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Research Article

Computation and Experimental Approaches to Uncover the Antidiabetic Potential of Insulin Leaf (*Tithonia diversifolia*) Extract Based on Flavonoid Constituents

Masniah [1,2]

Eko Suhartono [3,4*]

Fujiati [3]

Muhammad Ali Faisal [5]

Budu [6]

Mohammad Bakhriansyah [7]

Istiana [8]

Dewi Indah Noviana Pratiwi [9]

[1]Doctoral Program of Medical Sciences, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[2]Department of Ophthalmology, Brigadier General H. Hasan Basry Kandangan Regional Hospital, Kandangan, South Kalimantan, Indonesia
[3]Department of Biochemistry and Biomolecular, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[4]Health Research Centre for Wetland, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[5]Department of Ophthalmology, Universitas Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia
[6]Department of Ophthalmology, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia
[7]Department of Pharmacology, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[8]Department of Parasitology, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[9]Department of Clinical Pathology and Medical Laboratory, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

*email: esuhartono@ulm.ac.id; phone: +6281251126368

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Abstract

Insulin leaves (*Tithonia diversifolia*) are traditionally used by the community to lower blood sugar, although this has not been scientifically proven. *Tithonia diversifolia* is known to contain flavonoids, which are responsible for the antidiabetic activity of these leaves. Therefore, this research was conducted to investigate the antidiabetic effects of *T. diversifolia*, specifically through its flavonoid content. *Tithonia diversifolia* was extracted with 90% methanol and analyzed for flavonoid content by Liquid Chromatography-Mass Spectrometry (LC-MS). To investigate the antidiabetic activity, the sample extract was tested for its effect on the *in vitro* amylase enzyme and inhibition of glycated haemoglobin. Moreover, advanced techniques such as *in silico* analysis and molecular docking are used to elucidate the molecular mechanism behind the antidiabetic effects of *T. diversifolia*. The results of our study revealed that plant extracts, particularly those rich in flavonoids such as luteolin, genistein, and hispidulin, are potent inhibitors of the α-amylase enzyme and the formation of glycated haemoglobin. The Inhibition Concentration 50% (IC_{50}) values of flavonoids were lower than those of glibenclamide, suggesting their superior efficacy in stabilizing blood sugar levels. Docking analysis further confirmed the strong interactions between flavonoids and key enzyme residues and haemoglobin, while Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) predictions indicated favourable absorption and distribution profiles. Both experimental and computational approaches show the importance of flavonoids in the antidiabetic activity of *T. diversifolia* extracts, highlighting their contribution to its effectiveness.

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INTRODUCTION

The leaves of *Tithonia diversifolia*, locally known as the "insulin leaf" or Mexican sunflower, have a long history in traditional medicine in Kalimantan as a primary remedy for glycemic regulation. In Central Kalimantan, the Dayak Manyan tribe specifically uses these leaves to manage diabetes[1,2], a practice also observed among the Banjar tribe, which employs a traditional method of chewing the leaves to lower blood glucose levels effectively[3,4]. Comprehensive phytochemical investigations have begun to elucidate the molecular basis of these traditional practices. Research by Oluwamodupe *et al*.[5] used Gas Chromatography-Mass Spectrometry (GC-MS) to identify key constituents, including β-D-glucopyranoside, methyl palmitic acid (15.225%), a TMS derivative (10.98%), and methyl esters of hexadecenoic acid (8.75%). Complementary quantitative analyses by Robinson *et al*.[6] established that 100 g of the leaf matrix contains significant concentrations of alkaloids (1535 mg), tannins (540 mg), and flavonoids (851.67 mg), alongside a total phenolic content of 64.58 mg.

Among these diverse phytochemicals, flavonoids are increasingly viewed as the pivotal agents driving the observed antidiabetic activity. Al-Ishaq *et al*.[7] demonstrated that these polyphenolic compounds significantly reduce the risk of diabetes development by lowering systemic blood sugar levels. This bioactivity is structurally dictated; as Shamsudin *et al*.[8] noted, the anti-inflammatory and antidiabetic efficacy of flavonoids is attributed to specific structural motifs, including the C2–C3 double bond in the C-ring and hydroxyl substitutions at the C3', C4' (B-ring), and C5, C7 (A-ring) positions. The therapeutic potential of these compounds encompasses a sophisticated array of metabolic pathways. As detailed by Yen *et al*.[9], flavonoids modulate glucose transporters, stimulate glucose uptake via the AMP-activated protein kinase (AMPK) signaling pathway in skeletal muscle, and suppress hepatic glucose production. Furthermore, they provide a protective effect on pancreatic β-cells and inhibit critical enzymes, such as α-glucosidase, glucose-6-phosphate dehydrogenase (G6PD), and glucose-6-phosphatase (G6Pase), thereby reducing glycogenolysis, gluconeogenesis, and the formation of glycated hemoglobin.

