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Antidiabetic Activity of Ethyl Acetate Compounds from Gayam (*Inocarpus fagiferus*): In Silico and In Vivo Studies

Mus Ifaya*, Bai A. Ridwan, Putri S. Laugi, Apriliani P. Utami, Syawal Abdurrahman
Department of Pharmacy, Faculty of Science and Technology, Universitas Mandala Waluya, Kendari, Southeast Sulawesi, Indonesia

Abstract
Diabetes mellitus is characterized by high blood sugar levels. Although many drugs are available, blood glucose control remains challenging to achieve due to decreased beta cell function and side effects of long-term use. Gayam tree bark (*Inocarpus fagiferus*) has the potential to inhibit digestive enzymes, although the molecular mechanism of its antidiabetic effect has not been ascertained. This study aims to determine the antidiabetic activity of compounds from ethyl acetate extract, tested in silico and in vivo. This study used molecular docking and in vivo activity analysis. LC-MS analysis was used to determine the compounds in the ethyl acetate fraction of gayam bark. Through molecular docking and ADME-toxicology, compounds in the extract showed antidiabetic activity. The molecular docking analysis revealed, that Cochliophilin A had a lower binding energy than acarbose toward the α-amylase receptor÷ (-9.76 kcal/mol vs -8.95 kcal/mol). The ADME-Tox model predicted good skin permeability, absorption, and distribution for Cochliophilin A. The ethyl acetate extract showed antidiabetic activity in lowering blood sugar levels in rats on day 14, by 74% (IV), 72% (V), and 74% (VI), respectively. Collectively, these findings suggest that compounds in gayam bark are useful in treating diabetes.

Keywords: antidiabetic, *Inocarpus fagiferus*, mass spectrometry, molecular docking

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*Corresponding author:
mus.ifaya@umw.ac.id

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Aktivitas Antidiabetik Senyawa Etil Asetat dari Gayam (*Inocarpus fagiferus*): Studi *In Silico* dan *In Vivo*

Abstrak
Diabetes melitus ditandai dengan kadar gula darah tinggi. Meskipun banyak obat tersedia, kontrol glukosa darah masih sulit dicapai karena penurunan fungsi sel beta dan efek samping penggunaan jangka panjang. Kulit pohon gayam (*Inocarpus fagiferus*) berpotensi menghambat enzim pencernaan walaupun mekanisme molekuler efek antidiabetesnya belum dipastikan. Penelitian ini bertujuan menentukan aktivitas senyawa dari ekstrak etil asetat sebagai antidiabetes yang diuji secara *in silico* dan *in vivo*. Selain itu, penelitian ini menggunakan penambatan molekuler dan analisis aktivitas *in vivo*. Analisis LC-MS digunakan untuk menentukan kandungan senyawa dalam fraksi etil asetat kulit gayam. Melalui prediksi interaksi molekuler dan ADME-toksikologi, senyawa dalam kulit gayam menunjukkan aktivitas antidiabetes. Dalam studi interaksi penambatan molekuler, senyawa Cochliophilin A memiliki energi ikatan lebih rendah daripada akarbose pada reseptor α-amilase÷ (-9,76 kcal/mol dan -8,95 kcal/mol). Model ADME-Tox memprediksi permeabilitas kulit, absorpsi, dan distribusi yang baik untuk Cochliophilin A. Uji aktivitas kelompok (IV-VI) ekstrak etil asetat menunjukkan aktivitas antidiabetes menurunkan kadar gula darah pada tikus pada hari ke-14, masing-masing sebesar 74% (IV), 72% (V), dan 74% (VI). Secara keseluruhan, senyawa dalam kulit gayam bermanfaat dalam pengobatan diabetes.

