Trop J Nat Prod Res, November 2024; 8(11): 9079 - 9085

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In Silico Study of the Antidiabetic Effect of Ethyl Acetate Fraction of Banana Bract in Diabetic Rats

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ARTICLE INFO

Article history:
Received 12 June 2024
Revised 30 July 2024
Accepted 02 November 2024
Published online 01 December 2024

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion, action, or both. Hyperglycemic conditions alter cell morphology and the extracellular matrix, including MDA, CRP, Nf-kB, and insulin. Anthocyanins, a class of flavonoids, exhibit antidiabetic activity by binding to specific receptors. These compounds exert their effects by competitively binding at the active site of the α-glucosidase enzyme. This study aims to predict the interaction of anthocyanin compounds with MDA, CRP, Nf-kB, and insulin receptors *in silico*. This study was conducted using AutoDock Vina software supported by the Vega ZZ, PyMOL, and BIOVIA Discovery Studio Visualizer program to create visual profiles of MDA, CRP, Nf-kB, and insulin ligand proteins, which were compared with anthocyanin compounds. Meanwhile, pharmacokinetic predictions were conducted using pkCSM. The post-docking analysis revealed that the binding affinity of anthocyanin compounds, as indicated by ΔGbinding scores, was most significant against Nf-kB (-7.36 kcal/mol; 84.40%), MDA (-6.53 kcal/mol; 111.81%), CRP (-4.00 kcal/mol; 167.36%), and insulin proteins (-4.51 kcal/mol; 182.60%), surpassing the ΔGbinding scores of acarbose, which were -1.08 kcal/mol (12.38%), -2.63 kcal/mol (45.55%), -3.77 kcal/mol (157.74%) and -0.08 kcal/mol (3.04%), respectively. These findings suggested that flavonoids, particularly anthocyanin, from ethyl acetate fraction of banana bract (*Musa acuminata colla*) have the potential as natural therapeutic agents for the treatment of type 2 diabetes mellitus.

Keywords: Hyperglycemia, Type 2 diabetes mellitus, Molecular docking, Banana bract, α-glucosidase, Antidiabetic.

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia resulting from impaired insulin secretion, action, or both. [1] The etiology of diabetes is complicated,[2] involving the inability of the pancreas to produce sufficient insulin or the development of insulin resistance.[3] Insulin plays a critical role in facilitating glucose uptake by cells.[4] Hyperglycemia results from an accumulation of glucose in the bloodstream caused by either insufficient insulin or resistance to insulin.[5] Type 2 diabetes mellitus (T2DM) is the most prevalent form of the disease,[6] affecting approximately 422 million people worldwide, with the majority residing in low- to middle-income nations. The disease is responsible for 1.5 million deaths annually.[6]

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Citation: Hardono, H, Soetrisno, S, Purwanto, B, Wasita, B, Nurwati, I, Pamungkasari, EP. *In Silico* Study of the Antidiabetic Effect of Ethyl Acetate Fraction of Banana Bract in Diabetic Rats. Trop J Nat Prod Res. 2024; 8(11): 9079 – 9085. https://doi.org/10.26538/tjnpr/v8i11.16

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Acarbose is an effective medication for managing hyperglycemia, functioning as an α-glucosidase inhibitor.[7] It is frequently prescribed either as monotherapy or in combination with other oral antihyperglycemic agents.[8] However, its use has been associated with adverse effects such as hepatotoxicity and gastrointestinal discomfort, including excess gas, abdominal distention, meteorism, bloating, and diarrhea.[9] Among natural substances, polyphenols are particularly beneficial, noted for their anti-inflammatory and antioxidant properties.[10] A crucial component of polyphenols in T2DM treatment is anthocyanin, a class of flavonoids. [11] Flavonoid compounds exhibit antidiabetic activity by binding to specific receptors. Anthocyanins have a structure similar to acarbose and demonstrate antidiabetic properties. These compounds exert their effects by competitively binding at the active site of the α-glucosidase enzyme in the microvilli of the small intestine.[12,13]

