

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

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Abstract

Diabetes mellitus is characterized by high blood sugar levels. Though many drugs are available, blood glucose control is still 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 activity of compounds from ethyl acetate extract as antidiabetics, tested in silico and in vivo. The study used molecular docking and in vivo activity analysis. LC-MS analysis was used to determine the content of compounds in the ethyl acetate fraction of gayam bark. Through molecular interaction prediction and ADME-Toxicology, compounds in gayam bark showed antidiabetic activity. In the molecular docking interaction study, the Cochliophilin A compound had a lower binding energy than acarbose on the α -amylase receptor: -9.76 kcal/mol and -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, respectively by 74% (IV), 72% (V), and 74% (VI). Collectively, compounds in gayam bark are useful in treating diabetes.

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

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. Studi 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, docking molekuler, *Inocarpus fagiferus*., spektrometri massa

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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 died worldwide from diabetes mellitus, which is considered one of the ten leading causes of death in adults.¹ In 2019, 463 million people were reported to have DM, 9.3% of 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 diabetes mellitus 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 is thiazolidinediones, which are Peroxisome proliferator-activated gamma receptor (PPAR) agonists, and the biguanide metformin.⁴

Third, it contains an α -glucosidase inhibitor, which inhibits 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 also be used.⁶ In order to discover new drugs as an alternative treatment for diabetes mellitus, many drugs derived from natural ingredients have been developed. One of the native plants of Indonesia known for its antidiabetic properties is the bark of the gayam tree (*I. fagiferus*), which is empirically used by the community in Kulisusu District, North Buton Regency, for the treatment of diabetes. The local community uses it by boiling the water and then drinking it. Ethanol extract of gayam tree bark (*I. fagiferus*) is reported to contain main compounds such as phenolics, flavonoids, 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 from the ethyl acetate fraction of gayam tree bark (*I. fagiferus*) along with its molecular mechanisms *in silico* and *in vivo*. Thus, it is necessary to explore active compounds that have the potential as antidiabetics.

2. Materials and Methods

2.1. Tools

The hardware tools used were 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) with AMD Ryzen 7 5800H with Radeon Graphics (3.20 GHz) processor, and NVIDIA GeForce RTX 3050M Graphics Card (Taiwan) were used. The software used for binding energy analysis were AutoDock Tools 1.5.6,⁸ and the results of the molecular structure were visualized with Discovery Studio Visualizer. These results were then tested for *in vivo* activity using the Accu-Chek performance glucometer.

2.2. Materials

The gayam bark (*I. fagiferus*) was collected from Jampaka Village in Kulisusu District, North Buton Regency, Central Sulawesi Regency. For docking simulations, the two-dimensional structures of reference ligands (acarbose, test ligands, and compounds contained therein) and selected macromolecules, alpha-amylase receptors (PDF ID code: 4UAC; resolution of 1.6 Å) were obtained from the Protein Data Bank (PDB) (<http://www.rscb.org/pdb/>), and ADME-tox analysis was conducted through <https://biosig.lab.uq.edu.au/pk>. To perform the *in vivo* test, the materials used included distilled water, 96% ethanol, ethyl acetate, MgSO₄, 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

Determination 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 by the maceration method using 96% ethanol as the solvent, 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 with 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 Phenomenal® HPLC column (5 μ C8; 150 × 2 mm i.d.) was used for the analysis. The chemical components of the pure ethyl acetate extract of Gayam bark (*I. fagiferus*) were identified using LC-MS/MS. A full scan was performed from m/z 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 (N_2) was 350°C, the gas flow rate was 6 mL/min, and the nebulization pressure (N_2) 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 made as a native ligand.¹²

2.3.6. Ligand Preparation

The ligand structures of acarbose and test compounds derived from pure ethyl acetate extract of gayam bark (*I. fagiferus*) 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 a 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 in.¹³

2.3.8. Molecular Docking Simulation

Molecular docking simulation calculations 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) $x = 40$, $y = 40$, and the grid box size for the α -amylase receptor $x = 28.499$, $y = 65$. Interactions with biomacromolecules occurred in rigid conditions, and all binders were in stable conditions.¹⁴ The Discovery Studio visualizer shows 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 program was accessible to users at the Biosig Lab.¹⁶ The obtained chemical structures were converted into SMILE format using PubChem, and the resulting structures were then downloaded using the SMILE canonical link.

