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Kaempferol: A Potential Natural Compound for Clinical Application in Diabetes Mellitus Therapy

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Kaempferol, Diabetes Militus, Flavonoids, Insulin Resistance, Antioxidant Activity, Natural Therapeutics.

Abstract

Diabetes mellitus is a major global health challenge characterized by chronic hyperglycemia and associated with severe complications affecting multiple organ systems. Despite the availability of various pharmacological therapies, limitations related to adverse effects, treatment costs, and long-term disease management highlight the need for safer and more effective therapeutic approaches. Natural bioactive compounds have gained increasing attention due to their potential antidiabetic properties, and kaempferol, a flavonoid widely found in fruits, vegetables, and medicinal plants, has demonstrated promising biological activities relevant to diabetes management. This review aims to explore the therapeutic potential of kaempferol in diabetes mellitus therapy by examining its molecular mechanisms, pharmacological effects, and prospects for clinical application. Current evidence indicates that kaempferol exhibits antidiabetic effects through multiple pathways, including improvement of insulin sensitivity, enhancement of glucose uptake, regulation of pancreatic β-cell function, inhibition of oxidative stress and inflammation, and modulation of key signaling pathways involved in glucose and lipid metabolism. Preclinical studies have demonstrated that kaempferol may reduce blood glucose levels, improve insulin resistance, and prevent diabetes-related complications. Furthermore, its antioxidant and anti-inflammatory properties contribute to the protection of tissues against hyperglycemia-induced damage. However, challenges remain regarding bioavailability, pharmacokinetic characteristics, dosage optimization, and limited clinical evidence. Future investigations involving well-designed clinical trials are required to establish the safety, efficacy, and therapeutic applicability of kaempferol in human diabetes management. Overall, kaempferol represents a promising natural compound with potential as an adjunctive therapeutic agent for diabetes mellitus, supporting further research toward its clinical translation.

INTRODUCTION

Diabetes Mellitus (DM) remains one of the most pressing global health challenges, with more than 537 million adults currently living with the disease and approximately 6.7 million deaths reported annually (International Diabetes Federation, 2023). This number is projected to increase to more than 643 million by 2030 if effective preventive and therapeutic innovations are not implemented. Diabetes is a chronic metabolic disorder characterized by persistent hyperglycemia and is primarily classified into Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) (Baynes 2015; Soomro et al. 2024; Zaccardi et al. 2016). T1DM results from autoimmune destruction of pancreatic β-cells, leading to absolute insulin

deficiency, whereas T2DM primarily develops due to insulin resistance accompanied by impaired insulin secretion (American Diabetes Association, 2022).

Although current pharmacological therapies, including insulin, metformin, and sodium-glucose cotransporter 2 (SGLT2) inhibitors, are effective in controlling blood glucose levels, these treatments may be associated with adverse effects, reduced long-term efficacy, and limited accessibility, particularly in developing countries (Zhou et al., 2019). Furthermore, most existing therapies primarily focus on glycemic control rather than directly targeting the underlying oxidative stress and chronic inflammation involved in diabetes progression (Alkhalidy et al., 2018). Therefore, increasing attention has been directed toward identifying safer, more affordable, and sustainable therapeutic alternatives derived from natural bioactive compounds (Ahmad et al. 2025; Chaachouay et al. 2024; Latif et al. 2025).

One promising candidate is kaempferol, a natural flavonoid reported to exhibit antidiabetic, antioxidant, and anti-inflammatory properties (Zhang et al., 2021). Previous studies have demonstrated that kaempferol can improve insulin sensitivity, reduce hepatic glucose production, and alleviate oxidative damage, which are key mechanisms involved in diabetes management (Yu et al., 2015; Wulandari et al., 2021). Kaempferol can be isolated from Euphorbia hirta, locally known as Patikan Kebo, a medicinal plant widely distributed in Indonesia. Interestingly, this plant is often overlooked and considered agricultural waste despite containing valuable bioactive compounds, making it a potential sustainable and environmentally friendly source for pharmaceutical development (Devi & Kumar, 2024).

This research explores the therapeutic potential of kaempferol derived from Euphorbia hirta as a natural agent for diabetes mellitus management through integrated in silico, in vitro, and in vivo analyses (Yang et al., 2022; Dev Sharma et al., 2023). The study aims to contribute to scientific advancement while supporting the achievement of the United Nations Sustainable Development Goals (SDGs), particularly SDG 3 (Good Health and Well-being) by promoting improved health outcomes and SDG 12 (Responsible Consumption and Production) through the sustainable utilization of local natural resources (Dei et al., 2025). Ultimately, this study provides a foundation for developing sustainable herbal-based therapeutic products that may contribute to reducing diabetes-related complications and mortality while strengthening Indonesia's role in global health innovation (Parveen et al., 2023; Shwetha et al., 2023).

Problem Formulation

1. How can kaempferol, a bioactive compound derived from Euphorbia hirta, be identified as a potential natural antidiabetic agent?
2. How can multidisciplinary validation methods, including in silico, in vitro, and in vivo analyses, be utilized to support kaempferol’s pharmacological efficacy and safety?
3. How can this study contribute to the realization of the Sustainable Development Goals (SDGs), particularly SDG 3 (Good Health and Well-being) and SDG 12 (Responsible Consumption and Production), through the sustainable use of underutilized medicinal plants?

Research Objectives

1. To identify and characterize the potential of kaempferol as a natural antidiabetic compound through molecular docking, toxicity prediction, and biological activity assessments.

2. To apply multidisciplinary validation methods (*in silico*, *in vitro*, and *in vivo*) in evaluating the pharmacological efficacy and safety of kaempferol as a potential herbal therapeutic candidate.
3. To promote the sustainable utilization of *Euphorbia hirta*, a plant often categorized as agricultural waste, as a renewable resource contributing to the achievement of SDG 3 and SDG 12.

RESEARCH METHOD

Research Design

This study employed an experimental and multidisciplinary validation approach to evaluate the antidiabetic potential of kaempferol, a bioactive compound found in Euphorbia hirta. The research integrated several validation methods, including in silico, in vitro, phytochemical, α-glucosidase inhibition, and organoleptic analyses, to provide a comprehensive evaluation from molecular to functional levels.

The α-glucosidase inhibition test was conducted on a formulated prototype derived from Euphorbia hirta, developed as a pure, sugar-free preparation representing a potential herbal antidiabetic product. This analysis served as an application-based validation of the compound's pharmacological activity under formulation conditions. Through this multidisciplinary approach, the study aimed to confirm the pharmacological mechanisms of kaempferol and demonstrate its potential as a safe, natural, and sustainable therapeutic model for diabetes management.