Kata Kunci: antidiabetik, penambatan molekuler, *Inocarpus fagiferus*., Spektrometri Massa

1. Introduction

Increased blood glucose levels due to impaired insulin metabolism are the cause of a serious long-term (chronic) disease known as diabetes. In 2017, four million people worldwide died from diabetes mellitus (DM), which is considered one of the ten leading causes of death in adults.1 In 2019, 463 million people were reported to have DM, with 9.3% of representing the age group between 20 and 70 years. Estimates suggest that this disease will affect 578.4 million to 700.2 million people between 2030 and 2045.[2,3] Maintaining regular blood glucose levels is the goal of DM treatment. Diabetes can be treated with three types of drugs. The first group includes sulfonylureas, such as glibenclamide, glinides, insulin analogs, glucagon-like peptide 1 (GLP-1) agonists, and dipeptidyl peptidase-4 (DPP-4) inhibitors. The second group comprises, which are peroxisome proliferator-activated gamma receptor (PPAR) agonists, and the biguanide metformin.[4]

The third group, contains an α-glucosidase inhibitors, which inhibit this enzyme, thereby reducing the digestion and absorption of complex carbohydrates.[5] This makes it useful for treating type -2 DM. Although modern medicine can be used to treat DM, both orally and by injection, its high cost can cause problems. In addition to these drugs, traditional medicines derived from plants can be used.[6] In order to discover new drugs as an alternative treatment for DM, many drugs derived from natural ingredients have been investigated. One of the native plants of Indonesia known for its antidiabetic properties is the bark of the gayam tree (*(Inocarpus fagiferus*), which is traditionally used by the community in Kulisusu District, North Buton Regency, for the treatment of diabetes. By boiling it in water and then drinking it. The ethanol extract of gayam tree bark is reported to contain main compounds such as phenolics, flavonoids, and triterpenoids, which act as antioxidants and inhibit the formation of lipid peroxide by 63.04%.[7] However, there has been no research explaining the compounds present in the ethyl acetate fraction of gayam tree bark along with its molecular mechanisms *in silico* and *in vivo*. Therefore, it is necessary to explore their potential as antidiabetics agents.

2. Materials and Methods

2.1. Tools

The instruments employed included a Liquid Chromatography-Mass Spectrometry (LC-MS) system (Agilent Technologies 6400 Series Triple Quadrupole) for compound identification. For *in silico* testing, a computer with 8.00 GB RAM (7.40 GB usable), an AMD Ryzen 7 5800H processor with Radeon Graphics (3.20 GHz) processor, and an NVIDIA GeForce RTX 3050M Graphics Card (Taiwan) was used. The binding energy analysis were conducted using AutoDock Tools 1.5.6.[8], and the results of the molecular structure were visualized using Discovery Studio Visualizer. These results were then tested for *in vivo* activity using an Accu-Chek Performa glucometer.

2.2. Materials

The gayam tree bark (*I. fagiferus*) was collected from Jampaka Village in Kulisusu District, North Buton Regency, Southeast Sulawesi. For docking simulations, the two-dimensional structures of the reference ligand (acarbose), test ligands, and identified compounds were retrieved along with the selected macromolecule, alpha-amylase receptors (PDF ID code: 4UAC; resolution of 1.6 Å), from the Protein Data Bank (PDB) (http://www.rscb.org/pdb/). ADME-toxicty analysis was performed using an online platform at https://biosig.lab.uq.edu.au/pk. To perform the in vivo test, the materials used included distilled water, 96% ethanol, ethyl acetate, $MgSO_4$, acetone, methanol, n-hexane, alloxan monohydrate (Novomix®), acarbose (pharmaceutical grade), Swiss Webster mouse strain, sodium carboxy methyl cellulose (Na-CMC) (pharmaceutical grade), acetone pa, methanol pa, and chloroform pa.

2.3. Methods

2.3.1. Sample Preparation

Identification of the gayam bark plant (*I. fagiferus*) was carried out at the pharmacognosy and phytochemistry laboratory of Mandala Waluya University (082/09.3.01/VIII/2024), and samples were obtained from Jampaka Village, Kulisusu, Regency, North Buton Regency, Southeast Sulawesi Province.

2.3.2. Sample Extraction

Gayam bark powder (*I. fagiferus*) was obtained from 3 kilograms of samples prepared using the maceration method with 96% ethanol as the solvent, performed three times a day.[9] The thick ethanol extract of gayam bark had a yield of 19.49% and a weight of 584.86 grams.