2.3.10. Antidiabetic Activity

Before being treated, mice (*Mus musculus*), Swiss Webster strain, were fed for 18 hours and weighed. Then blood glucose levels (BGL) were measured by taking blood through a tail vein wound. 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, blood glucose levels 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 had a normal control (170 mg/kg.ip), group II had a negative control (Sodium-CMC 0.5%), group III had a glibenclamide control (170 mg/kg.ip), and group V had an ethyl acetate fraction (100 mg/kg BW) and group VI had an ethyl acetate fraction (300 mg/kg BW).

3. Result

3.1. Compounds Identified Using Liquid Chromatography-Mass Spectrometry

The analysis results can describe the compound content profile of the ethyl acetate extract from the separation of the ethanol extract of Gayam bark (*I. fagiferus*). The compound content can be observed from the chromatogram peaks, which have 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 showed 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 bond formed between acarbose and α -amylase receptor (Figure 1). Hydrogen bonds are formed between amino acids ASP109, GLN110, SER87, ASN191, LYS305, GLU86, SER85, and GLU246. The

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

closest residues in the acarbose- α -amylase receptor complex are TRP383, ASN157, TRP267, and TRP193 (Table 2), with a redocking bond energy value of -8.95 kcal/mol and an RMSD value of 0.57.

3.4. Docking Simulation of Acarbose and Test Ligand

Docking simulation was performed using AutoDock, with coordinate settings according to the interaction position of acarbose with the α -amylase receptor (4UAC). Analysis was performed to determine the value of binding energy and hydrogen bond formation with acarbose and the test ligand whose structure was

known from LC-MS (Table 2 and Figure 2).

A description of the interaction of standard acarbose compounds derived from gayam bark (*I. fagiferus*) with the α -amylase receptor (4UAC) has been obtained. Cochliophilin A has a low binding energy value to the α -amylase receptor (4UAC). Figure 2 shows the visualization of the docking results.

3.5. ADME-Tox Prediction

Pharmacokinetic analysis and ADME-Tox prediction were performed to identify compounds that can