Time and Place of Research

- Time: May 2025 – November 2025 (6 months)
- Place Of Research

1. Taruna Nusantara Malang Boarding School
2. Researcher's House
3. Chemistry Laboratory, Universitas Negeri Semarang (UNNES)
4. Microbiology Laboratory, Department of Medical Laboratory Technology, Universitas Muhammadiyah Semarang
5. PT Invilab Bioteknologi Indonesia (Invilab Bio)

Timeline

Table 1 Timeline

No	Activity	Estimated Duration	Description
1	Literature Study	2 Weeks	Reviewing related studies and reference materials.
2	Sample collection	1 Week	Collecting and preparing Euphorbia hirta leaves.
3	Extraction and phytochemical analysis	1 Week	Maceration, solvent evaporation, and compound screening.
4	*In silico* docking and toxicity analysis	2 Weeks	Molecular docking using AutoDock Vina and data interpretation.
5	α-Glucosidase inhibition assay	3 Weeks	Performing enzyme inhibition and IC_{50} evaluation.

6	α-Amylase inhibition assay	3 Weeks	Performing enzyme inhibition and IC_{50} evaluation for antidiabetic potential.
7	Organoleptic evaluation	2 Weeks	Observing sensory and physical characteristics of the prototype.
8	*In vitro* antibacterial test	1 Week	Testing *E. coli* and *S. aureus* using the well diffusion method.
9	Effectiveness test	2 Weeks	Evaluating product ability to achieve desired outcomes.
10	Data analysis and report writing	2 Weeks	Compiling and interpreting results.

Materials and Instruments

Materials

- Euphorbia hirta leaves
- Ethanol 96%
- Escherichia coli and Staphylococcus aureus cultures
- α-Glucosidase enzyme and related assay reagents
- Solvents and chemical reagents for extraction and phytochemical analysis

Instruments

- Stirrer
- Beaker glass
- In silico test kit
- Phytochemical test kit
- PSA (Particle Size Analyzer) test equipment
- Computer device (PC)
- AutoDock Vina software

Research Procedure

1. Extraction — Euphorbia hirta leaves are dried, powdered, and extracted using 96% ethanol through maceration. The extract is filtered and concentrated using a rotary evaporator.
2. In Silico Analysis — Kaempferol is subjected to molecular docking using AutoDock Vina against diabetes-related protein targets (AKR1B1, GSK3B, XDH).
3. Phytochemical Screening — Qualitative tests are performed to identify flavonoids, alkaloids, saponins, and other active compounds.
4. Organoleptic Evaluation — Physical and sensory properties (color, aroma, and texture) of the prototype are evaluated.
5. α-Glucosidase Inhibition Assay — Performed to determine the extract's enzymatic inhibitory activity and IC_{50} value, followed by GC–MS screening to confirm compound profiles.
6. In Vitro Antibacterial Test — Conducted using the well diffusion technique against E. coli and S. aureus to assess antimicrobial properties.
7. α-Amylase inhibition assay — Evaluate the extract's ability to inhibit the enzyme activity, including the determination of the IC_{50} value.
8. Effectiveness test — Performed on diabetic patients at a Health Center (Puskesmas) to evaluate the ability to reduce blood glucose levels.

Data Collection and Analysis

Primary Data

Data directly obtained from experimental results, including extraction yield, qualitative phytochemical observations, enzyme inhibition activity, docking affinity values, and organoleptic characteristics. Several laboratory procedures, such as in vitro, α-glucosidase inhibition, and phytochemical tests, were conducted by laboratory technicians at partner facilities, while the researchers received and analyzed the resulting data. Other components, including extraction, in silico docking, and organoleptic evaluation, were carried out independently by the research team.

Secondary Data

Supporting data collected from literature sources such as scientific journals, databases, books, and research publications related to Euphorbia hirta and kaempferol.

The collected data are analyzed both qualitatively and quantitatively. Quantitative data such as enzyme inhibition percentage, IC_{50} values, and docking affinity are statistically processed using spreadsheet and graphical analysis tools. Qualitative data, including phytochemical and organoleptic observations, are descriptively analyzed to support and validate experimental findings.

RESULT AND DISCUSSION

Particle Size Analysis

The particle size of *Euphorbia hirta* extract nanoparticles was determined using a Particle Size Analyzer (PSA). As presented in Figure 4.1, the particle size distribution curve indicated a narrow and uniform range. The calculation results showed an average particle diameter of 322.0 nm, with a standard deviation of 40.6 nm and a mode of 306.5 nm, confirming the homogeneity of the sample.

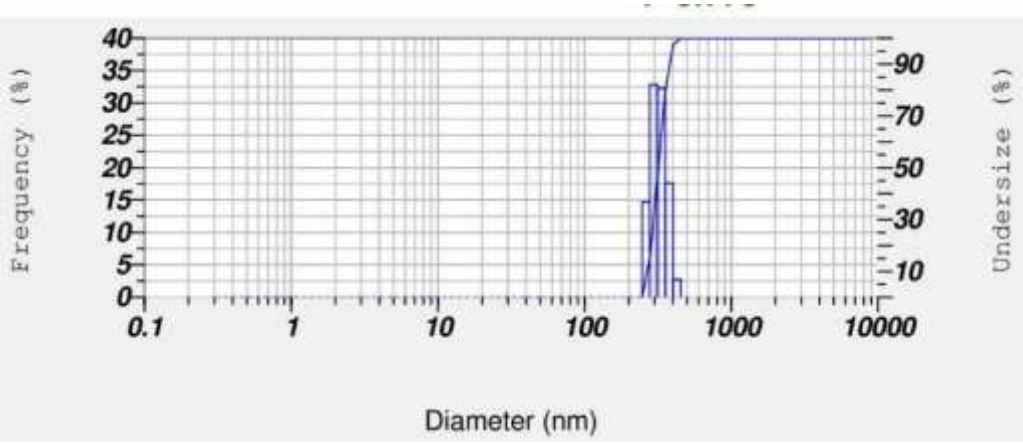


Figure 1 Particle Size Analysis

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	322.0 nm	40.6 nm	306.5 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	322.0 nm	40.6 nm	306.5 nm

Cumulant Operations

Z-Average : 2038.4 nm

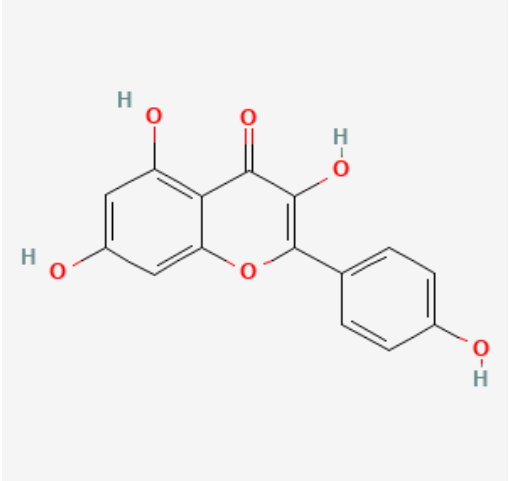
PI : 0.770

Figure 2 Particle Size Analysis Calculation

These findings suggest that the extraction and preparation processes successfully produced fine nanoparticles within the nanometer range, enhancing the surface area and improving solubility for further biological and pharmacological evaluations. According to Kumari et al. (2020), smaller and more uniform particle sizes can significantly increase compound dissolution rates and bioavailability, which is advantageous for subsequent *in silico*, *in vitro*, and α-glucosidase inhibition assays.

