Integrated In-Vitro and Silico Assessment of *Hydrocotyle Umbellata* Crude Extract: Salivary Amylase Inhibition, Molecular Docking, and Predictive ADMET Analysis for Anti-Diabetic Potential

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ABSTRACT

Diabetes mellitus remains a major global health concern, prompting the continued search for safe and effective natural agents capable of regulating postprandial hyperglycemia. This study evaluated the anti-diabetic potential of *Hydrocotyle umbellata* (Takip-Kohol) through in vitro α-amylase inhibition assays, complemented by molecular docking and in silico ADMET analysis to elucidate possible mechanisms and drug-likeness properties. An ethanolic crude extract of *H. umbellata* leaves was prepared via maceration followed by rotary evaporation and assessed at three dilution concentrations (1:2, 1:5, and 1:10 v/v), with all assays performed in triplicate to ensure reproducibility. The inhibitory activity against salivary α-amylase was quantitatively measured, and corresponding IC_{50} values were determined.

Results demonstrated a clear concentration-dependent inhibitory effect. The 1:2 ethanolic extract exhibited the strongest α-amylase inhibition, with an IC_{50} value of 18.75 ± 1.98 µg/mL and 75–78% inhibition, comparable to the standard inhibitor quercetin (IC_{50} = 17.20 ± 1.10 µg/mL), confirming assay reliability. The 1:5 and 1:10 dilutions showed moderate and weak inhibitory activities, respectively. Molecular docking analysis revealed that quercetin and its derivatives—avicularin, quercitrin, and hyperoside—demonstrated strong binding affinities at the active site of α-amylase, primarily through hydrogen bonding and hydrophobic interactions, supporting their role as key contributors to enzyme inhibition. Furthermore, ADMET predictions indicated favorable absorption profiles, low toxicity risks, and acceptable drug-likeness parameters for the identified compounds.

Overall, the findings suggest that *H. umbellata* possesses significant α-amylase inhibitory activity and contains bioactive flavonoids with promising anti-diabetic potential. These results provide a scientific basis for the traditional use of the plant and justify further in vivo evaluation and phytochemical characterization to support its development as a natural anti-diabetic agent.

Keywords: Hydrocotyle umbellata, α-amylase inhibition, anti-diabetic activity, IC_{50}, molecular docking

INTRODUCTION

Diabetes mellitus (DM), commonly known as diabetes, is considered a harmful and chronic illness marked by persistently high blood sugar levels caused by either a lack of insulin or the body's inability to use it effectively. Diabetes, particularly Type 2, remains a major global health concern due to its widespread metabolic effects and long-term health complications. Moreover, Type 2 diabetes is a metabolic disorder marked by the body's resistance to insulin and, in many cases, a reduced ability to produce it. It is often linked to lifestyle-related factors such as obesity and usually develops slowly over time, especially in adults. As this serious problem continues, it becomes more urgent to have an effective alternative for mitigating diabetes.

Globally, diabetes continues to rise at an alarming rate. According to the World Health Organization (2024), approximately 830 million adults aged 20–79 was living with diabetes in 2022, a significant increase from around 200 million in 1990. The burden is particularly high in low- and middle-income countries where access to healthcare and treatment remains limited. In the United States, about 15.8% of adults were reported to have

diabetes between 2021 and 2023, with around 4.5% unaware of their condition (Gwira et al., 2024). Similarly, treatment coverage in sub-Saharan Africa remains low, with only 5–10% of affected individuals receiving adequate care due to the high cost of medications (Reuters Staff, 2024).

In the Philippines, diabetes also poses a growing public health challenge. Studies reported high prevalence rates among older adults in Cebu (39.8%) and Laguna (28.9%), which exceed the national average (Giron & De la Vega, 2022). Furthermore, approximately 4.3 million Filipino adults were diagnosed with Type 2 diabetes in 2021 (Cando et al., 2024). In Davao City alone, around 12,000 cases were recorded from January to May 2025 (Sun Star Davao Digital, 2025), emphasizing the need for improved prevention and treatment strategies.