In recent years, *in silico* molecular docking has become an indispensable tool for characterizing molecular interactions between plant-derived flavonoids and therapeutic targets such as α-amylase and glycated hemoglobin. This computational approach facilitates screening for potential inhibitors and clarifies binding mechanisms at the amino acid level (such as hydrogen bonding and hydrophobic interactions), enabling the prediction of binding affinities and orientations within enzyme active sites[10]. To further investigate the antidiabetic potential of *T. diversifolia*, the present study employs an integrated strategy combining LC-MS profiling, *in vitro* bioassays, and *in silico* docking analysis. This hybrid methodology aims to elucidate the specific molecular interaction pathways through which *T. diversifolia* flavonoids target α-amylase and glycated hemoglobin, ultimately contributing to a more profound understanding of the species' capacity to mitigate diabetic activity.

MATERIALS AND METHODS

Materials

The biological matrix for this study consisted of *T. diversifolia* leaves harvested from Pengambangan Village, East Banjar District, Banjarmasin. Taxonomic authentication was strictly performed at the Plant Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat (No. 287/LB.LABDASAR/XII/2024). Chemical reagents used in the extraction and bioassays included 90% methanol, phosphate buffers (pH 7.0 and 7.4), 1% amylum, iodine indicator, and analytical-grade glibenclamide as a positive control. For the glycated hemoglobin assay, hemoglobin solution, gentamicin, and 2% glucose in 0.01 M phosphate buffer were employed. High-performance analytical equipment included Buchi® rotary evaporator, Thermo Scientific ACCELLA 1250 UHPLC system equipped with Hypersil Gold column (50 mm × 2.1 mm × 1.9 µm), and UV-Vis spectrophotometer. Computational resources involved the RCSB Protein Data Bank (https://www.rcsb.org/; PDB IDs 2QV4 and 5HY8), UCSF Chimera 1.19, CB-Dock (http://clab.labshare.cn/cb-dock/), BIOVIA Discovery Studio Visualizer, SwissADME (https://www.swissadme.ch/), and the ProTox-3.0 (https://tox.charite.de/) prediction platform.

Methods

Preparation and extraction of Tithonia diversifolia leaves

The harvested leaves underwent rigorous preparation, including sorting, cleaning, and washing under running water to remove impurities. The samples were subsequently dried in a controlled oven at 40°C before being processed into a fine powder. For the isolation of bioactive flavonoids and alkaloids, 100 g of the dried powder was macerated in 1 L of 90% methanol for 72 hours. The resulting mixture was filtered, and the filtrate was concentrated via a rotary evaporator at 37°C to obtain the crude methanolic extract[11].

Phytochemical characterization via LC-MS

The identification of flavonoid constituents was conducted at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta. A 0.1 mL aliquot of the *T. diversifolia* extract was analyzed using an ACCELLA 1250 UHPLC system. The separation was achieved on a Hypersil Gold column, meticulously controlled by a quaternary pump and a vacuum degasser. System control and data acquisition were performed using the Xcalibur 2.1 program to ensure precise metabolite profiling.

In vitro antidiabetic bioassays

The antidiabetic potential of the methanolic extracts of *T. diversifolia* leaves was evaluated through both α-amylase and glycated hemoglobin inhibition assays[12,13]. For the α-amylase inhibition analysis, 500 µL of a 1% amylum solution was combined with 500 µL of the extract at concentrations ranging from 50 to 400 ppm. To this mixture, 500 µL of the α-amylase enzyme and 500 µL of phosphate buffer (pH 7.0) were added. The solution was homogenized via vortexing and incubated at 40°C for 30 minutes, after which the reaction was stabilized by adding 50 µL of iodine indicator. The final absorbance was measured using a UV-Vis spectrophotometer at 593 nm. A parallel assessment was conducted using glibenclamide as a positive control at equivalent concentrations. The antidiabetic efficacy was quantified by determining the IC_{50} value, the concentration required to inhibit enzymatic activity by 50%[14].

The investigation into glycated hemoglobin inhibition involved adding 1 mL of hemoglobin solution and 5 µL of gentamicin to reaction tubes containing the extract at concentrations of 50-400 ppm. The glycation process was initiated by adding 1 mL of 2% glucose to 0.01 M phosphate buffer (pH 7.4), followed by a 24-hour incubation in the dark at room temperature. The resulting concentration of glycated hemoglobin was measured spectrophotometrically at a wavelength of 443 nm. Consistent with the enzymatic assay, glibenclamide served as the reference standard. The antidiabetic activity was similarly reflected in the IC_{50} value, which indicates the concentration required to inhibit the formation of glycated hemoglobin by 50%[15,16].