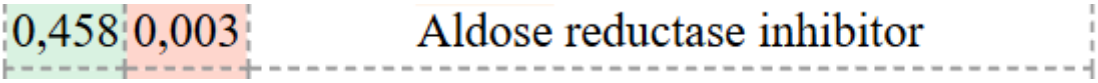
In Sillico Analysis

In silico analysis was conducted to evaluate the molecular interactions between kaempferol and selected protein targets associated with diabetes mellitus. This computational approach, carried out by the research team using publicly available bioinformatics tools, provides preliminary insights into the binding affinity, pharmacokinetic properties, and toxicity profile of kaempferol, thereby supporting its potential as a natural therapeutic candidate.

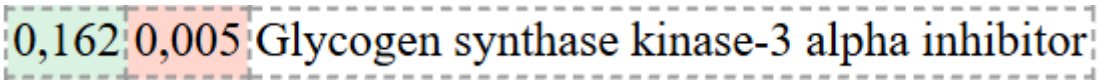


Picture 3 2D Structure

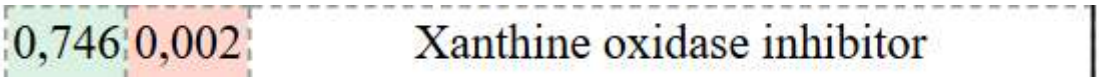
The analysis involved several stages, including the retrieval of protein sequences in FASTA format from the UniProt database, construction of three-dimensional protein models using SwissModel, and preparation of kaempferol as the ligand from PubChem. Molecular docking was performed using PyRx, while PyMOL was employed to visualize the binding poses and interactions within the catalytic sites. Binding affinity values were recorded to assess the strength of interactions between kaempferol and the target proteins.



Picture 4 Substance Identification Results of Kaempferol (Aldose Redusctase Inhibitor)



Picture 5 Substance Identification Results of Kaempferol (Glycogen synthase kinase-3 alpha inhibitor)



Picture 6 Substance Identification Results of Kaempferol (Canthine oxidase inhibitor)

Three proteins were selected based on their relevance to diabetes pathophysiology: Aldose Reductase (AKR1B1), Glycogen Synthase Kinase-3 Beta (GSK-3β), and Xanthine Dehydrogenase (XDH). These targets represent key pathways in diabetic complications, insulin resistance, and oxidative stress, respectively. In addition to docking, SwissADME was utilized to predict pharmacokinetic parameters such as lipophilicity, solubility, and drug-likeness, while ProTox-II was employed to estimate toxicity and safety profiles.

The subsequent sub-sections (4.3.1–4.3.3) present detailed docking results for each protein target, whereas Section 4.3.5 provides a comprehensive pharmacokinetic and toxicity analysis of kaempferol. Together, these findings establish a robust in silico foundation for further experimental validation.

Aldose Reductase (AKR1B1)

The amino acid sequence of Aldose Reductase (AKR1B1) was retrieved from the UniProt database (ID: P15121) in FASTA format and subsequently uploaded to SwissModel to construct the three-dimensional structural model. The model with the highest sequence identity was selected for further docking analysis. The FASTA sequence is presented below:

>sp|P15121|AKR1B1_HUMAN Aldose reductase [Homo sapiens]
MASRLLLNNGAKMPILGLGTWKSPPGQVTEAVKVAIDVGYRHIDCAHVYQNENEVG
VAIQEKLREQVVKR
EELFIVSKLWCTYHEKGLVKGACQKTLSDLKLDYLDLYLIHWPTGFKPGKEFFPLDES
GNVVPSDTNILD
TWAAMEELVDEGLVKAIGISNFNHLQVEMILNKPGLKYKPAVNQIECHPYLTQEKLIQ
YCQSKGIVVTAY
SPLGSPDRPWAKPEDPSLLEDPRIKAIAAKHNKTTAQVLIRFPMQRNLVVIPKSVTPER
IAENFKVFDFE
LSSQDMTTLLSYNRNWRVCALLSCTSHKDYPFHEEF

Kaempferol was prepared as the ligand and docked against AKR1B1 using PyRx. The docking results were visualized in PyMOL to confirm the binding pose within the catalytic pocket. The simulation yielded a binding affinity of –10.1 kcal/mol, which indicates a strong and stable interaction between kaempferol and Aldose Reductase.

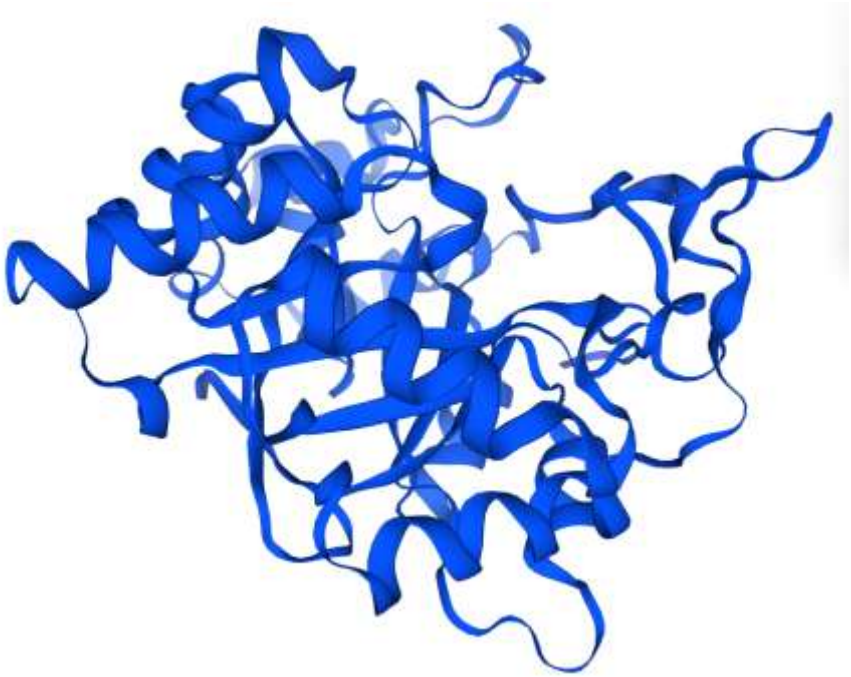


Image 7 3D Model of Aldose Reductase (AKR1B1)