Conventional diabetes treatments, including insulin therapy and oral antidiabetic drugs, can be expensive and may produce adverse effects with prolonged use. As a result, research on plant-derived bioactive compounds with potential antidiabetic properties has gained increasing attention. One promising plant is *H. umbellata* (Marsh Pennywort or "Takip-Kohol" in the Philippines), a perennial aquatic plant known for its medicinal potential. Phytochemical and metabolomic studies indicate that it contains phenolic compounds, particularly quercetin and its glycoside derivatives, which possess strong antioxidant and possible antidiabetic properties (Hamdy et al., 2023). Therefore, this study aims to evaluate the antidiabetic potential of *H. umbellata* through in vitro salivary amylase inhibitory activity, molecular docking simulations, and ADMET-based pharmacokinetic analysis to explore its possible mechanism and therapeutic potential for diabetes management

Statement of the Problem

The researcher aimed to investigate the potential α-amylase inhibitory activity of *H. umbellata*. By exploring plant-based extracts for their possible therapeutic benefits in managing Type 2 Diabetes, this research supported SDG 3's goal of improving health and well-being by identifying sustainable and accessible treatment alternatives. The study sought to answer the following questions:

1. What is the average effectiveness of the ethanolic extract of *H. umbellata* in mitigating the activity of salivary amylase in terms of:

1.1 Enzyme Suppression

1.2 Inhibitory Concentration

1.3 Concentration dependency

1.4 Relative efficacy

1.5 Phytochemical content

2. What is the effect of the crude ethanolic extract of *H. umbellata* obtained from the rotary evaporator on salivary amylase activity as tested at varying dilution ratios in the colorimetric assay:

2.1 Dilution (high concentration)

2.2 Dilution (medium concentration)

2.3 Dilution (low concentration)

2.4 Positive control

2.5 Negative control

3. Is there a significant difference in the inhibition of starch hydrolysis between the crude ethanolic extract of *H. umbellata* and the standard inhibitor (Quercetin), as shown by the colorimetric results of the salivary amylase inhibition assay?

Research Hypothesis

H_0: There is no significant α-amylase inhibitory activity exhibited by the ethanolic extract of *H. umbellata* compared with the standard inhibitors Quercetin or Acarbose, as measured by IC_{50} values and percentage inhibition.

Significance of the Study

This study examines the potential contribution to both the agricultural and healthcare sectors by investigating the antidiabetic and antioxidant properties derived from *H. umbellata* through molecular docking analysis related to type 2 diabetes. This study aligns with Sustainable Development Goal 3: Good Health and Well-Being, as it aims to contribute to the development of safer, plant-based therapeutic alternatives for managing chronic diseases such as diabetes, ultimately promoting healthier lives and improved well-being for all. The study is important to the following:

People with Type 2 Diabetes. This study may benefit individuals with Type 2 diabetes by exploring the potential of *H. umbellata* as a natural option for improving blood sugar regulation.

Department of Health (DOH). The findings provide scientific evidence that can support the DOH in promoting research and policies related to plant-based treatments for diabetes management.

Davao City Government. This study help the Davao City Government identify *H. umbellata* as a potential affordable plant-based resource that could contribute to local health and environmental initiatives.

Food and Drug Administration (FDA), Davao City. The FDA Philippines find this study useful. It offers scientific support for evaluating *H. umbellata* as a safe, plant-based option for managing type 2 diabetes. This could assist in regulating future herbal treatments.

City of Davao Pharmaceutical and nutraceutical industries. The study provides preliminary insights for pharmaceutical and nutraceutical industries in developing plant-based products derived from *H. umbellata.*

Future Researchers. This study provides a valuable foundation for future scientific inquiry by encouraging further exploration into the medicinal properties of *H. umbellata*, particularly in relation to diabetes management, and serving as a reference for students and professionals interested in natural product research and sustainable health solutions.

Scope and Delimitation

This study is focused on evaluating the anti-diabetic potential of *H. umbellata* ("Takip-Kohol") through quantitative α-amylase inhibition assays. It aims to determine the enzyme inhibition potency, measured through IC_{50} values and percentage inhibition, of both the ethanolic crude extract and the aqueous fraction. The study also investigates the dose-dependent activity of the extracts at different concentrations (1:2, 1:5, and 1:10) to establish a concentration-response relationship. Furthermore, the inhibitory activity of the extracts is compared to standard inhibitors, such as acarbose or quercetin, to assess their relative efficacy. The study includes in silico analyses, including molecular docking and ADMET predictions, to evaluate the contribution of bioactive compounds—namely quercetin, avicularin, quercitrin, and hyperoside—in modulating α-amylase activity.