2.3.3. Extract Fractionation

Liquid-liquid extraction was used to remove chlorophyll from the ethanol extract, using a 1:1 ethanol ratio. The mixture was stirred and left to stand for a day, until a precipitate formed. The chlorophyll-free filtrate (3 L) was then separated from the precipitate using

ethyl acetate (1:1). The ethyl acetate phase was then dried by adding $MgSO_4$ powder, and the mixture was filtered.[10]

2.3.4. Identification Using LC-MS

A Phenomenex® HPLC column (5µ C8; 150 × 2 mm i.d.) was used for the analysis. The chemical components of the pure ethyl acetate extract of gayam bark were identified using LC-MS/MS. A full scan was conducted over an m/z range up to 1200 at a source temperature of 140°C. Samples (0.5 g) were diluted with methanol and filtered through a 0.22 µm HPLC column. In MS, the dry gas temperature (N2) was 350°C, the gas flow rate was 6 mL/min, and the nebulization pressure (N2) was set to 25 psi.[11]

2.3.5. Protein Preparation

The active form of α-amylase receptor (4UAC), which binds acarbose, was selected from the PDB, with a mutation value of zero. Using AutoDock Tools, the 3D protein structure was downloaded as a PDB file, and acarbose was designated as the native ligand.[12]

2.3.6. Ligand Preparation

The ligand structures of acarbose and test compounds were derived from the pure ethyl acetate extract of gayam bark through the purification method. The two-dimensional (2D) structures of acarbose and test compounds were created using ChemDraw 8.0, which were then converted into three-dimensional (3D) structures.

2.3.7. Validation of Molecular Docking

The acarbose, as the native ligand, was re-docked to the α-amylase receptor, and the water and acarbose ligand residues from the protein were removed by changing the position of the grid boxes.[13]

2.3.8. Molecular Docking Simulation

Molecular docking simulations were performed using Chem3D Ultra 8.0 with the MM2 semi-empirical computational method to minimize energy consumption by optimizing the geometry of their 3D structure. For docking, each ligand was bound to the receptor in PDBQT format using tether coordinates (grid center) at x = 40 and, y = 40, and the grid box size for the α-amylase receptor at x = 28.499, y = 65. Interactions with biomacromolecules occurred in rigid conditions, and all binders were in stable conditions.14 The Discovery Studio Visualizer showed examples of hydrogen bonds, hydrophobic bonds, and long-range bonds.[15]

2.3.9. Prediction of Absorption, Distribution, Metabolism, Release, and Toxicity (ADME Tox).

The ADME-Tox SAR analyses were performed using the online platform provided by the Biosig Lab.[16] The obtained chemical structures were converted into SMILES format using PubChem, and the resulting structures were then downloaded using the SMILES canonical link.

2.3.10. Antidiabetic Activity

Before being treated, Swiss Webster mice (*Mus musculus*), were fed for 18 hours and weighed. Blood glucose levels (BGL) were measured by taking blood samples from the tail vein. The glucotrip that had been prepared before treatment was exposed to the blood that came out. Furthermore, for seven consecutive days, each group was given normal, negative, positive, and extract preparations orally.17 In addition, BGL were measured every day starting from the first day of raw material administration. After the mouse tails were cut, blood sugar levels were measured. Group I serves as the normal control (170 mg/kg.ip), group II the negative control (Sodium-CMC 0.5%), group III received glibenclamide (170 mg/kg.ip), group V received the ethyl acetate fraction (100 mg/kg BW), and group VI received the ethyl acetate fraction (300 mg/kg BW).

3. Result

3.1. Compounds Identified Using Liquid Chromatography-Mass Spectrometry

The analysis revealed the compound profile of the ethyl acetate extract from the separation of the ethanol extract of gayam tree bark (*I. fagiferus*). The compound profile can be observed from the chromatogram peaks, with different molecular weights. The molecular weights of the compounds obtained are listed in Table 1.

3.2. Preparation of Protein Receptor

By using chemical bonds, the α-amylase receptor (4UAC) interacted with organic materials. Figure 1 illustrates the position of the native ligand (acarbose) superimposed on the α-amylase receptor resulting from re-docking.