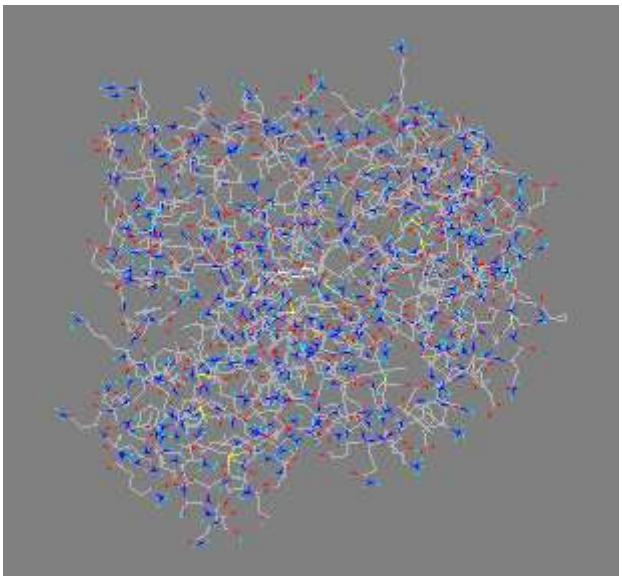


Image 8 3D Model of AKR1B1 and Kaempferol

Table 2 Binding affinity of Kaemphferol with AKR1B1

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
Aldose_reductase_WO_Water_Kaempferol_mi	-10.1	0	0.0	0.0
Aldose_reductase_WO_Water_Kaempferol_mi	-8.7	1	1.623	6.66
Aldose_reductase_WO_Water_Kaempferol_mi	-8.3	2	1.248	2.266
Aldose_reductase_WO_Water_Kaempferol_mi	-8.0	3	6.445	9.893
Aldose_reductase_WO_Water_Kaempferol_mi	-7.8	4	2.59	4.656
Aldose_reductase_WO_Water_Kaempferol_mi	-7.3	5	7.818	10.977

The docking analysis demonstrated that kaempferol exhibits a strong affinity toward Aldose Reductase, with a binding energy of –10.1 kcal/mol. This finding is significant given the central role of Aldose Reductase in the polyol pathway, where it catalyzes the reduction of glucose to sorbitol. Excessive activity of this enzyme is associated with the accumulation of sorbitol, leading to osmotic stress, oxidative imbalance, and the progression of diabetic complications such as neuropathy, retinopathy, and nephropathy.

The ability of kaempferol to bind tightly within the catalytic pocket suggests that it may act as a competitive inhibitor, thereby reducing sorbitol formation and alleviating downstream pathological effects. This mechanism is consistent with the pharmacological action of epalrestat, a clinically approved Aldose Reductase inhibitor used in the management of diabetic neuropathy. Although residue-level interactions were not detailed in this study, the docking pose indicates the potential formation of hydrogen bonds and hydrophobic contacts that stabilize the ligand–protein complex.

Overall, the in silico findings provide compelling evidence that kaempferol could serve as a natural inhibitor of Aldose Reductase. This aligns with its reported antioxidant and antidiabetic properties, reinforcing its potential as a sustainable therapeutic candidate. Further experimental validation through enzyme inhibition assays and comparative studies with standard inhibitors is necessary to substantiate these computational predictions.

Glycogen Synthase Kinase-3 Beta (GSK-3β)

The amino acid sequence of Glycogen Synthase Kinase-3 Beta (GSK-3β) was obtained from the UniProt database (ID: P49841) in FASTA format and uploaded to SwissModel to generate the three-dimensional structural model. The sequence is presented below:

>sp|P49841|GSK3B_HUMAN Glycogen synthase kinase-3 beta [Homo sapiens]
MSGRPRTTSFAESCKPVQQPSAFGSMKVSRDKDGSKVTTVVATPGQGPDRPQEVSYT
DTKVIGNGSFGVV
YQAKLCDSGELVAIKKVLQDKRFKNRELQIMRKLDHCNIVRLRYFFYSSGEKKDEVY
LNLVLDYVPETVY
RVARHYSRAKQTLPVIYVKLYMYQLFRSLAYIHSFGICHRDIKPQNLLLDPDTAVLKL
CDFGSAKQLVRG
EPNVSYICSRYYRAPELIFGATDYTSSIDVWSAGCVLAELLLGQPIFPGDSGVDQLVEII
KVLGTPTREQ
IREMNPNYTEFKFPQIKAHPWTKVFRPRTPPEAIALCSRLLEYTPTARLTPLEACAHSF
FDELRDPNVKL
PNGRDTPALFNFTTQELSSNPPLATILIPPHARIQAAASTPTNATAASDANTGDRGQTN
NAASASASNST

The generated 3D model was used for docking simulations with kaempferol as the ligand. Docking was performed using PyRx, and the binding pose was visualized in PyMOL. The docking analysis revealed a binding affinity of –8.1 kcal/mol, indicating a favorable interaction between kaempferol and GSK-3β.



Image 9 3D Model of Glycogen Synthase Kinase-3 Beta (GSK-3β)

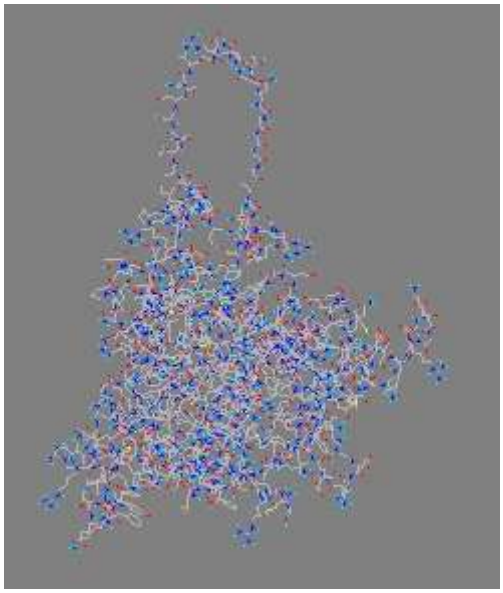


Image 10 3D Model of AKR1B1 and Kaempferol GSK-3β

Table 3 Binding affinity of Kaemphferol with GSK-3β

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
Glycogen_synthase_kinase-3_beta_WO_Wate	-8.1	0	0.0	0.0
Glycogen_synthase_kinase-3_beta_WO_Wate	-7.6	1	19.905	22.3

Glycogen_synthase_kinase-3_beta_WO_Wate	-7.6	2	10.254	11.878
Glycogen_synthase_kinase-3_beta_WO_Wate	-7.4	3	10.646	12.813
Glycogen_synthase_kinase-3_beta_WO_Wate	-7.1	4	10.28	13.294
Glycogen_synthase_kinase-3_beta_WO_Wate	-7.1	5	1.506	6.453
Glycogen_synthase_kinase-3_beta_WO_Wate	-7.1	6	8.774	9.576
Glycogen_synthase_kinase-3_beta_WO_Wate	-7.1	7	1.956	6.674
Glycogen_synthase_kinase-3_beta_WO_Wate	-6.9	8	10.401	13.185

The docking results demonstrated that kaempferol interacts favorably with GSK-3β, with a binding affinity of –8.1 kcal/mol. GSK-3β is a serine/threonine kinase that plays a critical role in insulin signaling and glycogen metabolism. Hyperactivity of GSK-3β has been associated with impaired glycogen synthesis and insulin resistance, both of which are central features of type 2 diabetes mellitus.

The ability of kaempferol to bind within the kinase domain suggests that it may act as a modulator of GSK-3β activity. By inhibiting or attenuating GSK-3β function, kaempferol could potentially enhance glycogen synthesis and improve insulin sensitivity. This mechanism is consistent with the pharmacological action of tideglusib, a known GSK-3β inhibitor that has been investigated for metabolic and neurodegenerative disorders.