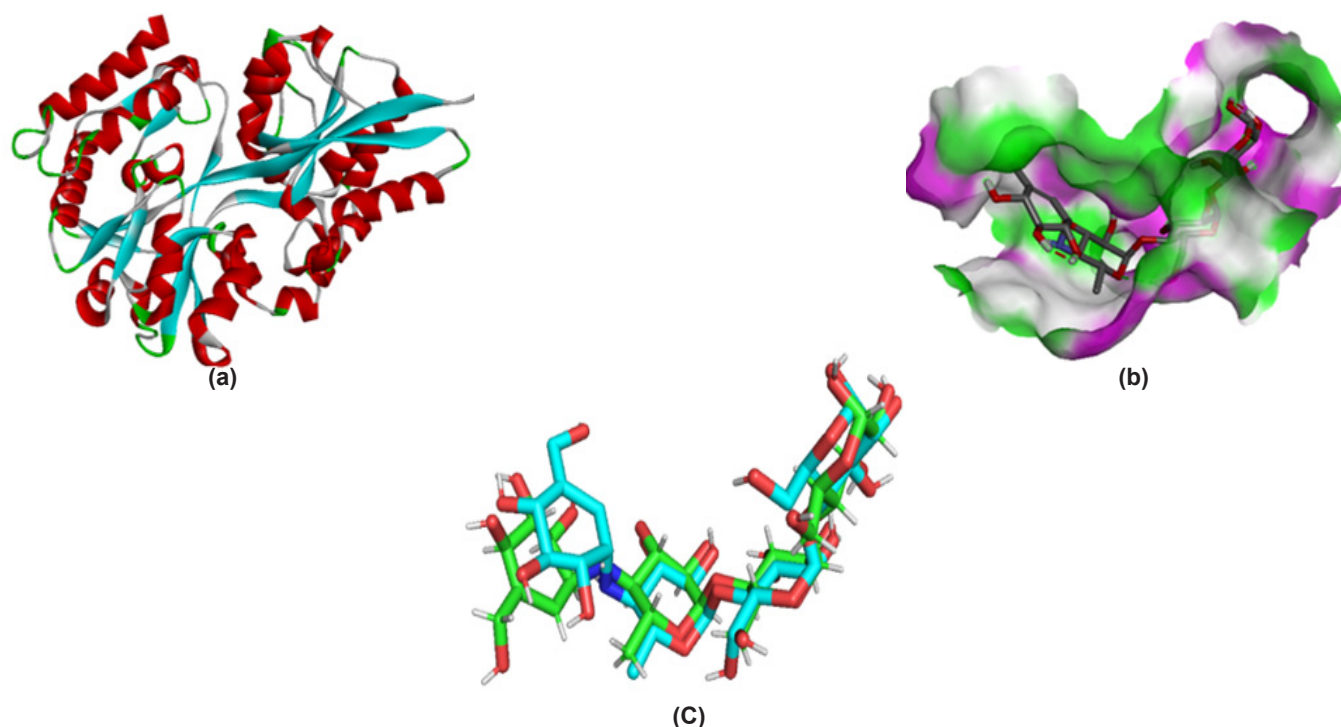
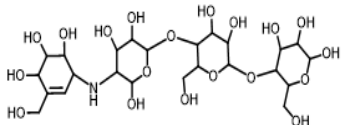
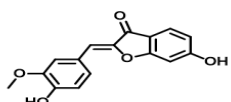
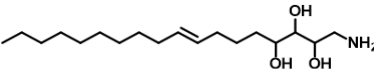
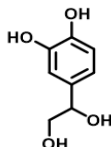
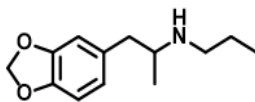
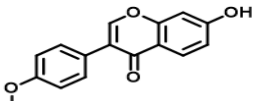
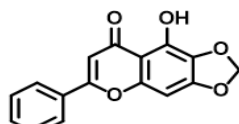
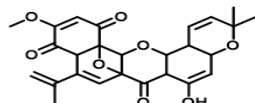
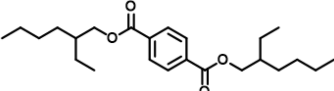
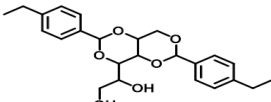
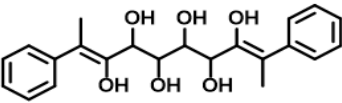
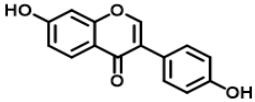


Figure 1. (a) Structure of α -amylase receptor (4UAC). (b) shows a representation of the interaction between the α -amylase receptor (4UAC) and acarbose, with the white clouds representing the hydrophobic interactions. (c) the figure shows an overlay of the acarbose pose docked with the cocrystallized 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, TRP383	
Daidzein	-8.61	2.88, 2.68	GLU246, ASN191	TRP193	

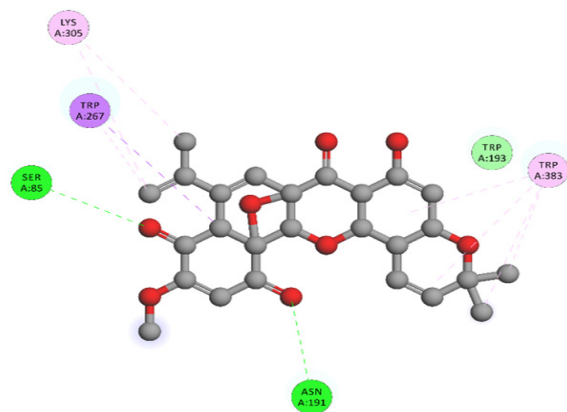


Figure 2. The 2D visualization from the molecular binding between the Cochliophilin and the α -amylase.

act as drugs; the predicted values for anti-diabetic compounds were shown in Table 3.