Computational docking and ADMET profiling

In silico investigations began with the preparation of target proteins, α-amylase (PDB ID 2QV4) and glycated hemoglobin (PDB ID 5HY8), by removing water molecules and heteroatoms using Chimera 1.19. Ligands identified through LC-MS were similarly prepared and subjected to blind docking via the CB-Dock. Binding affinities were quantified using Vina scores, which represent the change in free energy (ΔG). The resulting 2D and 3D molecular interactions were visualized through BIOVIA Discovery Studio Visualizer[17]. Furthermore, the pharmacological potential of the identified flavonoids was assessed through ADME predictions using SwissADME and toxicity profiling (hepatotoxicity, neurotoxicity, nephrotoxicity, and cardiotoxicity) via the ProTox-3 platform[18,19].

Data analysis

The antidiabetic efficacy of the extracts was quantitatively assessed by calculating the IC_{50} values[14]. These values were derived from the linear regression of the inhibition percentage versus the log-concentration of the extract. For the docking analysis, Vina scores were used to rank ligand binding strength, with lower scores indicating higher binding affinity. All experimental data were processed to ensure statistical significance, and the *in silico* results were validated against the observed *in vitro* inhibition trends to provide a comprehensive mechanism of action.

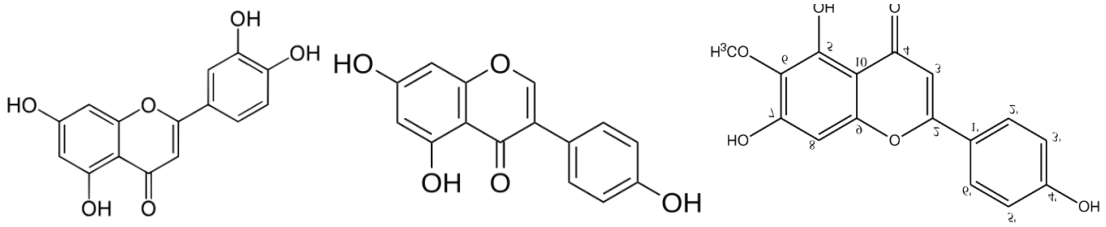
RESULTS AND DISCUSSION

The analytical characterization of the methanolic extract of *T. diversifolia* leaves using LC-MS is presented in **Figure 1** and detailed in **Table I**, with corresponding chemical architectures identified as luteolin, genistein, and hispidulin (**Figure 2**). Qualitative profiling revealed that hispidulin represents the most abundant flavonoid constituent at 2.159%, followed by luteolin (0.465%) and genistein (0.137%). Luteolin, detected with a molecular mass of 286.04748 g/mol and a retention time of 10.825 minutes, is recognized in the field of phytochemistry for its potent antioxidant properties and its ability to enhance insulin sensitivity by protecting pancreatic β-cells[20]. The presence of hispidulin and genistein further underscores the extract's potential to stabilize blood glucose and mitigate oxidative cellular damage.

Figure 1. Chromatogram of the methanol extract of *T. diversifolia* leaves.

Table I. Bioactive compounds identified in *R. stylosa* through data mining.

No.	Name	Formula	Mass	RT (min)	Area (%)
1	Luteolin	$C_{15}H_{10}O_6$	286.04748	10.825	0.465
2	Genistein	$C_{15}H_{10}O_5$	270.05269	11.798	0.137
3	Hispidulin	$C_{16}H_{12}O_6$	300.06286	11.825	2.159



a **b** **c**

Figure 2. Chemical structure of (**a**) luteolin, (**b**) genistein, and (**c**) hispidulin.

The presence of the identified flavonoids within the *T. diversifolia* extract provides a robust molecular foundation for its traditional use in stabilizing blood glucose levels. This therapeutic potential is corroborated by *in vitro* assessments detailed in **Figure 3**, which show that the extract demonstrated significantly greater potency in inhibiting α-amylase than glibenclamide. With an IC_{50} value of 109.861 ppm against glibenclamide's 318.214 ppm, the extract effectively modulates the enzymatic breakdown of starch into simple sugars like glucose. By binding to the active site of α-amylase, these flavonoids block substrate interaction, thereby slowing glucose release into the bloodstream and mitigating postprandial hyperglycemic spikes[21,22]. Mechanistically, α-amylase utilizes specific amino acid residues to cleave glycosidic bonds; however, the introduction of luteolin and hispidulin facilitates the formation of hydrogen bonds and hydrophobic interactions with these residues. This binding induces conformational shifts in the enzyme that inhibit catalysis, directly contributing to glycemic stability[23,24]. Unlike glibenclamide, which acts as a secretagogue by stimulating pancreatic insulin secretion, *T. diversifolia* extract offers a distinct advantage by suppressing carbohydrate digestion at the source.