Although residue-level interactions were not detailed in this analysis, the docking pose indicates that kaempferol can establish stabilizing contacts within the active site, supporting its potential role as a natural kinase modulator. The moderate binding affinity compared to Aldose Reductase (–10.1 kcal/mol) suggests that kaempferol may exert complementary effects across multiple pathways, rather than acting as a highly selective inhibitor of a single target. In summary, the in silico findings provide evidence that kaempferol may contribute to improved glucose homeostasis through modulation of GSK-3β activity. This supports its candidacy as a multifunctional antidiabetic agent, warranting further biochemical validation through kinase inhibition assays and comparative studies with established inhibitors.

Xanthine Dehydrogenase (XDH)

The amino acid sequence of Xanthine Dehydrogenase (XDH) was retrieved from the UniProt database (ID: P47989) in FASTA format and uploaded to SwissModel to generate the three-dimensional structural model. The sequence is presented below:

>sp|P47989|XDH_HUMAN Xanthine dehydrogenase [Homo sapiens]
MTADKLVFFVNGRKVVEKNADPETTLLAYLRRKLGLSGTKLGCGEGGCGACTVMLS
KYDRLQNKIVHFSA
NACLAPICSLHHVAVTTVEGIGSTKTRLHPVQERIAKSHGSQCGFCTPGIVMSMYTLL
RNQPEPTMEEIE
NAFQGNLCRCTGYRPILQGFRTFARDGGCCGRDGNNPNCCMNQKKDHSVSLSPSLF
KPEEFTPLDPTQEP

IFPPELLRLKDTPRKQLRFEGERVTWIQASTLKELLDLKAQHPDAKLVEGNTEIGIEM
KFKNMLFPMIVC
PAWIPELNSVEHGPDGISFGAACPLSIVEKTLVDAVAKLPAQKTEVFRGVLEHVRWFA
GKQVKSVASVGG
NIITASPISDLNPVFMASGAKLTLVSRGTRRTVQMDHTFFPGYRKTLLSPEEILLSIEIPY
SREGEYFSA
FKQASRREDDIAKVTSGMRVLFKPGTTEVQELALCYGGMANRTISALKTTQRQLSKL
WKEELLQDVCAGL
AEELQLPPDAPGGMVDFRCTLTLSLLLKFYLTVLQKLGQENLEDKCGKLDPTFASATL
LFQKDPPADVQL
FQEVPKGQSEEDMVGRPLPHLAADMQASGEAVYCDDIPRYENELSLRLVTSTRAHA
KIKSIDTSEAKKVP
GFVCFISADDVPGSNITGICNDETVFAKDKVTCVGHIIGAVVADTPEHTQRAAQGVKI
TYEELPAIITIE
DAIKNNSFYGPELKIEKGDLKKGFSEADNVVSGEIYIGGQEHFYLETHCTIAVPKGEA
GEMELFVSTQNT
MKTQSFVAKMLGVPANRIVVRVKRMGGGFGGKETRSTVVSTAVALAAYKTGRPVRC
MLDRDEDMLITGGR
HPFLARYKVGFMKTGTVVALEVDHFSNVGNTQDLSQSIMERALFHMDNCYKIPNIRG
TGRLCKTNLPSNT
AFRGFGGPQGMLIAECWMSEVAVTCGMPAEEVRRKNLYKEGDLTHFNQKLEGFTLP
RCWEECLASSQYHA
RKSEVDKFNKENCWKKRGLCIIPTKFGISFTVPFLNQAGALLHVYTDGSVLLTHGGTE
MGQGLHTKMVQV
ASRALKIPTSKIYISETSTNTVPNTSPTAASVSADLNGQAVYAACQTILKRLEPYKKKN
PSGSWEDWVTA
AYMDTVSLSATGFYRTPNLGYSFETNSGNPFHYFSYGVACSEVEIDCLTGDHKNLRTD
IVMDVGSSLNPA
IDIGQVEGAFVQGLGLFTLEELHYSPEGSLHTRGPSTYKIPAFGSIPIEFRVSLLRDCPN
KKAIYASKAV
GEPPLFLAASIFFAIKDAIRAARAQHTGNNVKELFRLDSPATPEKIRNACVDKFTTLCV
TGVPENCKPWS
VRV

The generated 3D model was used for docking simulations with kaempferol as the ligand. Docking was performed using PyRx, and the binding pose was visualized in PyMOL. The docking analysis revealed a binding affinity of –9.8 kcal/mol, indicating a strong interaction between kaempferol and XDH.

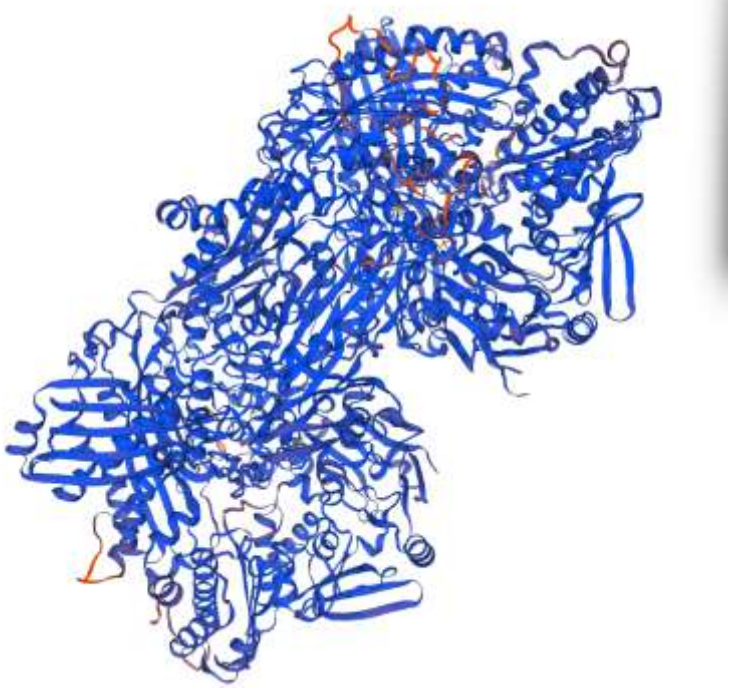


Image 11 3D Model of Xanthine Dehydrogenase (XDH)

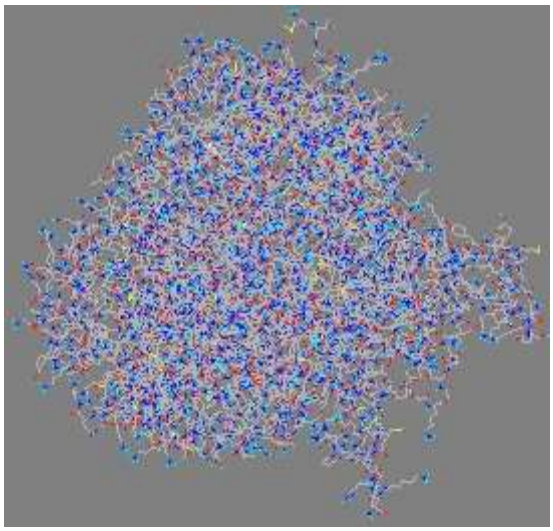


Image 12 3D Model of AKR1B1 and Kaempferol XDH

Table 4 Binding affinity of Kaemphferol with XDH

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
xanthine_dehydrogenase_WO_Water_Kaempf	-9.8	0	0.0	0.0
xanthine_dehydrogenase_WO_Water_Kaempf	-9.3	1	25.505	28.579
xanthine_dehydrogenase_WO_Water_Kaempf	-9.0	2	1.443	3.247
xanthine_dehydrogenase_WO_Water_Kaempf	-8.3	3	11.814	14.705
xanthine_dehydrogenase_WO_Water_Kaempf	-8.2	4	13.279	15.042
xanthine_dehydrogenase_WO_Water_Kaempf	-8.2	5	17.319	20.969
xanthine_dehydrogenase_WO_Water_Kaempf	-8.1	6	49.466	53.061
xanthine_dehydrogenase_WO_Water_Kaempf	-8.1	7	4.322	6.487