3.3. Validation of Molecular Docking Method

Discovery Studio Visualizer was used to analyze the bonds formed between acarbose and α-amylase receptor (Figure 1). Hydrogen bonds were formed between amino acids, including ASP109, GLN110,

Table 1. Compounds Contained in the Fraction of Gayam Tree Bark (*I. fagiferus*) Identified by Liquid Chromatography-Mass Spectrometry

No.	Compound	Observed m/z
1.	(2Z)-6-hydroxy-2-[(4-hydroxy-3methoxyphenyl)methylidene]	285.07581
2.	Phytosphingosine	317.29330
3.	3,4-dihydroxyphenylglycol	170.05788
4.	3,4-MDPA	221.14168
5.	7-hydroxy-3-(4-methoxyphenyl)-4Hchromen-4-one	268.07351
6.	Cochliophilin A	282.05291
7.	12-hydroxy-17-isopropenyl-20methoxy-8,8-dimethyl-3,9,23trioxahexacyclo	462.13164
8.	Bis(2-ethylhexyl) terephthalate	390.27689
9.	Bis(4-ethylbenzylidene)sorbitol	414.20435
10.	Bis(methylbenzylidene)sorbitol	386.17307
11.	Daidzein	254.05811
12.	Deacetylvindoline	414.21575

SER87, ASN191, LYS305, GLU86, SER85, and GLU246. The closest nearest residues in the acarbose–α-amylase receptor complex were TRP383, ASN157, TRP267, and TRP193 (Table 2), with a binding energy of -8.95 kcal/mol and an RMSD value of 0.57.

3.4. Docking Simulation of Acarbose and Test Ligand

Docking simulations were performed using AutoDock, with coordinate settings according to the interaction position of acarbose with the α-amylase receptor (4UAC). The analysis was performed to determine the value of binding energy and hydrogen bond formation between acarbose and the test ligands whose structure were identified through LC-MS (Table 2 and Figure 2).

The interaction between standard acarbose compounds derived from gayam bark (I. fagiferus) and the α-amylase receptor (4UAC) exhibited varying affinities. Cochliophilin A had a low binding energy toward the α-amylase receptor (4UAC). Figure 2 presents the visualization of the docking results.