Banana bracts, which are abundant yet underutilized in Indonesia, are rich in anthocyanins. Although banana bracts are frequently being discarded as waste, they are actually a rich source of potassium, vitamins A, C, and E, fatty acids, and minerals. In addition, bananas are rich in bioactive compounds such tannins, alkaloids, flavonoids, and saponins, which known for their antioxidant properties. The high concentration of phytochemicals in banana bracts suggests potential for improving health.[14,15] Ethyl acetate fraction is a method used to isolate compounds, such as anthocyanins, from banana bracts. Hyperglycemic conditions lead to changes in cell morphology and the extracellular

matrix, including malondialdehyde (MDA), C-reactive protein (CRP), nuclear factor-kappa B (Nf-kB), and insulin. The byproduct of lipid peroxidation is used as a biomarker for oxidative stress.[16] High blood glucose levels can trigger inflammatory responses in the body, resulting in the release of proinflammatory cytokines, which contribute to insulin resistance.[16]

Computational modeling can be used to conduct research on the potential applications of herbal ingredients and drug discovery.[17] One such technique is called "molecular docking," which predicts how ligands bind to receptors, making it particularly useful in the development of drugs from natural substances. [18] Computational modeling can enhance efficiency, reduce errors, and increase productivity. Therefore, this study used a computational approach to investigate the potential interactions of bioactive compounds, namely anthocyanins, present in the banana bract fraction for the treatment of T2DM using *in silico* methods.

Materials and Methods

This study used an Asus i5 laptop for receptor preparation and docking validation, employing AutoDock4 Tools (v1.5.6) from The Scripps Research Institute's Molecular Graphics Laboratory. The Vega ZZ application (version 3.0, Istituto di Chimica Farmaceutica e Tossicologica "Pietro Pratesi", Università degli Studi di Milano) was downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The structural datasets used in this study included the following: PDB ID: 4DN5 for nuclear factor kappa B (Nf-κB); PDB ID: 6VJ3 for the malondialdehyde (MDA) PD protein; PDB ID: 1B09 for the C-Reactive Protein (CRP) protein; and PDB ID: 1ZT3 for the insulin protein. These datasets were obtained from the Protein Data Bank website (www.rcsb.org). The docking results for the interaction of the Nf-kB, MDA, CRP, and insulin proteins were visualized using BIOVIA Discovery Studio Visualizer and the SwissADME program (http://www.swissadme.ch). Toxicity predictions were conducted using the online pkCSM tool (http://structure.bioc.cam.ac.uk/pkcsm).

The fractionation of the banana bracts was performed in three stages using water, n-hexane. and ethyl acetate by the separation funnel method.[19] The quantification of anthocyanins was carried out using spectrophotometry, specifically the pH differential method, at wavelengths of 500 and 700 nm.[20]

Assembly of the Protein and Ligands

The BIOVIA Discovery Studio Visualizer software was used to visualize the Nf-kB, MDA, CRP, and insulin proteins. The first step involved separating the proteins from their original ligands and receptor residues, after which all files were saved. For ligand preparation, the SMILES representations of anthocyanin compounds were input into the Vega ZZ software to optimize their three-dimensional structure. The compounds were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov), saved in PDB format, and subsequently converted to PDBQT format using the AutoDock program.

