visualizing the interaction shows that betanin forms several hydrogen bonds with amino acid residues around the receptor's active pocket (Figure 2). The presence of hydroxyl groups (-OH) and polar groups in the betanin structure allows for the formation of numerous hydrogen interactions, thereby increasing the stability of the protein-ligand complex. Additionally, carbon hydrogen bonds and hydrophobic interactions such as Pi-cation interactions were also observed, supporting the stability of the bond. In the docking complex, unfavorable interactions were also found, including unfavorable donor-donor interactions and unfavorable positive-positive interactions. These interactions indicate that there are donor atom positions or positive charges that are too close together, which can cause electrostatic repulsion (25). However, the existence of some of these unfavorable interactions does not significantly decrease the stability of the complex because it is still balanced by the numerous hydrogen bonds formed.

Biologically, these results indicate that betanin has the potential to act as a modulator of the PPAR-γ receptor. Activation of the PPAR-γ receptor is known to enhance insulin sensitivity through the regulation of glucose and lipid metabolism (26). PPAR-γ also plays a role in inhibiting the inflammatory process and oxidative stress, which are major factors in the development of type 2 diabetes mellitus (27,28). The ability of betanin to bind to the active site of PPAR-γ indicates a potential antidiabetic effect through a mechanism of increased insulin sensitivity. Therefore, betanin is believed to have the ability as a natural partial agonist of PPAR-γ.

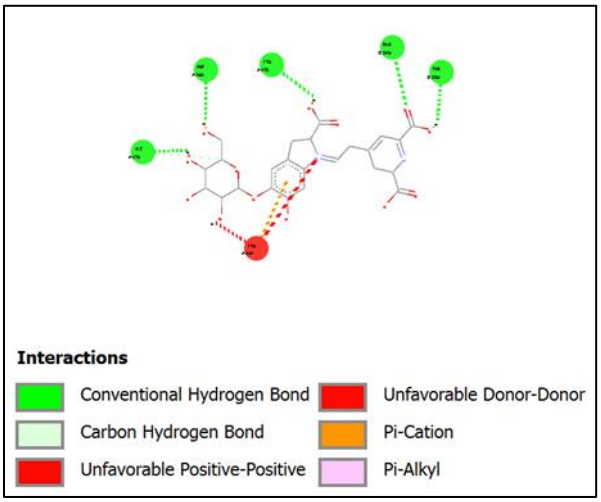


Figure 2 Interaction between betanin and the PPAR-γ receptor

The antioxidant activity of betanin in previous studies supports the docking results in this research. Betanin is the main betalain pigment in beets (*Beta vulgaris* L.) that has the ability to scavenge free radicals and reduce the formation of ROS (16,29). A decrease in ROS can help reduce pancreatic β-cell damage and improve insulin signaling pathways (30). The combination of antioxidant activity and interaction capability with PPAR-γ makes betanin a potential candidate for natural compounds to be developed as preventive agents or alternative therapies for type 2 diabetes mellitus.

Although the docking results show promising potential, this study still has limitations because it was only conducted in silico. Therefore, further research involving molecular dynamics simulations, in vitro, and in vivo tests is needed to evaluate the stability of the complex and to more comprehensively confirm the biological activity of betanin against the PPAR-γ receptor.

3.2. Prediction Results of Betanin Compound Toxicity from *B. vulgaris*

Toxicity and ADME testing were conducted using three virtual laboratories, including pK-CSM, Pro-Tox, and Swiss ADME. The betanin compound has a complex structure with an aromatic ring and polar groups. Betanin has a molecular weight of 550.47 g/mol, exceeding the ideal threshold of Lipinski's rule. Lipinski's rule of five states that poor absorption or permeability of a compound involves a molecular weight >500 Da, hydrogen bond donors >5, log P >5, and hydrogen bond acceptors from nitrogen and oxygen atoms >10 (31). Based on the Log P value, it can be interpreted that betanin has low lipophilicity, supporting diffusion through lipid membranes, but is hindered by its size and polarity (Table 2). This is due to its high TPSA value, resulting in poor membrane permeability (31–33). Based on water solubility values, this compound has fairly good water solubility due to the numerous polar groups. However, this does not guarantee good systemic absorption. From a pharmacokinetic perspective, betanin is predicted to have low oral absorption capability, does not cross the blood-brain barrier, does not inhibit several major metabolic isoenzymes, and has a low risk of drug interactions. Meanwhile, from a drug likeness prediction, betanin does not meet general criteria because it violates three Lipinski rules, with predicted low bioavailability and being less ideal as an oral drug candidate.