3.5. ADME-Tox Prediction

Pharmacokinetic analysis and ADME-tox prediction

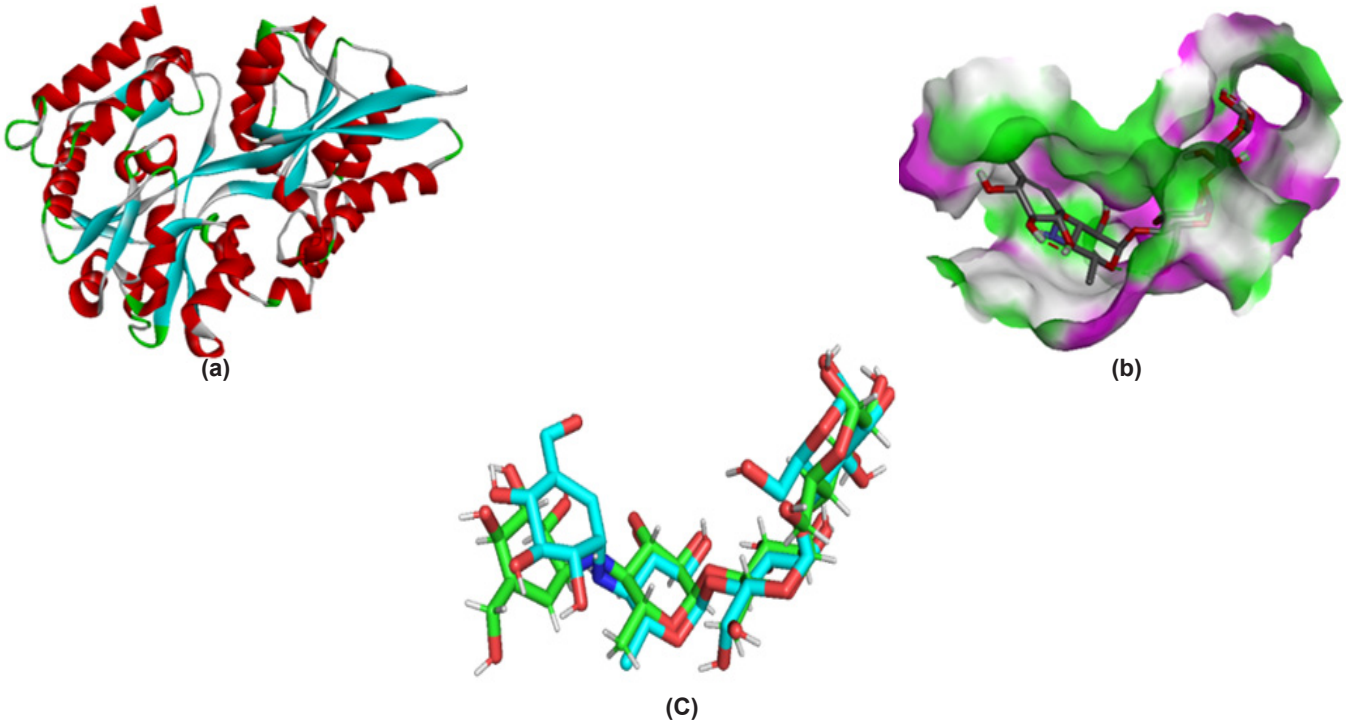
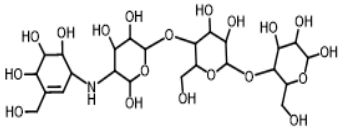
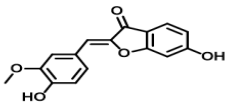
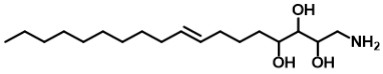
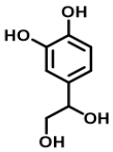
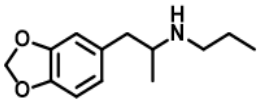
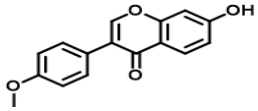
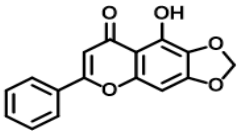
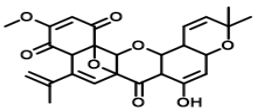
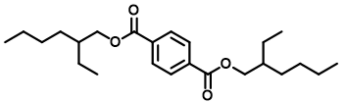
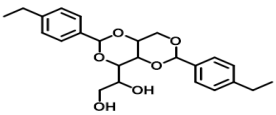
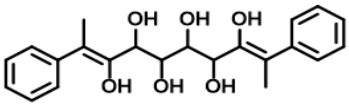
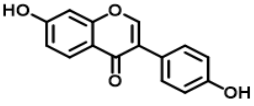


Figure 1. (a) Structure of α-amylase receptor (4UAC). (b) Representation of the interaction between the α-amylase receptor (4UAC) and acarbose, with the white clouds representing the hydrophobic interactions. (c) An overlay of the acarbose pose docked with the cocrystallized ligand 4UAC. The native ligand acarbose is shown in green before docking and in blue after docking.

Table 2. Docking simulation results.

IUPAC Name	Binding Energy (kcal/mol)	Hydrogen Bond Distance (Å)	Amino Acids that Bind	Nearest Amino Acid Residue(s)	Structure
Native ligand acarbose	-8.95	1.73, 2.54, 2.83, 2.44, 2.65, 2.22, 1.88 and 2.65	ASP109, GLN110, SER87, ASN191, LYS305, GLU86, SER85, GLU246	TRP383, ASN157, TRP267, TRP193	
(2Z)-6-hydroxy-2-[(4-hydroxy-3methoxyphenyl) methylidene	-6.28	1.86, 2.09	ASP109, SER87	TRP193	
Phytosphingosine	-4.85	1.96, 1.97, 2.30, 2.04	GLU86, SER85, LYS305, ASN157	ALA107, TRP193, TRP267	
3,4-dihydroxyphenylglycol	-7.48	2.85, 2.05	SER87, ASP109	TRP193, LYS305, ALA107, TRP267, TRP383	
3,4-MDPA	-8.03	2.14, 2.01, 2.26	ASP109, ASN191, ASN264	TRP193	
7-hydroxy-3-(4-methoxyphenyl)-4Hchromen-4-one	-8.05	2.07, 2.00, 2.26	ASN157, LYS305, SER85	TRP383, TRP193	
Cochliophilin A	-9.76	2.83, 2.73	SER85, ASN191	TRP267, LYS305, TRP383,	
12-hydroxy-17-isopropenyl-20methoxy-8,8-dimethyl-3,9,23trioxahexacyclo	-7.6	2.08, 1.96	ASN157, LYS305	TRP383, ALA107, TRP267	
Bis(2-ethylhexyl) terephthalate	-8.4	1.96, 2.19	ASN157, ASN191	PRO113, LYS90, TRP383, TRP267	
Bis(4-ethylbenzylidene) sorbitol	-7.22	2.25, 2.20, 1.90	GLU86, LYS305, ASN157	TRP267, ALA107, TRP193, TRP383	
Bis(methylbenzylidene) sorbitol	-8.38	1.92, 2.18, 2.31, 2.00, 2.20	GLU246, ASN264, TRP267, ASN191, ASP109	TRP193, TRRP383	
Daidzein	-8.61	2.88, 2.68	GLU246, ASN191	TRP193	