Docking Parameter Validation

AutoDock version 1.5.6 was employed to validate the parameters in this study, which included redocking the original ligands of the following proteins: MDA (PDB ID: 6VJ3), insulin (PDB ID: 1ZT3), CRP (PDB ID: 1B09), Nf-κB (PDB ID: 4DN5), and CRP (PDB ID: 1B09). Grid parameters and root mean square deviation (RMSD) values were obtained from this process. RMSD measures the size of the docking zone, or the average distance between the docking pose and the crystallographic ligand pose, with a value less than 2 Å indicating a close alignment. The determination of the ligand binding location during the docking process required careful consideration of the grid box layout, which was based on the ligands bound to the protein macromolecules during the downloading process.[21]

Molecular Docking

AutoDock Vina version 1.5.6 was employed to carry out the molecular docking procedure. The ligand and receptor files were properly prepared and saved in PDBQT format, were stored in the same directory, namely in the "vina" folder within the C drive. The Command Prompt (CMD) software was used to execute the program within the designated folder to initiate the molecular docking process. The “vina.exe --config conf” command was employed to open the Vina executable configuration file, retrieve the RMSD metric, and obtain the docking score. Subsequently, the “txt-log log.txt” command was run to compile all necessary documents for the docking procedure.

The PDBQT files for the Nf-kB, MDA, CRP, and insulin proteins were recorded in a new file named “conf.txt”, with ligands appropriately labeled. The values within the designated grid box corresponded to the x, y, and z axes in PDBQT format. Using the Vina command, the RMSD values of the molecular docking process were calculated. The “txt-log log” program was executed via the CMD to run the configuration file. The RMSD values of all configurations were found to be less than 2 Å. An RMSD value if less than 2 Å was considered valid, indicating a successful docking process, while values greater than 2 Å were deemed invalid.[22]

Display of Molecular Docking Information

The data from the molecular docking were visualized using the BIOVIA Discovery Studio Visualizer (v20.1) across three experimental iterations. Additionally, the results were analyzed using the PyMOL software, which provides 3D visualization features to assess the interactions between test compounds and proteins. This procedure involved evaluating the bonds formed between the ligands and proteins to observe the occupied surface area. The visualization results were then utilized to illustrate the interactions between the proteins and tested ligands.

Evaluation of Toxicity and Pharmacokinetics Parameters

Using the SwissADME online tool and pkCSM, the pharmacokinetic characteristics and toxicity of anthocyanins were predicted based on the Lipinski’s rule.[23] Table 4 provides a detailed description of the ADME features that were acquired, including absorption, distribution, metabolism, and excretion.

Results and Discussion

The grid box configuration was crucial in the validation of the molecular docking approach. A key step in this validation was determining the interactions between the ligands and amino acids at the designated target protein binding sites. To ensure that the test compounds and the original ligands were included in the grid box, the grid box was resized to fit both of them. The grid box settings were determined by the grid size and grid center axes. For this study, the grid box coordinates for the original ligands Nf-kB, MDA, CRP, and insulin were set as follows: x: -8.074, y: 29.309, z: -3.518 with a box size of 40 x 40 x 40; x: 15.326, y: 3.803, z: 13.121 with a box size of 40 x 48 x 42; x: 139.03, y: 171.723, z: 34.896 with a box size of 40 x 40 x 40; and x: -27.874, y: -7.671, z: 16.942, with a box size of 40 x 40 x 40, respectively.

These values were calculated based on the positions of the standard ligand molecules within the Nf-kB, MDA, CRP, and insulin proteins as listed in the Protein Data Bank (PDB) files. The binding interactions between anthocyanin compounds and the MDA, CRP, Nf-kB, and insulin receptors were studied *in silico*. The BIOVIA DS Visualizer tool was used to prepare the proteins and ligands. Ligand preparation involved optimizing the three-dimensional structure of anthocyanin compounds using the Vega ZZ program. Pharmacokinetics were predicted using pkCSM models, while molecular docking applications were used to evaluate drug design and protein affinity. The docking score, which measures binding affinity, was determined through the molecular docking results.