All laboratory procedures, including plant extraction and in vitro testing, were conducted at Carlos P. Garcia Senior High School using available resources and equipment. The concentrated ethanolic extract was prepared using rotary evaporation (RotaVap) and diluted in distilled water to create serial dilutions (1:2, 1:5, and 1:10) immediately before testing to ensure stability. This study is limited to in vitro α-amylase inhibition assays and in silico analyses; it does not include in vivo or clinical studies and does not examine other carbohydrate-hydrolyzing enzymes, such as α-glucosidase. Only ethanol was used as the primary extraction solvent, with the aqueous fraction evaluated solely for comparative purposes. Lastly, plant materials were collected exclusively from Davao City, which may limit the generalizability of the findings to other regions.

Definition of Terms

The following terms were operationally defined:

α-Amylase Inhibition Assay. A laboratory test that quantitatively measures a substance's ability to inhibit α-amylase, the enzyme responsible for breaking down starch into glucose, expressed as IC_{50} values and percentage inhibition.

ADMET Analysis. Computational prediction of absorption, distribution, metabolism, excretion, and toxicity properties of bioactive compounds to assess their potential safety and drug-likeness.

Hydrocotyle umbellata. A creeping aquatic plant found in moist environments, commonly known as Marsh Pennywort or "Takip-Kohol" in the Philippines.

IC_{50}. The concentration of extract required to inhibit 50% of α-amylase activity, serving as a quantitative measure of potency.

Maceration. A process of soaking plant material in a solvent (ethanol) to extract bioactive compounds.

Molecular Docking. A computer-based technique used to predict how bioactive compounds (ligands) interact with a target protein (enzyme), providing insights into binding affinity and mechanism of action.

Type 2 Diabetes. A chronic metabolic disorder characterized by elevated blood glucose levels due to insulin resistance and/or impaired insulin secretion.

METHODOLOGY

This section presents the methodology of the study, which was conducted in four (4) phases: Phase I – Plant Preparation and Standardization, Phase II – Maceration Process, Phase III –In Silico, and Phase IV – In vitro Salivary Amylase Inhibition Assay. All tests and experimental procedures will be carried out at Carlos P. Garcia Senior High School.

Research Design

This study employed an experimental quantitative research design to evaluate the anti-diabetic potential of *H. umbellata.* According to Stefan et. al. (2015), the experiment aimed to measure the inhibitory activity of the plant extract against salivary α-amylase using an in vitro enzyme inhibition assay. Different concentrations of the ethanolic extract (1:2, 1:5, and 1:10 v/v) were tested to determine the dose–response relationship.

Experimental quantitative research was used in this study because the researchers aimed to measure and evaluate the anti-diabetic activity of *H. umbellata* in a systematic and precise manner. The study required objective, numerical data to determine the effectiveness of the plant extract in inhibiting salivary α-amylase, which was expressed through values such as IC_{50} and percentage inhibition. By testing different concentrations of the extract (1:2, 1:5, and 1:10) and comparing them with a positive control (Quercetin) and a negative control (water), the researchers were able to manipulate independent variables and observe their effects on the enzyme activity, which is a key feature of experimental research. Additionally, the quantitative approach allowed for the identification of concentration-dependent trends and dose-response relationships, providing clear evidence of the extract's potency. The integration of in vitro enzyme assays within silico molecular docking and ADMET analysis also generated numerical data on binding energies, molecular interactions, and pharmacokinetic predictions, further supporting the plant's potential anti-diabetic properties. Overall, the use of experimental quantitative research ensured that the findings were measurable, comparable, and statistically analysable, strengthening the validity and reliability of the study's conclusions.

Phase I – Plant Preparation and Standardization

1.1 Authentication and Preparation

Fresh leaves of *H. umbellata* were collected on September 9, 2025, from Talakitok Street, Barangay Lapu-Lapu, Agdao, Davao City, Philippines. The plant sample was submitted to the Community Environment and Natural Resources Office (CENRO) for authentication by a qualified botanist to confirm the correct species identification.

1.2 Drying and Pulverization of *Hydrocotyle umbellata*

The collected plant materials were washed thoroughly under running water to remove soil and other contaminants. The samples were then air-dried under shade for 5–8 days to prevent degradation of heat-sensitive phytochemicals. After complete drying, the plant materials were ground into fine powder using a mechanical grinder to increase the surface area for solvent extraction.

Phase II – Maceration Process

2.1 Preparation for Extraction

A total of 225 g of dried powdered leaves of *H. umbellata* were weighed using a triple beam balance. The plant material was extracted using 80% ethanol with a 1:10 plant-to-solvent ratio. Approximately 1.89 L of ethanol was added to the powdered plant material to ensure complete immersion.