Beyond enzymatic inhibition, *T. diversifolia* extract provides a comprehensive approach to diabetes management by significantly hindering the formation of glycated hemoglobin (HbA1c), as illustrated in **Figure 4**. The extract exhibits a lower IC_{50} value (247.331 ppm) than glibenclamide (391.080 ppm), indicating superior efficacy in preventing the non-enzymatic glycation of hemoglobin. This glycation process typically involves glucose reacting with free amino groups on *N*-terminal valine or internal lysine residues (such as Lys-61) to produce a Schiff base, which subsequently undergoes an Amadori rearrangement to form HbA1c, a critical biomarker for long-term glycemic control[25,26].

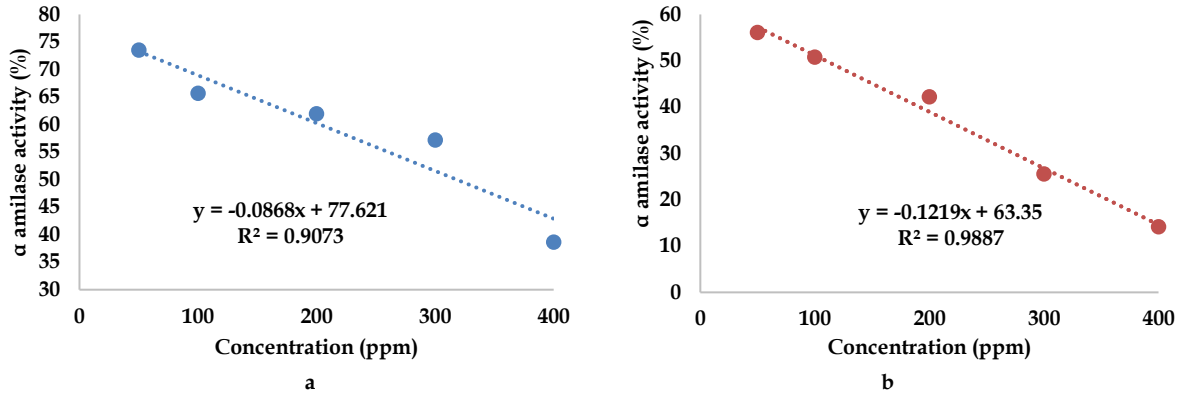


Figure 3. Inhibition of α-amylase activity by (**a**) glibenclamide with IC_{50} of 318.214 ppm and (**b**) *T. diversifolia* leaf extract with IC_{50} of 109.861 ppm.

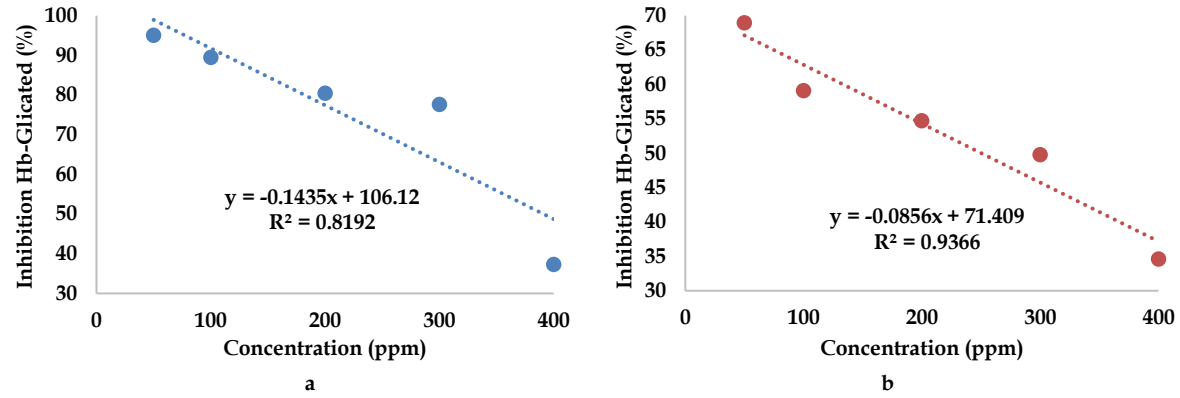


Figure 4. Inhibition of HbA1c by (**a**) glibenclamide with IC_{50} of 391.080 ppm and (**b**) *T. diversifolia* leaf extract with IC_{50} of 247.331 ppm.

The flavonoid constituents, including luteolin, genistein, and hispidulin, appear to interact directly with these glycosylation sites. By forming hydrogen bonds with valine and lysine residues and establishing π- π stacking interactions with aromatic residues like phenylalanine or tyrosine, these compounds effectively shield the protein from glucose attachment[27]. This multi-site binding capability explains the extract's robust performance compared to glibenclamide. While glibenclamide remains a standard therapy due to its ability to close ATP-sensitive potassium channels in pancreatic β-cells and boost insulin secretion, its molecular-level inhibition of protein glycation is less efficient than that of the flavonoid-rich *T. diversifolia* extract[28]. Consequently, the extract serves as a promising multi-targeted candidate for managing both acute postprandial glucose and chronic diabetic complications.