The molecular docking results indicated that the ΔGbinding scores from Nf-kB with anthocyanin test ligands were -8.72 kcal/mol (84.40%). When combined with anthocyanin test ligands, MDA showed a value

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

of -5.85 kcal/mol (111.81%), CRP showed a value of -2.40 kcal/mol (167.36%), and insulin showed a value of -2.47 kcal/mol (182.60%). In comparison to the test ligands, the binding affinity binding scores of the original ligands with acarbose were lower. The initial ligand ΔGbinding scores for Nf-kB, MDA, CRP, and insulin were -1.08 kcal/mol (12.38%), -2.66 kcal/mol (45.55%), -3.77 kcal/mol (157.74%), and -0.08 kcal/mol (3.24%), respectively (Table 1).

Table 1: Molecular Docking Results of Banana Bracts, Acarbose, and Anthocyanin

Ligand	Protein	Compound	ΔGbinding Score (kcal/mol)	Percentage	RMSD
Banana bract					
	Nf-kB	Original ligand	-8.72		2.40
	Anthocyanin	Test ligand	-7.36	84.40	
	Acarbose	Original ligand	-1.08	12.38	
	MDA	Original ligand	-5.85		1.078
	Anthocyanin	Test ligand	-6.53	111.81	
	Acarbose	Original ligand	-2.66	45.55	
	CRP	Original ligand	-2.40		1.421
	Anthocyanin	Test ligand	-4.00	167.36	
	Acarbose	Original ligand	-3.77	158.74	
	Insulin	Original ligand	-2.47		0
	Anthocyanin	Test ligand	-4.51	182.60	
	Acarbose	Original ligand	-0.08	3.04	

The results indicated a binding affinity between anthocyanin molecules and acarbose. Anthocyanins belong to the flavonoid group and share biosynthetic pathways and structural features with other flavonoid subgroups, such as flavanones and isoflavones.[13] This structural similarity enable anthocyanins to inhibit the alpha-glucosidase enzyme in the small intestine.[24] This enzyme is crucial for the breakdown of carbohydrates into glucose. By inhibiting α-glucosidase, anthocyanins can slow glucose absorption, thereby reducing postprandial blood sugar spikes. Moreover, anthocyanins possess potent antioxidant properties that can mitigate oxidative stress commonly associated with diabetes complications.[25] By alleviating oxidative stress, anthocyanins may protect pancreatic cells and other tissues from damage. Additionally, anthocyanins enhance insulin sensitivity and regulate glucose metabolism, improving the body's ability to utilize glucose effectively and aiding in blood sugar control. [16] Their anti-inflammatory properties further contribute to reducing systemic inflammation, thereby enhancing blood sugar regulation and lowering the risk of diabetes-related complications. [26]

To observe variations in the crystallographic binding orientations on the active side of proteins in various poses with the same ligand, the RMSD values were calculated. An RMSD value of less than 2 Å was considered valid. In this study, the RMSD values for Nf-kB, MDA, CRP, and insulin were 2.40 Å, 1.078 Å, 1.421 Å, and 0 Å, respectively. These values indicated that the original ligand and the test ligand interacted correctly with the MDA, CRP, and insulin proteins. On the contracy, an RMSD value of greater than 2 Å suggested significant deviations and increased prediction errors. Therefore, it is not advisable to use ligand-receptor interactions with such RMSD values as references *in silico*. [27] In this study, acarbose, a typical medicinal molecule, had an RMSD value of 0 Å (< 2 Å) during molecular docking. The visualization of molecular docking results allowed for the identification of test ligand-target protein interactions. Amination of Nf-kB, MDA, CRP, and insulin proteins by anthocyanin resulted in the formation of amino acid residues on hydrogen bonds (Table 2). In particular, Nf-kB proteins formed non-hydrogen bonds with Pro624, Cys622, and Ser621. MDA proteins formed hydrogen bonds with Thr199, Gln97, and Asn67, and non-hydrogen bonds with His98, Leu198, Val143, Glu106, His119, His94, Thr200, Trp209, Val121, Ile91, Phe131, and Glu69. CRP proteins formed a hydrogen bond with Gln150 and non-hydrogen bonds with Ser74, Asn61, Glu81, Phe66, and Asp140. Insulin proteins formed non-hydrogen bonds with Ser186, Val183, and Leu187.