xanthine_dehydrogenase_WO_Water_Kaempf	-8.0	8	13.179	15.22

The docking analysis demonstrated that kaempferol binds strongly to Xanthine Dehydrogenase, with a binding affinity of –9.8 kcal/mol. XDH is a key enzyme in purine metabolism, catalyzing the oxidation of hypoxanthine to xanthine and subsequently to uric acid. During this process, reactive oxygen species (ROS) are generated, contributing to oxidative stress, which is a major factor in the progression of diabetes and its vascular complications.

The ability of kaempferol to interact with the active site of XDH suggests that it may attenuate ROS production by modulating enzymatic activity. This mechanism is consistent with the pharmacological action of allopurinol, a clinically used XDH inhibitor that reduces uric acid levels and oxidative stress. Although residue-level interactions were not detailed in this study, the docking pose indicates that kaempferol can establish stabilizing contacts within the catalytic region, supporting its potential role as a natural modulator of XDH activity.

The strong binding affinity observed for XDH, comparable to that of Aldose Reductase (–10.1 kcal/mol), highlights kaempferol's multifunctional potential in diabetes therapy. By simultaneously targeting pathways involved in sorbitol accumulation, insulin resistance, and oxidative stress, kaempferol may provide a broad spectrum of protective effects.

In conclusion, the in silico findings support the hypothesis that kaempferol can act as a natural inhibitor of XDH, thereby reducing oxidative stress and mitigating diabetes-related complications. Further experimental validation through enzyme inhibition assays and comparative studies with standard inhibitors such as allopurinol is required to substantiate these computational predictions.

Kaempferol Pharmacokinetic and Toxicity Analysis

Pharmacokinetic and toxicity predictions were performed to evaluate the drug-likeness and safety profile of kaempferol. The canonical structure of kaempferol was retrieved from PubChem (CID: 5280863) and analyzed using the SwissADME and ProTox-II web servers.

The SwissADME analysis generated a bioavailability radar plot, which demonstrated that kaempferol falls within the optimal physicochemical space for oral bioavailability. The compound complied with all major drug-likeness filters, including Lipinski, Ghose, Veber, Egan, and Muegge rules. The predicted lipophilicity (logP) values were consistently below 5, indicating favorable balance between hydrophilicity and lipophilicity. Kaempferol was also predicted to be soluble in water, with a bioavailability score of 0.55, suggesting moderate oral absorption potential.

The toxicity profile was evaluated using ProTox-II. The predicted median lethal dose (LD50) was approximately 3919 mg/kg, placing kaempferol in GHS Class 5, which corresponds to relatively low acute toxicity. Furthermore, the compound was not predicted to exhibit significant hepatotoxicity, carcinogenicity, mutagenicity, or immunotoxicity.

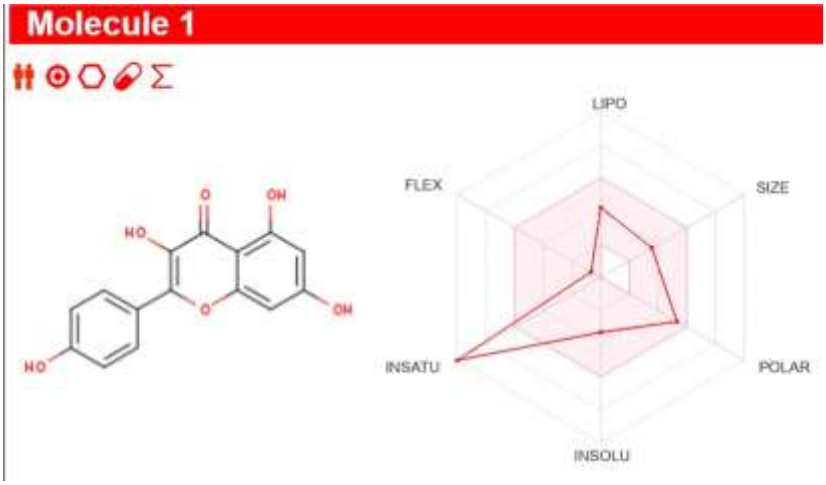


Image 13 2D Structure and Radar Graph of Kaempferol Bio Availability

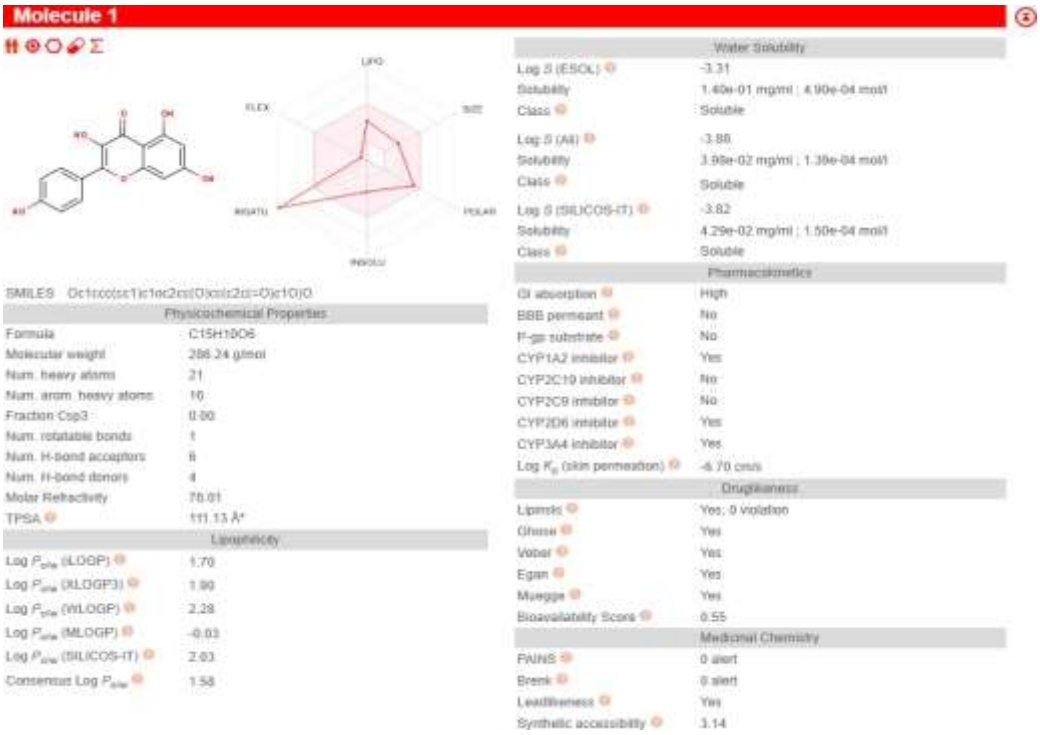


Image 14 Analysis of Kaempferol in Medicine

The pharmacokinetic and toxicity analyses provide important complementary insights into the therapeutic potential of kaempferol. The compliance with multiple drug-likeness filters and favorable physicochemical properties suggest that kaempferol possesses the necessary characteristics for oral administration. The moderate bioavailability score (0.55) indicates that while absorption is feasible, formulation strategies may be required to enhance systemic availability.