Molecular docking was utilized to elucidate the atomic-level interactions between the identified flavonoids and the α-amylase enzyme, as well as their role in inhibiting the formation of glycated hemoglobin. This *in silico* analysis facilitates the identification of active residues involved in critical hydrogen bonding and hydrophobic interactions that drive inhibitory efficacy. The molecular interactions characterizing the binding of these compounds to the α-amylase enzyme are presented in **Figure 5**. These docking simulations provide a detailed illustration of the nuanced binding modes observed across the test ligands. As shown in **Figure 5a**, glibenclamide establishes significant contact with residues His45, Pro44, and Phe43 via hydrogen bonds and hydrophobic interactions with several leucine residues. While these contacts enable glibenclamide to stabilize itself within the active site, its inhibitory potency remains largely dependent on relatively weak hydrophobic interactions compared to the flavonoid constituents[28].

In contrast, genistein (**Figure 5c**) shows promising potential to suppress amylase activity by forming a higher frequency of hydrogen bonds with residues such as Ser110, complemented by hydrophobic interactions with Phe, Val, and Leu. This unique interaction pattern suggests that the aromatic scaffold and flexible hydroxyl groups of genistein allow it to effectively obstruct the enzymatic active site by engaging with both polar and nonpolar residues, thereby exhibiting greater inhibitory potential than glibenclamide[29]. Similarly, luteolin and hispidulin (**Figures 5b** and **5d**) maintain robust bonds with key active residues. Hispidulin interacts with numerous hydrophobic residues and forms critical hydrogen bonds that stabilize the

ligand-protein complex. Luteolin stands out due to its highly stable hydrogen-bonding network involving His and Ser residues in proximity to the catalytic site. The ability of these flavonoids to form strong hydrogen bonds via their aromatic hydroxyl groups enhances their inhibitory activity against enzymes. Collectively, these interaction patterns explain why genistein, hispidulin, and luteolin possess the potential to outperform glibenclamide as α-amylase inhibitors by reaching a broader array of key residues within the enzyme's binding pocket[10].