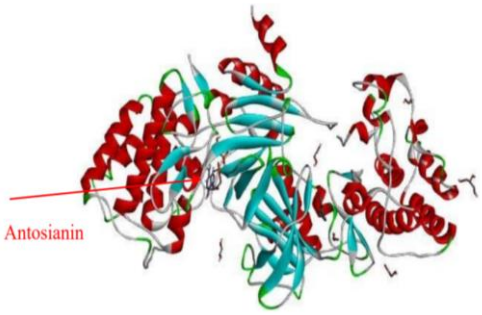
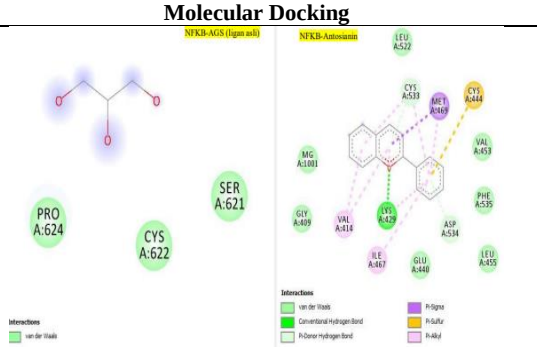
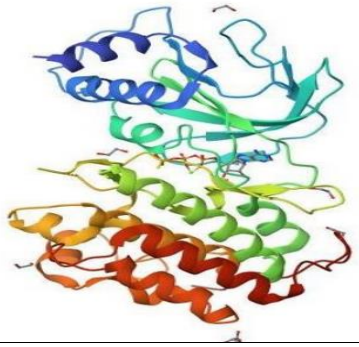
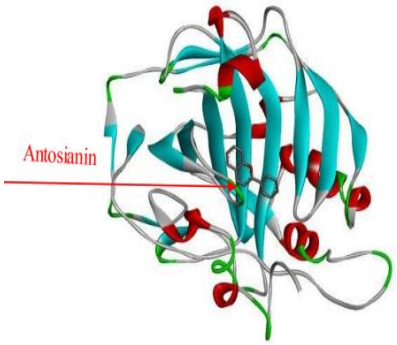
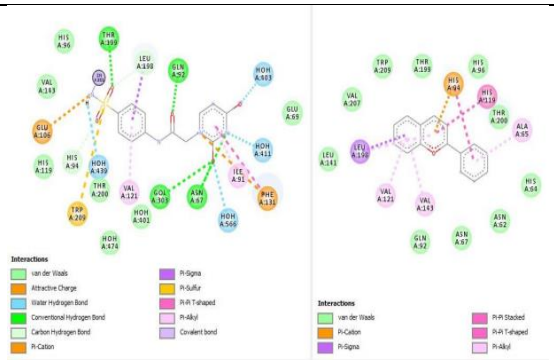
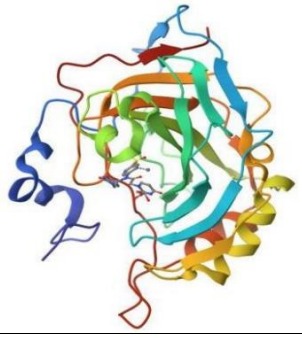
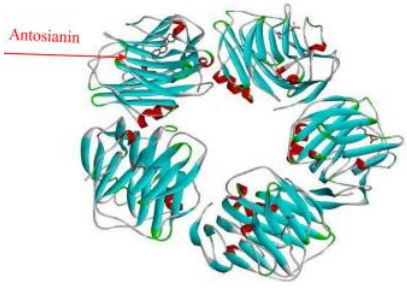
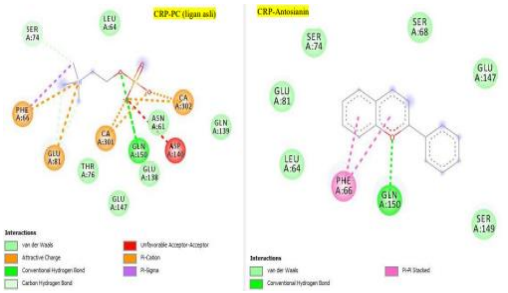
According to binding affinity and RMSD values of less than 2 Å, anthocyanins may exhibit anti-diabetic properties comparable with conventional treatments such as acarbose. Lipinski's rule states that a compound with a molecular weight of less than 500 g/mol (207.25 g/mol), a log P value of less than 5.5 hydrogen donors (0), and fewer than 10 hydrogen acceptor bonds are considered good candidates for pharmaceuticals.[28,29] Using pkCSM to predict the pharmacokinetic profile of drug absorption (Table 3), the anthocyanin compounds demonstrated strong absorption potential, as indicated by a log Kp value of -5.07. This suggested their viability as candidates for novel drug development. Additionally, predictions regarding the distribution of anthocyanin compounds indicated their ability to cross the blood-brain barrier (BBB), as affirmed by a positive BBB result. The BBB is crucial in regulating the influx and efflux of substances essential for brain and neural metabolic activities, making its integrity and functionality vital for maintaining the homeostasis of the brain's microenvironment.[30]