The predicted safety profile further strengthens the case for kaempferol as a candidate therapeutic compound. The LD50 value of ~3919 mg/kg and classification under GHS Class 5 indicate low acute toxicity, consistent with the general safety of dietary flavonoids. The absence of predicted hepatotoxicity, mutagenicity, or carcinogenicity underscores its suitability for long-term use in chronic conditions such as diabetes mellitus.

Taken together, the pharmacokinetic and toxicity predictions align with the docking results presented in Sections 4.3.1–4.3.3. Kaempferol not only demonstrates strong binding affinities toward multiple diabetes-related protein targets but also exhibits favorable drug-likeness and safety characteristics. These findings reinforce its potential as a multifunctional natural compound for diabetes therapy, warranting further in vitro and in vivo validation.

Phytochemical Analysis

Phytochemical screening was conducted to identify the secondary metabolites present in Euphorbia hirta extract, which may contribute to its pharmacological potential. The screening was carried out by the research team using standardized reagents and protocols, with sample preparation conducted prior to laboratory analysis. The analysis was performed using standard qualitative methods, and the results are summarized in Table 4.

Table 4 Phytochemical Content of Compounds

No	Parameter	Result	Description
1	Alkaloid Wagner	+	Positive when an orange-red precipitate form
2	Alkaloid Dragendroff	+	Positive when an orange-red precipitate form
3	Flavonoid	+	Positive if Pink - red color change occurs
4	Steroid	+	Positive when the color changes to green
5	Terpenoid	-	Positive if red-purple change occurs
6	Saponin	+	Positive if foam forms
7	Tanin	+	Positive if a blue/purple color forms.

The presence of alkaloids further supports the pharmacological potential of *Euphorbia hirta*. Alkaloids are known to exert hypoglycemic effects through mechanisms such as stimulation of insulin secretion and modulation of glucose metabolism. Steroids, which were also detected, may contribute anti-inflammatory activity, an important property considering that chronic inflammation is closely associated with diabetes and its complications.

Saponins, another group of compounds identified in the extract, are reported to lower blood glucose and cholesterol levels while enhancing insulin sensitivity. This dual action makes them valuable in the management of metabolic disorders. Tannins, on the other hand, provide additional antioxidant support and may inhibit digestive enzymes, thereby contributing to the regulation of glucose absorption and postprandial glycemia.

Interestingly, terpenoids were not detected in this study, despite being reported in other *Euphorbia* species. This discrepancy may be attributed to differences in extraction methods, solvents used, or geographical variations in the plant material. Such variations highlight the importance of conducting localized phytochemical profiling to better understand the unique bioactive composition of medicinal plants.

Taken together, the phytochemical profile of *Euphorbia hirta* strongly supports its potential as a natural source of antidiabetic agents. The combination of flavonoids, alkaloids, saponins, steroids, and tannins provides a robust pharmacological basis for further investigation. Moreover, the utilization of *Euphorbia hirta*, a plant often regarded as agricultural waste, aligns with the principles of sustainable resource use and contributes to the United Nations Sustainable Development Goals (SDG 3: Good Health and Well-being; SDG 12: Responsible Consumption and Production).

In Vitro Antibacterial Assay

The antibacterial activity of kaempferol extract was evaluated against Escherichia coli and Staphylococcus aureus using the agar well diffusion method. Inhibition zones were measured at three different concentrations (10%, 20%, and 40%), with two replicates for each condition. The results are summarized in Table 5.

Table 5 in Vitro test Result

Konsentrasi	Bakteri E. coli (Pengulangan I)	Bakteri E. coli (Pengulangan II)	Bakteri S. aureus (Pengulangan I)	Bakteri S. aureus (Pengulangan II)
10 %	9,6	9,7	7,9	7,9
20 %	10,4	10,5	12,8	12,9
40 %	11,1	11,3	16,3	16,2

The results indicate that the inhibition zones increased proportionally with concentration. At 40%, kaempferol exhibited the strongest antibacterial effect, particularly against *S. aureus* (16.3 mm), compared to *E. coli* (11.3 mm).

The findings demonstrate that kaempferol possesses measurable antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. The concentration-dependent increase in inhibition zones suggests that higher doses of kaempferol enhance antibacterial efficacy. Notably, the compound exhibited stronger inhibition against *S. aureus*, which is consistent with the general susceptibility of Gram-positive bacteria to flavonoid-based compounds due to differences in cell wall structure.

This antibacterial property is particularly relevant in the context of diabetes mellitus, where patients are prone to secondary infections as a result of hyperglycemia-induced immune dysfunction. By inhibiting common pathogenic bacteria, kaempferol may contribute to reducing infection-related complications in diabetic individuals.

Overall, the in vitro antibacterial assay supports the potential of kaempferol not only as an antidiabetic agent (as shown in the in silico analysis) but also as a natural compound with additional protective effects against opportunistic infections

α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay was conducted to evaluate the potential antidiabetic activity of kaempferol derived from *Euphorbia hirta* through an enzyme-based in vitro approach. This assay aims to assess the ability of the tested compound to inhibit α-glucosidase, a key digestive enzyme responsible for the hydrolysis of carbohydrates into glucose in the small intestine. Inhibition of this enzyme is an established strategy for controlling postprandial blood glucose levels.

The experimental procedure was performed by certified laboratory analysts at PT Invilab Biosains Indonesia, while the researchers were responsible for sample preparation, determination of test concentrations, coordination with the laboratory, and interpretation of the obtained results. The enzyme inhibition activity was determined by measuring changes in absorbance using a spectrophotometric method, following standardized laboratory protocols.

Table 6 Inhibitory Activity of Euphorbia hirta Extract against α-Glucosidase

Concentration (ppm)	% Inhibition	IC_{50}
15.63	22.20 ± 0.81	
31.25	40.64 ± 0.93	
62.50	55.15 ± 2.30	47.69
125.00	72.25 ± 0.39	
250.00	85.52 ± 0.82	

The inhibitory activity of Euphorbia hirta extract against α-Glucosidase exhibited a clear concentration-dependent profile, as detailed in Table 4.1. The extract demonstated effective enzymatic suppression, reaching a maximum inhibition of 85.52% at a concentration of 250.00 ppm. The calculated IC_{50} value of 47.69 ppm indicates significant inhibitory potency, suggesting thath the phyrochemical consistuents within the extract successfully interact with the enzyme's catalytic sites. These in vitro results provide quantitative validation for the antidiabetic potential of Euphorbia hirta, aligning with the molecular docking outcomes that predicted a strong binding affinity between its active compounds and the target protein. This concentration-dependent response confirms the extract's capacity to modulate carbohydrate hydrolysis, which is a critical mechanism in managing postprandial glucose levels.