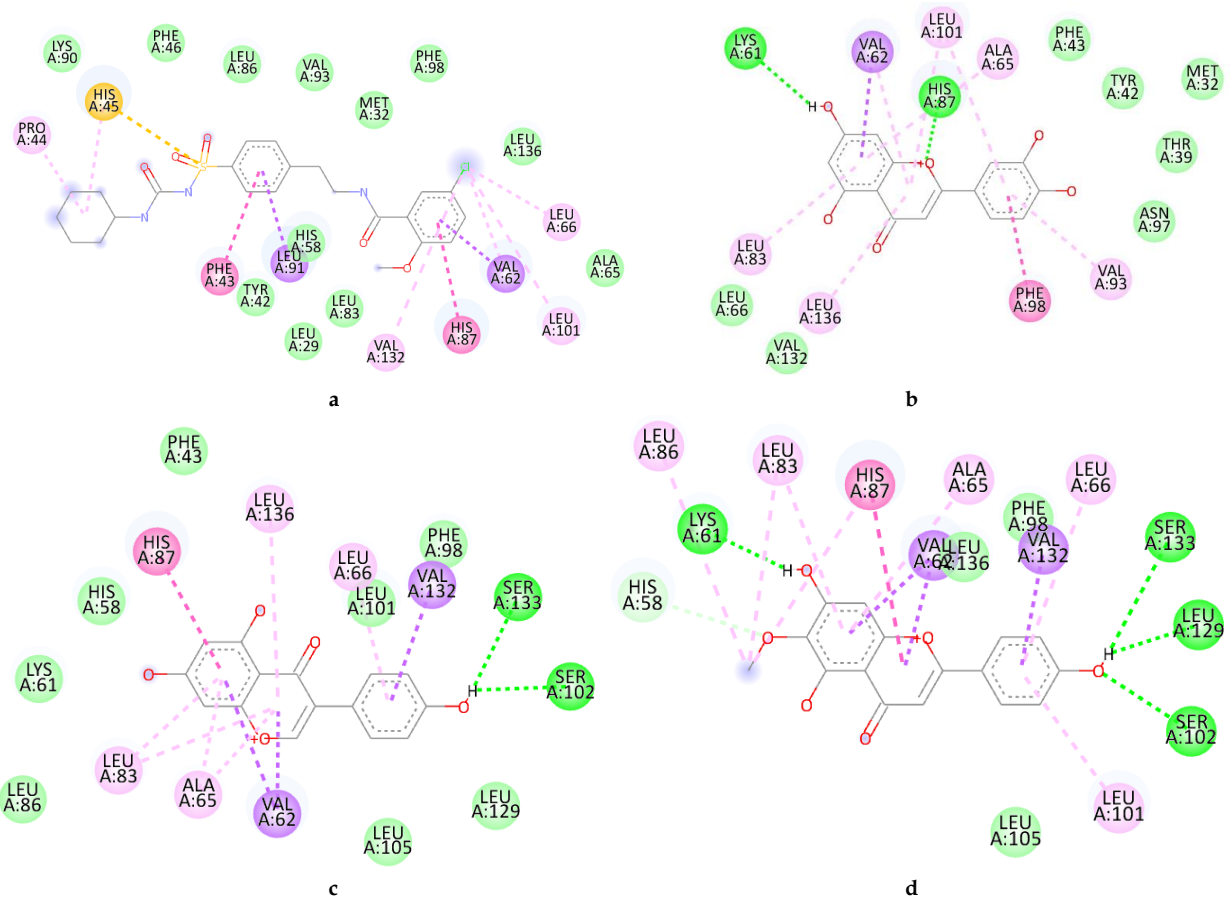


Figure 5. Interaction of α-amylase enzyme with (**a**) glibenclamide ΔG = -8.1 kJ/mol, (**b**) luteolin ΔG = -9.1 kJ/mol, (**c**) genistein ΔG = -8.3 kJ/mol, and (**d**) hispidulin ΔG = -8.8 kJ/mol.

Beyond enzymatic targets, these flavonoids also interact with hemoglobin proteins to mitigate the formation of glycated hemoglobin, as visualized in **Figure 6**. The docking results for glibenclamide with hemoglobin (**Figure 6a**) reveal hydrogen-bond interactions with residues such as His45, as well as hydrophobic contacts with Phe and Leu within the binding pocket. This orientation allows glibenclamide to occupy the pocket and limit glucose access; however, because hydrophobic interactions dominate these bonds, the complex stability is moderate compared to that of the flavonoids. Luteolin (**Figure 6b**) exhibits notably diverse interactions, forming hydrogen bonds with polar residues such as His and Lys while simultaneously engaging with surrounding hydrophobic residues. This multi-modal stabilization increases the residence time of luteolin within the hemoglobin pocket, enhancing its efficacy in blocking glucose entry. A comparable pattern is observed for genistein (**Figure 6c**), which forms strong hydrogen bonds with His and Asn residues, effectively partially obstructing the hemoglobin active site and preventing the formation of glycated hemoglobin, a pivotal marker of chronic diabetes[27]. Furthermore, hispidulin (**Figure 6d**) exhibits complex binding, characterized by extensive hydrogen bonding and hydrophobic interactions with Leu, Val, and Ala residues. This combination ensures a highly stable positioning within the binding pocket, significantly augmenting its potential to inhibit glycation compared to glibenclamide. In summary, the superior interaction stability of luteolin, genistein, and hispidulin with hemoglobin explains the impressive effectiveness of *T. diversifolia* leaf extract in suppressing glycated hemoglobin formation compared with synthetic therapeutic agents.