The detoxification enzyme cytochrome P450, which is found in the liver, aids in the oxidation processes that occur during the metabolic processes of a compound. This enzyme works by oxidizing external materials, such as drugs.[31] It is expected that CYP1A2 and CYP2D6 enzymes will be inhibited during the metabolism of anthocyanin compounds. Additionally, a log Kp value greater than -2.5 cm suggested good skin permeability, according to the excretion experiments of the anthocyanin compounds. The AMES test was used *in silico* to predict the chemical activity and mutagenicity of the ethyl acetate fraction of the banana bract.

The toxicity prediction results indicated that the fraction was unlikely to contain mutagenic compounds, with no evidence of hepatotoxicity or skin allergies. Toxicity was assessed using the LD50 parameter, which measures the acute oral toxicity in rats to establish relative toxicity levels. The term "LD50" refers to the dosage at which 50% of test animals die. The findings of the toxicity prediction are presented in Table 5, where the maximum dose that could be given to test animals for genistein is 2.76 mol/kg, and the LD50 value for anthocyanin compounds is 1.848 mol/kg. It was estimated that the highest tolerable dosage is 0.609 mg/kg per day.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: 3D Visualization of Molecular Docking between Ligands

Ligand	3D Visualization	Molecular Docking
Anthocyanin		
Nf-kB		
Anthocyanin		
MDA		
Anthocyanin		