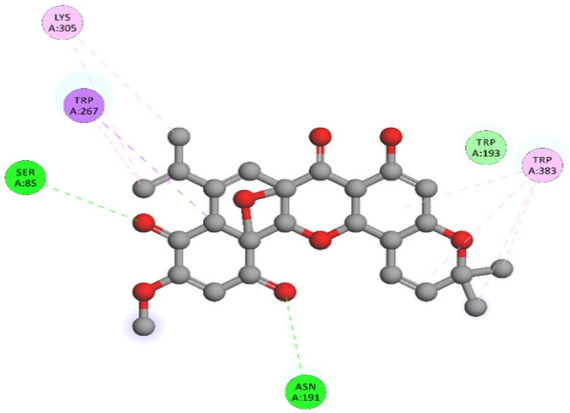


Figure 2. The 2D visualization of the molecular binding between Cochliophilin A and the α-amylase.

were performed to identify compounds with drug-like propertiess. The predicted values for the anti-diabetic compounds are summarized in Table 3.

3.6. Antidiabetic Activity of Gayam Bark Fraction

All rats in this study had their blood glucose levels measured while in a fasting state. The average fasting blood glucose levels were as follows: 99.67 ± 0.57 mg/dL for the normal control group, 188.3 ± 1.52 mg/dL for the negative control group (treated with 0.5% Na CMC), and 121.67 ± 29.02 mg/dL for the positive control group (treated with glibenclamide). For treatment groups IV, V, and VI, the levels were 93.67 ± 6.80 mg/dL, 130 ± 1.73 mg/dL, and 112.33 ± 41.10 mg/dL, respectively. Additionally, diabetic rats were induced with alloxan monohydrate at a dose of 170 mg/kg body weight over a 48-hour period (Figure 3).

4. Discussion

Based on the data obtained, the compound, Bis(2-

Table 3. Absorption and Distribution Prediction Values.

Test	Code	Compound	
		Acarbose	Cochliophilin A
Absorption	1	-1.482	-3.87
	2	-0.481	1.049
	3	4.172	96.069
	4	-2.735	-3.261
Distribution	5	-0.836	-0.72
	6	0,3506944	0,1076389
	7	-1.717	-0.181
	8	-6.438	-1.915
Metabolism	9	No	Yes
	10	No	No
Excretion	11	0,2972222	0,1340278
Toxicity	12	No	Yes
	13	0,3020833	0,6576389
	14	No	No
	15	Yes	No
	16	2.449	2.451
	17	No	No
	18	No	No
		16.823	1.237

Note: The model names and measurements are as follows: (1): aqueous solution (log mol/L), (2): Caco-2 permeability (log Papp at 10–6 cm/s), (3): intestinal absorption (human) (% absorbed, log value%), (4): skin permeability (log Kp), (5): volume distribution at steady state (human) (Vdss, log L/kg), (6): unbound fragments (human) (Fu), (7): BBB permeability (log BB), (8): CNS permeability (log PS), (9): CYP3A4 substrate (yes/no), (10): CYP2C9 inhibitor (yes/no), (11): total clearance (log mL/min/kg), (12): AMES toxicity (yes/no), (13): maximum tolerated dose (human) (log mg/kg/day), (14): hERG I inhibitor (yes/no), (15): hERG II inhibitor (yes/no), (16): acute toxicity in rats (LD) (mol/kg), (17): hepatotoxicity (yes/no), (18): skin sensitization (yes/no), and (19): minor toxicity (log mM).