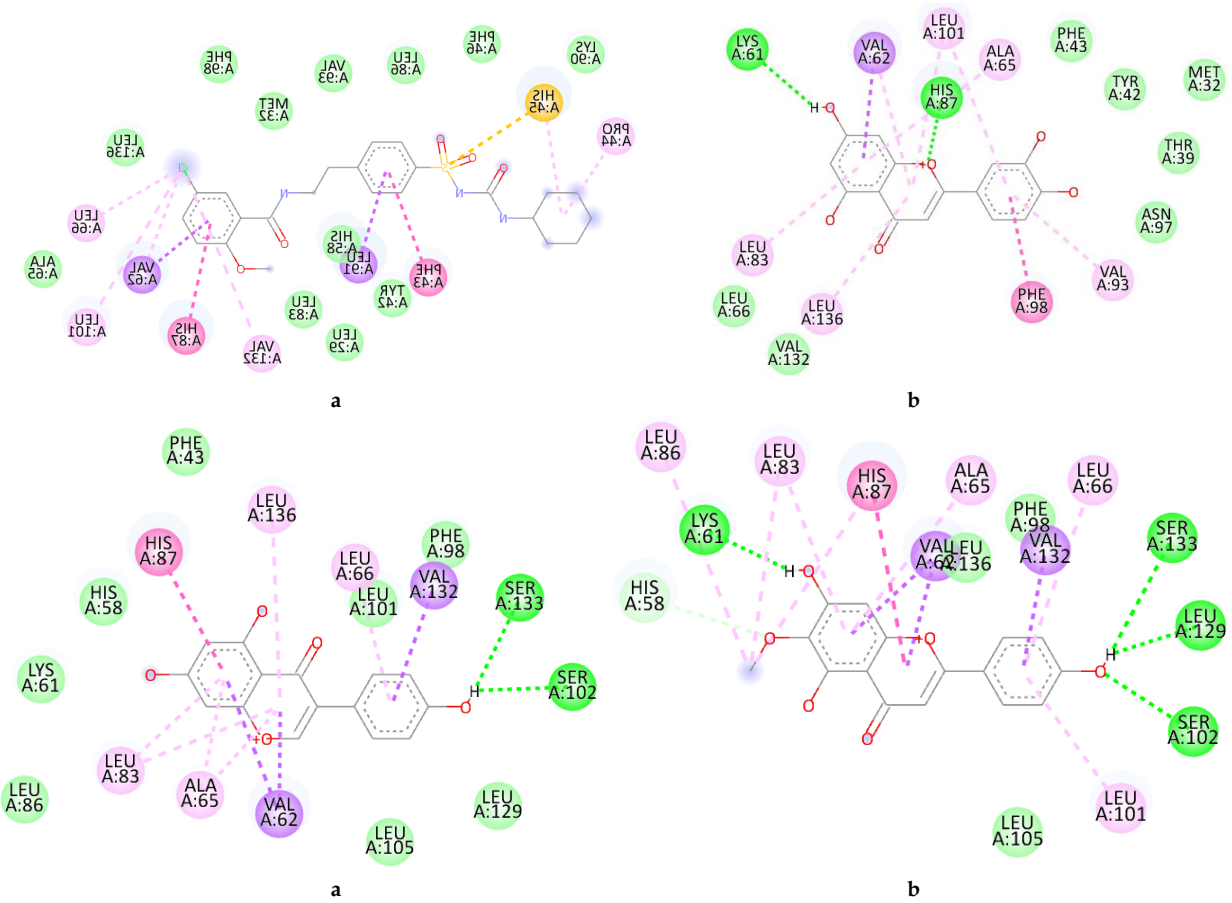


Figure 6. Interaction of glycated hemoglobin enzyme with (**a**) glibenclamide ΔG = -8.1 kJ/mol, (**b**) luteolin ΔG = -8.3 kJ/mol, (**c**) genistein ΔG = -8.0 kJ/mol, and (**d**) hispidulin ΔG = -8.0 kJ/mol.

The implementation of ADME analysis serves as a critical predictive tool for characterizing the absorption, distribution, metabolism, and excretion of potential therapeutic agents. These pharmacokinetic data are pivotal for comparing the clinical efficacy and safety profiles of synthetic standards, such as glibenclamide, with those of natural flavonoid constituents. Such evaluations enable a rigorous assessment of their viability as superior antidiabetic candidates, with the predictive values for this study summarized in **Table II**. The log P values presented in **Table II** elucidate the practical implications of lipophilicity for both glibenclamide and the identified flavonoids: luteolin, genistein, and hispidulin. As a measure of lipid solubility, the log P value is a primary determinant of a compound's pharmacokinetic behavior; glibenclamide's high log P of 3.64 suggests a strong lipophilic character that facilitates membrane penetration but may also predispose the compound to sequestration in adipose tissue. In contrast, the flavonoids exhibited log P values of 2.2-2.5, demonstrating an optimal balance between aqueous and lipid solubility, likely enhancing their stability and absorption in physiological environments.

Regarding intestinal uptake, the flavonoid compounds exhibited higher absorption percentages than the synthetic standard. Genistein demonstrated the highest intestinal absorption at 87.1%, followed by hispidulin at 84.6% and luteolin at 81.1%, whereas glibenclamide showed a comparatively lower rate of 71.7%. These findings suggest that flavonoids are more readily assimilated within the human gastrointestinal tract. However, Caco-2 permeability, a key model for human intestinal epithelial transport, showed significant variation among the flavonoids; genistein showed the highest permeability (1.207), while hispidulin exhibited the lowest (-0.045), indicating distinct transport mechanisms across the intestinal barrier. Notably, all tested compounds showed low blood-brain barrier (BBB) permeability, confirming that their pharmacological activity is predominantly sequestered in peripheral tissues, which aligns with the therapeutic requirements for managing systemic glycemic levels.

The distribution and metabolic profiles also displayed distinct differences between the natural and synthetic ligands. Luteolin exhibited the highest volume of distribution (VDss = 1.153 log L/kg), suggesting extensive tissue penetration, whereas glibenclamide remained more localized with a VDss of -0.218 log L/kg. From a metabolic perspective, none of the compounds functioned as substrates or inhibitors of CYP2D6, indicating a significantly reduced risk of metabolic drug-drug

interactions. Furthermore, the total systemic clearance of flavonoids, particularly luteolin and hispidulin, was higher than that of glibenclamide. This accelerated elimination rate suggests a reduced risk of long-term bioaccumulation, potentially positioning these natural flavonoids as safer alternatives for chronic diabetic management.

Table II. ADME prediction.