3.6. Antidiabetic Activity of Gayam Bark Fraction (*I. fagiferus*)

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, $130 \pm$

1.73 mg/dL, and 112.33 ± 41.10 mg/dL, respectively. Additionally, diabetic rats were induced using 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, a compound, Bis(2-ethylhexyl) terephthalate ester, is identified. In contrast, the results of the study showed that sorbitol ester Bis(4-ethylbenzylidene) functions. 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,

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

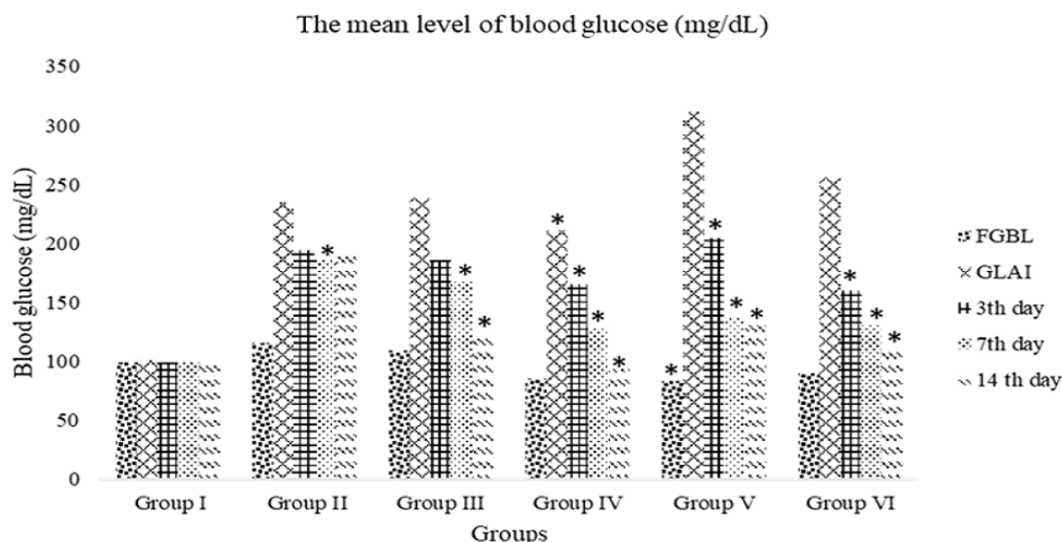


Figure 3. Effect of group (I-VI) of Gayam (*I. fagiferus*) stem bark in alloxan-induced Diabetic Mice

*Significantly different ($p < 0.05$). (GLAI: glucose level after alloxan exposure; FBGL: fasting blood glucose level). The sum of the data retrieved from five experiments $\pm$ SD.

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 is the result of an intramolecular esterification process involving hydroxycarboxylic acids, which occurs spontaneously in cases where the formed ring has five or six atoms. Flavonoids such as Cochliophilin A, Daidzein, and 7-hydroxy-3-(4-methoxyphenyl)-4-Hchromen-4-one are 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 Deacetylindoline, 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 diabetes mellitus. Flavonoid compounds have also shown potential as herbal antidiabetic agents in the management of diabetes.²⁰ Study by Liao et al.²¹ found that alkaloid compounds were 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 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 Ramirez et al.²²

Based on the results, the free binding energy (ΔG) for acarbose was -8.47 kcal/mol, and for Cochliophilin A was -9.76 kcal/mol. This indicates that, when compared to the common acarbose compound, the binding energy of Cochliophilin A to the α -amylase is lower. Furthermore, the amino acid residues closest to the hydrogen bonds formed in the α -amylase acarbose complex are 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 native ligands show that GLU plays an important role in the receptor protein binding site. The binding site of a protein is where molecules and ions, also known as ligands, interact, thereby impacting the protein's function. 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 will be clinically relevant.²⁵ Cochliophilin A had a log Vdss value of 1.183. Still, acarbose had a log Vdss value of -0.836 in pkCSM analysis, so 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 will allow 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.

With a modified method, the ethyl acetate extract of gayam bark (*I. fagiferus*) increases hair growth in male mice.¹⁷ In the study, the acarbose group, Na-CMC group, 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 (*I. fagiferus*) 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. Bioactive compounds in Gayam bark: The extract likely contains phytochemicals (possibly flavonoids, alkaloids, or tannins) that may have hypoglycemic properties 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 skin has a binding energy of -9.76 kcal/mol to the α -amylase enzyme.

Conflict of Interest

The authors declare no conflicts of interest.

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