Table 2 Toxicity Prediction Results using Swiss ADME

	Characteristics	**Results**
Physicochemical properties	Molecular weight	550.47 g/mol
	TPSA	247.11 Å^2
Lipophilicities	Nilai consensus Log P	-1.50
Water solubility	ESOL	-2.05 (soluble)
	Ali	-3.44 (soluble)
	SILICOS	1.78 (soluble)
Pharmacokinetics	GI absorption	Low
	BBB permeability	No
	P-gp substrate	No
	Inhibitor CYP (1A2, 2C9, 3A4)	No
Drug likeness	Lipinski	3 violations
	Bioavailability	0.11

The pk-CSM profile confirms the findings of Swiss ADME. Based on the pK-CSM results, the pharmacokinetic properties of betanin, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), are known. The prediction results indicate that betanin has sufficient water solubility, with a value of -2.852 log mol/L, consistent with the Swiss ADME output which rates it as "soluble" (Table 3). Betanin has no probability of being absorbed by the human intestine, aligning with Swiss ADME's classification as weak, which impacts oral bioavailability. P-glycoprotein is not affected by betanin during the absorption process. In the distribution phase, the volume of distribution is categorized as low at -1.341 log L/kg, suggesting the compound tends to remain in plasma with only a small portion distributed to tissues. Betanin does not have the ability to permeate the blood-brain barrier or the central nervous system. Based on metabolism predictions, betanin is not predicted to be a substrate or inhibitor of metabolic enzymes such as CYP3A4, thereby reducing pharmacokinetic risks associated with it. Meanwhile, the excretion capability, based on predicted clearance, is classified as moderate with a value of 2.88 mL/min/kg.

From the toxicity perspective, betanin is non-mutagenic based on the AMES test. The predicted tolerated dose for humans is 0.599 log mg/kg/day. In animal testing predictions using rats, an LD50 of 2.477 log (mol/kg) was obtained, which falls into the moderate toxicity category, and the Lowest Observed Adverse Effect Level (LOAEL) is 3.477 log (mg/kg bw/day). Furthermore, the prediction results indicate no toxicity to the liver, although this differs from toxicity

predictions in Pro-Tox. This discrepancy may be due to differences in datasets, QSAR parameters, and the model's ability to predict metabolite bioactivation, thus requiring further verification through in vitro studies. Based on the Pro-Tox model predictions, the compound has the potential to cause hepatotoxicity and immunotoxicity but does not show potential for nephrotoxicity, mutagenicity, carcinogenicity, cytotoxicity, or clinical and nutritional toxicity. The related molecular pathways involving PPAR-γ, MMP, and p53 are indicated as inactive, suggesting no significant interactions that could trigger further toxicity.

Table 3 Pharmacokinetic Properties of Betanin using pK-CSM

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	-2.852	Log mol/L
	Intestinal absorption	0	%
	P glycoprotein substrate	No	yes/no
Distribution	VDSS	-1.341	log L/kg
	BBB Permeability	-1.847	log BB
	CNS Permeability	-4.742	log PS
Metabolism	CYP3A4 substrate	No	yes/no
	CYP3A4 inhibitor	No	yes/no
Excretion	Total clearance	0.46	log ml/min/kg
Toxicity	AMES Toxicity	No	yes/no
	Max. Tolerated dose	0.599	log mg/kg/hari
	Oral Rat Acute Toxicity	2.477	log (mol/kg)
	Oral Rat Chronic Toxicity	3.477	log (mg/kg_bw/day)
	Hepatotoxicity	No	yes/no

Furthermore, the oral prediction toxicity outcome using Pro-Tox 3.0 indicates that betanin falls into toxicity class 4 (>300–2000 mg/kg body weight), thus categorized as a hazardous substance if ingested in large amounts, but not classified as a highly toxic substance (34,35). This is because the predicted oral LD50 of betanin is 305 mg/kg. The average similarity and prediction accuracy of the model are 45.17% and 54.26%, respectively.

4. Conclusion

This finding indicates that betanin is capable of forming stable and strong bonds at the active site of the PPAR-γ receptor with a binding affinity value of -8.9 kcal/mol. The betanin compound has the potential as a natural antidiabetic candidate through a mechanism of modulating the PPAR-γ receptor, which is related to increased insulin sensitivity and reduced oxidative stress in type 2 diabetes mellitus conditions. Betanin also has an LD50 ranging from 305 mg/kg, categorizing it as toxicity class 4. However, further research through molecular dynamics studies, in vitro, and in vivo experiments is necessary to verify these findings.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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