Pharmacokinetic category	Parameter	Glibenclamide	Luteolin	Genistein	Hispidulin
Lipophilicity	log P	3.6417	2.2824	2.5768	2.5854
Absorption	Caco-2 permeability (log Papp in 10^{-6} cm/s)	0.709	0.096	1.207	-0.045
	Intestinal absorption (human) (%)	71.775	81.130	87.135	84.654
Distribution	BBB permeability (log BB)	-1.010	-0.907	-1.086	-1.120
	VDss (human) (log L/kg)	-0.218	1.153	0.011	0.370
Metabolism	CYP2D6 substrate	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No
Excretion	Total clearance (log mL/min/kg)	-0.155	0.495	-25.296	0.531
	Renal OCT2 substrate	No	No	No	No

Toxicity prediction is a critical step in evaluating a compound's safety before advancing it in the drug development pipeline. This computational analysis facilitates the identification of potential adverse effects on vital organ systems, including the liver, kidneys, nervous system, and heart. By delineating these toxicological profiles, natural and synthetic compounds can be rigorously compared to select the most viable candidates for antidiabetic therapy. The results of this study, summarized in **Table III**, play a decisive role in guiding the selection of safe therapeutic agents. Among the compounds analyzed, genistein exhibited a distinct profile, with predicted hepatotoxicity and neurotoxicity. These findings are likely attributable to the complex pharmacological activity of genistein as a phytoestrogen, which may modulate the function of hepatic cells and neurons at specific concentrations. Although genistein demonstrates favorable absorption and bioavailability, its potential toxicity risks necessitate a cautious approach to its application as a natural antidiabetic agent, requiring further investigation to establish a safe therapeutic window[30].

Table III. Predicted organ-specific toxicity profiles of glibenclamide and identified flavonoids.

Toxicity parameter	Glibenclamide	Luteolin	Genistein	Hispidulin
Hepatotoxicity	No	No	Yes	No
Neurotoxicity	No	No	Yes	No
Nephrotoxicity	No	No	No	No
Cardiotoxicity	No	No	No	Yes

Hispidulin warrants significant attention due to its predicted cardiotoxic potential, despite showing no adverse effects on the liver, kidneys, or nervous system. This specific effect is likely mediated by interactions with cardiac ion channels or receptors, which can influence cardiovascular rhythm or contractile force[24]. Nevertheless, hispidulin remains a noteworthy candidate for antidiabetic drug development, particularly if these toxicological mechanisms can be mitigated through strategic dose adjustments or structural modifications. When comparing the overall safety profiles, luteolin emerges as the most favorable candidate, exhibiting no predicted toxicity across the assessed parameters. Conversely, genistein requires the most scrutiny due to its broader toxicity profile, highlighting the importance of comprehensive safety screening in the characterization of *T. diversifolia* derivatives[31].

CONCLUSION

This study demonstrates that the methanolic extract of *T. diversifolia* leaves possesses potent antidiabetic properties, as evidenced by *in vitro* assays showing its superior ability to inhibit the α-amylase enzyme and prevent the formation of glycated hemoglobin. By achieving significantly lower IC_{50} values than the synthetic standard glibenclamide, the extract demonstrates a high capacity to stabilize blood sugar levels through dual modulation of carbohydrate digestion and non-enzymatic protein glycation. These findings are further supported by molecular docking simulations, which revealed high-affinity interactions between the identified flavonoids (luteolin, genistein, and hispidulin) and the catalytic residues of the target proteins. Additionally, ADME predictions indicate a favorable pharmacokinetic profile characterized by efficient intestinal absorption and optimal distribution to peripheral tissues. By successfully integrating experimental and

computational methodologies, this research underscores the pivotal role of specific flavonoids in the therapeutic efficacy of *T. diversifolia* leaf extract, highlighting its potential as a robust and multi-targeted agent for the management of diabetes.

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AUTHORS' CONTRIBUTION

Conceptualization: Eko Suhartono, Fujiati, Muhammad Ali Faisal, Mohammad Bakhriansyah, Istiana, Dewi Indah Noviana Pratiwi
Data curation: Masniah
Formal analysis: Masniah
Funding acquisition: Masniah
Investigation: Masniah
Methodology: Eko Suhartono
Project administration: Eko Suhartono
Resources: Masniah, Eko Suhartono
Software: Eko Suhartono
Supervision: Eko Suhartono, Fujiati, Muhammad Ali Faisal, Budu, Mohammad Bakhriansyah, Istiana, Dewi Indah Noviana Pratiwi
Validation: Eko Suhartono, Fujiati, Muhammad Ali Faisal, Mohammad Bakhriansyah, Istiana, Dewi Indah Noviana Pratiwi
Visualization: Masniah, Eko Suhartono
Writing - original draft: Masniah
Writing - review & editing: Eko Suhartono, Fujiati, Muhammad Ali Faisal, Budu, Mohammad Bakhriansyah, Istiana, Dewi Indah Noviana Pratiwi

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declared no conflict of interest related to this research.

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