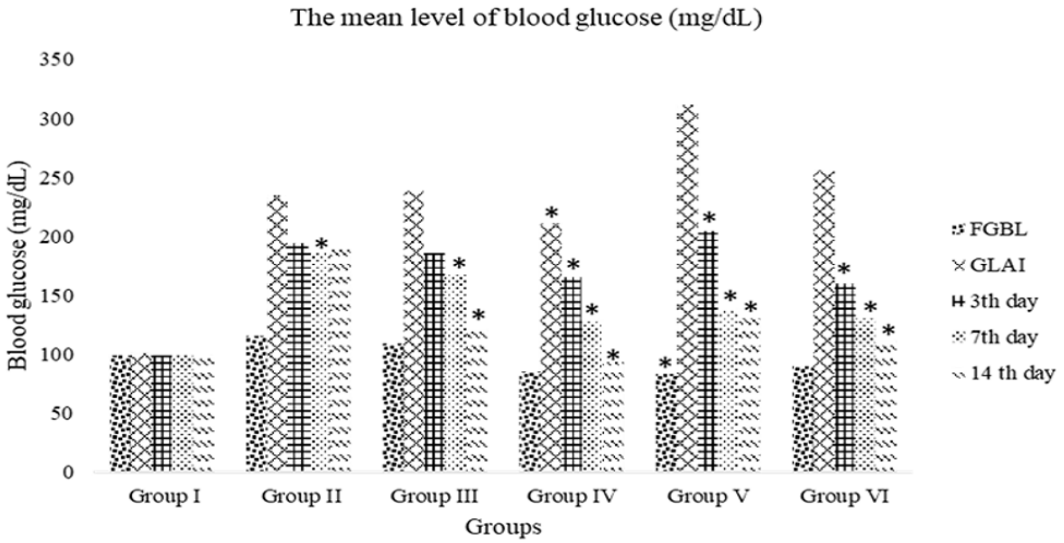


Figure 3. Effect of of gayam (*I. fagiferus*) bark fractions (Groups I-VI) on blood glucose levels in alloxan–induced diabetic mice
* Significantly different ($p < 0.05$). (GLAI: glucose level after alloxan exposure; FBGL: fasting blood glucose level.). Data represent the mean ± SD of five experiments.

ethylhexyl) terephthalate, an ester derivative, was identified. In addition, this study revealed the presence of Bis(4-ethylbenzylidene)sorbitol. Esters are compounds derived from carboxylic acids that undergo oxidation reactions at the hydroxyl group. In this case, the carboxylic acid group is in the form of phenylpropanoid, which is a derivative of cinnamic acid. Cinnamic acid comes from L-phenylalanine through a deamination reaction, and hydrogenation of cinnamic acid produces p-coumaric acid, which is an intermediate compound in the formation of phenylpropanoid derivatives, such as p-hydroxy synapse.

This process results from an intramolecular esterification involving hydroxycarboxylic acids, which occurs spontaneously in cases where the formed ring has five or six atoms. Flavonoids such as Cochliphilin A, Daidzein, and 7-hydroxy-3-(4-methoxyphenyl)-4-H-chromen-4-one were also present. They are derived from the enzyme chalcone synthase, which produces the yellow chalcone naringenin from the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA.

This enzyme regulates the rate of flavonoid production. However, the ingredients in the alkaloid group, such as phytosphingosine and deacetylvindoline, are derived from the amino acid tyrosine, which is oxidized in the aromatic ring to produce dopamine compounds.[18] According to Sen et al.,[19] alkaloid compounds function as hypoglycemic and antihyperglycemic agents of ethanol, and flavonoid compounds have the ability to function as herbal antidiabetic drugs in the treatment of DM. Flavonoid compounds have also shown potential as herbal antidiabetic agents in the management of diabetes.[20] A study by Liao et al.[21] found that alkaloid compounds serve as biomarkers of type 2 diabetes and are related to the hypoglycemic effects of sitagliptin.