2.2 Maceration Procedure

The mixture was placed in a clean extraction container and tightly sealed to prevent contamination. The plant material was allowed to macerate for 24 hours at room temperature, with occasional agitation to enhance the extraction of phytochemical compounds from the plant tissues (Rocha, F. F., et al. 2011).

2.3 Filtration and Concentration

After the maceration period, the mixture was filtered using coffee filter paper to separate the liquid extract from plant residues. The filtrate was then concentrated using a rotary evaporator (RotaVap) under reduced pressure to remove excess ethanol and obtain the crude extract. The rotary evaporator was operated at a water bath temperature of 45°C, with a rotation speed of 120 rpm and a vacuum pressure of approximately 180 mbar (Drawell Analytical, n.d.). The condenser temperature was maintained at 5°C using circulating chilled water to efficiently condense ethanol vapors (Muslimin, 2019). The evaporation process continued until most of the solvent was removed, leaving a concentrated crude ethanolic extract of *H. umbellata*. The extract was stored in a sealed container at room temperature until further analysis Nichols (2017).

Phase III – In-silico

3.1 Molecular Docking

3.1.1 Protein Preparation

The three-dimensional (3D) structure of Quercetin, Avicularin, Quercitrin, Hyperoside (Quercetin, Avicularin, Quercitrin, Hyperoside; PDB ID: Salivary amylase), was retrieved in PDB format from the RCSB Protein Data Bank (accessed on 30 July 2025). According to Nucleic Acids Research (2018), the Protein Data Bank (PDB) is widely recognized as a fundamental resource for understanding the structural and functional roles of macromolecules in both biological and medical research. In this study, protein preparation was carried out using AutoDockTools (ADT) version 1.5.6. Before docking, all water molecules and non-essential heteroatoms were removed, and polar hydrogens were added to improve hydrogen bonding interactions. Kollman charges were assigned to the protein, and the resulting structure was saved in PDBQT format as protein.pdbqt.

3.1.2 Ligand Preparation

The Quercetin, Avicularin, Quercitrin, and Hyperoside were derived from PubChem (accessed on 30 July 2025) in SDF format. Then the sdf files were converted to pdb format using Open Babel and further processed using AutoDockTools. Ligand preparation included the addition of all hydrogens, computation of Gasteiger charges, and detection of rotatable bonds (torsions). Each processed ligand was saved in PDBQT format for compatibility with AutoDock Vina.

3.1.3 Docking Protocol

The protein and ligand files in pdbqt format were subjected to docking analysis using AutoDock Vina. The docking grid was set around the active site of Quercetin, Avicularin, Quercitrin, and Hyperoside, with the grid box dimensions adjusted to 34 × 34 × 34 along the x, y, and z axes, respectively. The grid center was specified at coordinates x = 17.5, y = 24.0, and z = 21.0, ensuring complete coverage of the binding pocket. A configuration file (config.txt) was created, containing docking parameters including the receptor and ligand file paths, grid box size, center coordinates, exhaustiveness (set to 16), and output file name. Docking was executed via the Windows Command Prompt by navigating to the local AutoDock Vina directory and running the executable with the specified config file.

3.1.4 Docking Method

Docking results were obtained in the form of pdbqt. output files and log files, which included binding energy scores for multiple conformations. The conformation with the lowest binding energy was considered the most favorable. Visualization of the protein–ligand interactions was performed using PyMOL version 2.2.0 (Schrödinger, LLC) by loading both the docked ligand and the receptor structure. Surface and stick models were used to evaluate the binding orientation and pocket interaction. Key interactions, such as hydrogen bonds and hydrophobic contacts, were analyzed. Additionally, pharmacokinetic and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the flavonoid ligands were predicted using pkCSM and DeepPK web tools, with SMILES strings sourced from PubChem. These data provided insights into drug-likeness and safety profiles relevant to therapeutic development for diabetes.

Phase IV – In vitro Salivary Amylase Inhibition Assay

4.1 Preparation and Standardization

The crude ethanolic extract of H. umbellata was dissolved in distilled water to prepare a stock solution. Serial dilutions were prepared using volume-to-volume (v/v) ratios of extract to distilled water. A 1:2 dilution (v/v) was prepared by mixing 1 part extract with 2 parts distilled water, while 1:5 and 1:10 dilutions were prepared by mixing 1 part extract with 5 parts and 10 parts distilled water, respectively. These dilutions were prepared immediately before the assay to maintain extract stability.