ISSN 2616-0684 (Print)
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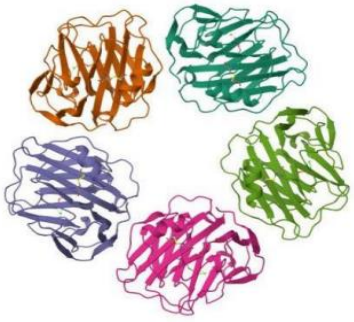
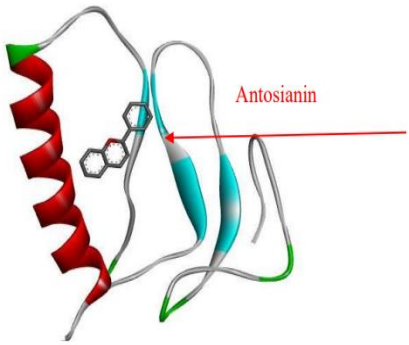
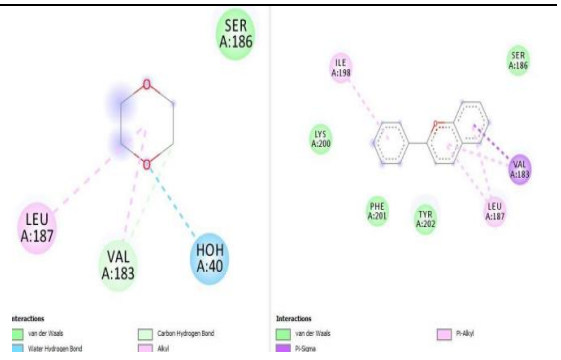
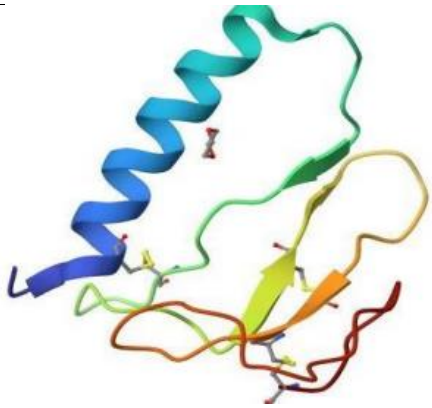
CRP		
Anthocyanin		
Insulin		

Table 3: Molecular Properties of pkCMS

Compound	Formula	Molecular Weight (g/mol)	H-bond Acceptor < 10	H-bond Donor < 5	Log P < 5
Anthocyanin	C15H11O+	207.25	0	0	4.38

Table 4: Prediction of Pharmacokinetic Characteristics

Banana bract		
Absorption	GI absorption	High
	TPSA	13.14 A^2
	Bioavailability	0.55
D. Distribution	BBB permeant	Yes
	P-gp substrate	Yes
M. Metabolism	CYPI A2 inhibitor	Yes
	CYP2C 19 inhibitor	No
	CYP2C 9 inhibitor	No
	CYP2D 6 inhibitor	Yes
	CYP3A 4 inhibitor	No
E. Excretion	Log Kp	-5.07 cm/s

The digestive tract's absorption process is facilitated by the prediction of ADME molecules with a high GIA. Compounds can alter the distribution process and bioavailability because they can pass through the brain barrier and are hydrophobic (P-gp substrate).

Table 5: Toxicity Prediction Results

Model Name	Anthocyanin
AMES Toxicity	No
Max tolerated doses (human)	0.44 mg/kg
Herg I inhibitor	No
Herg II inhibitor	No
Acute oral toxicity (LD50) IN rats	1.848 mol/kg
Chronic oral toxicity in rats	1.118 log mg/kg/bb
hepatotoxicity	No
Skin sensitization	No
Minnow toxicity	0.172 mM

Anthocyanins are safe at lower human doses, do not inhibit hERG1, and are safe at oral and renal levels in rats. Hepatotoxicity, increased toxicity, and lack of allergy-causing properties

Conclusion

The ethyl acetate fraction of banana bracts (*Musa Acuminata Colla*) contains anthocyanin compounds with docking scores that are comparable to those of conventional medications, such as acarbose. Based on the results of molecular docking, three target macromolecules that had the best ΔGbinding values against compounds from banana bracts were obtained, namely MDA, CRP, and insulin. Of the six target macromolecules of anthocyanin compounds, some had lower or higher ΔGbinding values compared to the original ligands of each target macromolecule. Anthocyanin compounds from the ethyl acetate fraction of the banana bracts were found to be non-toxic. These findings are promising for drug discovery efforts utilizing natural sources of anthocyanin compounds. Future in vitro and in vivo studies are recommended to further investigate the clinical potential of these compounds as antidiabetic agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to express their gratitude to Aisyah Foundation Lampung that have provided financial support for this study. The authors also extend their gratitude to the head of the Laboratory of the Faculty of Pharmacy at the University of Muhammadiyah Surakarta in Indonesia, for granting the necessary study permissions and providing the required facilities.

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