Lipinski's rule (also known as the Rule of Five) was used to filter and design ligand selection for use in the target protein binding process. In this study, the re-docking of the natural ligand acarbose to α-amylase (4UAC) was carried out, revealing a hydrogen bond distance ranging from 1.73 to 2.83 Å in the interaction between acarbose and amino acid residues such as ASP109, GLN110, SER87, and ASN191. Acarbose, as the native ligand, was re-docked to the target protein and repositioned to its original coordinates at the α-amylase receptor. The re-docked acarbose had a binding energy of -8.95 kcal/mol, as stated in the findings by Ramirez et al.[22]

Based on the results, the free binding energy (ΔG) of acarbose was -8.47 kcal/mol, and that of Cochliophilin A was -9.76 kcal/mol. These finding indicate that, when compared to the common acarbose compound, the binding energy of Cochliophilin A toward the α-amylase is lower. Furthermore, the amino acid residues closest to the hydrogen bonds formed in the α-amylase acarbose complex were TRP618, ALA284, ASP282, ASP616, MET519, ASP404, HIS674, ARG600, PHE649, and TRP376, with binding energy values of 1.73, 2.54, 2.83, 2.44, 2.65, 2.22, 1.88, and 2.65 Å, respectively. Since they are nonpolar amino acid residues, they are unable to form hydrogen bond interactions either inside or outside the protein receptor.[23] However, simulations with the native ligand showed that GLU plays an important role in the receptor protein binding site where molecules and ions, also known as ligands, interact, thereby

impacting the protein's function. Additionally, there may be improvements with the TRP376 residue.

The percentage of plasma protein binding (PB) affects the frequency of dosing, but not the daily dose.[24] Changes in plasma protein binding affect distribution. Suppose a drug has a percentage of bound plasma (PB) of more than 85%, a volume of distribution (Vd), and a small safety margin. In that case, drug interactions related to the distribution process become clinically relevant.[25] Cochliophilin A had a log Vdss value of 1.183, whereas acarbose had a log Vdss value of -0.836 in pkCSM analysis. Therefore, the low volume of distribution could be used to predict the reaction volume at the total dose required in blood plasma, or Vdss. *In vitro*, oral drug absorption (MDCK) can be predicted using the Caco-2 model and the Madin-Darby kidney cell.

The permeability of single-cell monolayer Caco-2 coatings can also be used to predict oral drug absorption (MDCK) after oral administration. A larger surface area allows the drug to interact with Caco-2 cells with stronger hydrogen bonds, resulting in a low Caco-2 permeability value.[26] According to the pkCSM results, the Papp value of -0.481 for acarbose and 0.1.049 for Cochliophilin A in this study indicated that Cochliophilin A had a greater capacity to adsorb Caco-2 than acarbose.[25] In addition, the Papp value, higher than 8 × 10^6 cm/s, indicated that the material had a significant capacity to adsorb Caco-2.

Using a modified method, the ethyl acetate extract of gayam bark (*I. fagiferus*) increased hair growth in male mice.17 In the study, the acarbose group, Na-CMC, and test groups (IV-VI) showed the lowest blood glucose levels. Groups IV and VI specifically had significantly lower values (93.67 ± 6.80 and 112.33 ± 41.10) on the fourteenth day ($p < 0.05$). After alloxan administration intraperitoneally, the gayam bark extract affected blood glucose levels in groups IV and VI. While groups II, III, and IV-VI initially showed increased blood glucose levels, groups IV-VI subsequently demonstrated a 74% decrease in blood sugar levels by day 14. The gayam extract likely contains phytochemicals (possibly flavonoids, alkaloids, or tannins) that may have hypoglycemic and antioxidant properties. Gayam bark compounds may protect pancreatic β-cells from oxidative damage caused by alloxan, enabling partial recovery of insulin production.

5. Conclusion

Based on this study, the presence of ester, alkaloid, flavonoid, and phenylpropanoid compound groups was identified in gayam bark extract. To support this study, additional experimental studies are needed. Gayam bark extract (*I. fagiferus*) significantly lowered blood sugar levels ($p < 0.05$) at a dose of 100 mg/kgBW. In silico studies show that the Cochliophilin A compound from gayam had a binding energy of -9.76 kcal/mol toward the α-amylase enzyme.

Conflict of Interest

The authors declare no conflicts of interest.

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