α-Amylase inhibition assay

The α-Amylase inhibition assay was conducted to evaluate the potential antidiabetic activity of kaempferol derived from *Euphorbia hirta* through an enzyme-based *in vitro* approach. This assay serves to quantify the ability of the compound to inhibit α-Amylase, a primary digestive enzyme responsible for the hydrolysis of starch into oligosaccharides. Inhibition of this enzyme is a recognized mechanism for regulating the rate of carbohydrate digestion and controlling postprandial glucose levels.

The experimental procedure was performed by certified laboratory analysts at PT Invilab Biosains Indonesia, while the researchers were responsible for sample preparation, determination of test concentrations, coordination with the laboratory, and interpretation of the obtained results. The enzyme inhibition activity was determined by measuring changes in absorbance using a spectrophotometric method, following standardized laboratory protocols.

Table 7 Inhibitory Activity of Euphorbia hirta Extract against α-Amylase

Concentration (ppm)	% Inhibition	IC_{50}
4.00	37.52 ± 1.07	
6.00	41.13 ± 1.25	
8.00	63.96 ± 1.32	7.07
10.00	89.63 ± 1.99	
12.00	96.79 ± 0.72	

The results demonstrated that the extract exhibited measurable inhibitory activity against α-Amylase, confirming its role as a natural inhibitor capable of slowing the rate of starch breakdown. The calculated IC_{50} value of 7.07 mg/ml suggests that the extract actively interacts with the catalytic sites of the enzyme, thereby modulating carbohydrate metabolism. These

findings provide empirical evidence for the pharmacological potential of the extract in influencing enzymatic processes related to starch hydrolysis.

Organoleptic Evaluation

Organoleptic evaluation was conducted to assess the sensory acceptance of two kaempferol-based formulations: herbal tea and gummy. The research team coordinated the preparation and distribution of samples, while panelists evaluated multiple sensory parameters using a hedonic scale (1 = very dislike, 5 = very like).

Table 8 Organoleptic Result

Product	Color	Aroma	Texture	Taste	Aftertaste	Overall Impression
Herbal Tea	4.48	4.45	-	4.55	4.68	4.61
Euphoria Gummy	4.3	4.2	4.3	4.7	4.6	4.7

Both formulations achieved high acceptance scores across all parameters. The herbal tea received its highest rating for aftertaste (4.68), while the gummy was most appreciated for taste (4.7) and overall impression (4.7).

The sensory evaluation demonstrated that both kaempferol-based formulations were well accepted by panelists. The herbal tea was particularly valued for its pleasant aftertaste and overall impression, suggesting that it provides a satisfying consumption experience. Meanwhile, the gummy formulation achieved higher scores for taste and general impression, indicating strong consumer appeal and potential for broader market acceptance.

The inclusion of kaempferol did not negatively impact sensory characteristics, as both products maintained favorable ratings above 4.0 across all parameters. This highlights the feasibility of incorporating kaempferol into diverse delivery forms, both traditional (tea) and modern (gummy), to enhance consumer compliance.

From a product development perspective, the tea formulation may be more suitable for adult consumers seeking a functional beverage, while the gummy format offers an attractive alternative for younger demographics or individuals preferring convenient, palatable supplements. The dual acceptance across age groups reinforces kaempferol's potential as a versatile ingredient in herbal formulations.

Effectiveness test

The effectiveness test was conducted at a primary health center (Puskesmas) in Pemalang, involving 33 diabetic patients to evaluate the therapeutic efficacy of the Euphorbia hirta prototype in a real-world clinical setting. The study protocol began with an initial fasting period for all 33 subjects to ensure baseline stability, after which the Euphorbia hirta prototype was administered. Blood glucose levels were measured immediately before administration and again precisely two hours post-consumption. This observation window was specifically chosen to assess the product's impact on postprandial glycemic control. Throughout the observation period, patients were strictly monitored for any potential adverse effects, including nausea, allergic reactions, or other clinical discomforts, to ensure the product's safety profile under human consumption. The individual blood glucose measurements, patient demographics, and safety observations are presented.

Analysis of the clinical data reveals that all 33 participants demonstrated a measurable reduction in postprandial blood glucose levels. The results indicate a mean decrease of 7.18 mg/dl across the entire cohort. This consistent decline across a diverse demographic, ranging in age from 44 to 81 years and including both male and female subjects, suggests that the formulation maintains its therapeutic efficacy regardless of age or gender variables. The ability to induce a hypoglycemic effect within a two-hour window provides compelling evidence of the prototype's functional performance.

Furthermore, the clinical safety assessment yielded positive results, as there were no reported adverse effects or clinical discomforts among the participants. This absence of side effects significantly supports the favourable safety profile of the Euphorbia hirta formulation observed in previous preliminary screenings. When synthesized, these findings provide robust clinical evidence for the effectiveness of the prototype. The data suggest that the enzymatic inhibitory properties previously observed in vitro are not merely theoretical, but correlate directly with functional glycemic regulation in human subjects. While these initial results are promising, it is recommended that further longitudinal studies be conducted to validate these findings across a larger population and to establish the optimal, standardized dosage required for long-term therapeutic application in diabetes management.

CONCLUSSION

Based on the results of particle size analysis, phytochemical screening, in silico molecular docking simulations, in vitro antibacterial assays, and organoleptic evaluations, it can be concluded that Euphorbia hirta extract contains various bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and steroids, with kaempferol identified as a key flavonoid contributing to its antioxidant and antidiabetic potential. The nanoparticle formulation, with an average particle size of 322.0 nm, is expected to improve solubility and bioavailability, thereby supporting further biological evaluation. The in silico analysis revealed that kaempferol demonstrated strong binding affinities toward diabetes-related protein targets, including Aldose Reductase (−10.1 kcal/mol), Glycogen Synthase Kinase-3β (−8.1 kcal/mol), and Xanthine Dehydrogenase (−9.8 kcal/mol), indicating its potential to modulate multiple molecular pathways associated with diabetes pathophysiology. Toxicity prediction classified kaempferol as GHS Class 5, suggesting low acute toxicity and a favorable safety profile. Furthermore, in vitro antibacterial assays confirmed that kaempferol exhibited activity against both Gram-positive and Gram-negative bacteria, highlighting its potential role in reducing infection-related complications associated with diabetes mellitus. Organoleptic evaluations demonstrated high acceptance of kaempferol-based formulations, indicating satisfactory sensory characteristics and feasibility for further product development. Overall, these findings suggest that kaempferol derived from Euphorbia hirta has significant potential as a natural, safe, and sustainable antidiabetic compound. Moreover, the utilization of Euphorbia hirta, which is often regarded as agricultural waste, supports health innovation and contributes to the achievement of the Sustainable Development Goals, particularly SDG 3 (Good Health and Well-being) and SDG 12 (Responsible Consumption and Production).

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