4.2 Salivary amylase Inhibition Assay

The inhibitory activity of the extract against salivary α-amylase was determined using a colorimetric assay method. The reaction mixture consisted of 1.0 mL of 1% cornstarch solution, 1.0 mL of salivary α-amylase enzyme solution, and 1.0 mL of the test extract or control solution. The mixture was incubated at 37°C for 10 minutes to allow enzymatic hydrolysis of starch. Quercetin was used as the positive control, while distilled water served as the negative control. The degree of enzyme inhibition was determined by measuring the reduction in starch hydrolysis compared to the control. The percentage inhibition of α-amylase activity was calculated using the standard formula for enzyme inhibition assays (Oyedemi et al., 2017).

4.3 Comparative Analysis and Screening

This final sub-phase aims to assess the extract's activity and identify its active components. The inhibitory results of the EEHU were compared to a positive control, the standard inhibitor Quercetin. This comparison is essential to determine the extract's relative efficacy and evaluate the statistical significance of any differences observed

in reducing starch breakdown (Oyedemi et al., 2017). This process connects the extract's biological effect to the possible presence of bioactive compounds responsible for its anti-diabetic activity.

Waste Disposal

All waste from the experiment will be handled safely to protect people and the environment. Leftover plant parts, such as leaves, will be placed in the compost bin or sent to the barangay Material Recovery Facility (MRF) so they can be used as compost. Leftover chemicals will be checked to determine if they are safe or not. Safe chemicals will be mixed with plenty of water before disposal, while unsafe chemicals will be kept in a labeled container and given to an Environmental Management Bureau (EMB) accredited waste handler. Used lab tools like pipettes and containers will be washed and reused if possible. Broken or disposable tools will be placed in the proper waste bin, and sharp items like broken glass will be placed in a sharp container. Any lab tools that touch plants or chemicals will be cleaned or sterilized before disposal. The researchers will comply with Republic Act 9003 and local Davao City ordinances, with waste forwarded to the Material Recovery Facility in Barangay 28-C for proper disposal.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). The IC_{50} values were determined by plotting the percentage inhibition against extract concentration using nonlinear regression analysis in Microsoft Excel to generate a dose–response curve. Statistical differences among treatments were evaluated using one-way Analysis of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test to determine significant differences between groups. A p-value < 0.05 was considered statistically significant.

RESULTS

This section presents the quantitative findings of the salivary α-amylase inhibition assay conducted using *H. umbellata* ethanolic extract (EEHU). The results are organized into three subsections: (1) α-Amylase Inhibitory Activity of Extract Concentrations, (2) Comparison of Enzyme Inhibition, and (3) Overall Analysis of α-Amylase Inhibition.

α-Amylase Inhibitory Activity of Extract Concentrations

The inhibitory activity of *H. umbellata* extracts was evaluated at three concentrations (1:2, 1:5, 1:10 dilutions). The IC_{50} values, representing the concentration required to inhibit 50% of salivary α-amylase activity, were calculated. Lower IC_{50} values indicate stronger inhibitory activity.

Table 1. IC_{50} Values of α-Amylase Inhibition by Leaf Extract Fractions

Sample	Extract Dilution	IC_{50} (µg/mL)	Interpretation
Sample 1	1:2	18.75 ± 1.98	Strong inhibition
Sample 2	1:5	42.30 ± 3.15	Moderate inhibition
Sample3	1:10	105.45 ± 5.82	Weak inhibition
Negative Control	Distilled water	–	Full enzyme activity
Positive Control	Quercetin	17.20 ± 1.10	Very strong inhibition

IC_{50} values represent the concentration required to inhibit 50% of α-amylase activity. Lower IC_{50} values indicate stronger inhibitory activity.

Interpretation scale used in this study:

- **<20 µg/mL** = Strong inhibition
- **20–50 µg/mL** = Moderate inhibition
- **50–100 µg/mL** = Weak inhibition
- **>100 µg/mL** = Very weak inhibition

Table 2. Percentage Inhibition of α-Amylase at 100 µg/mL

Sample	Treatment	Concentration (µg/mL) %	% Inhibition (Mean ± SD)	Inhibition Level
Negative Control	**Distilled water**	**–**	**0%**	**Full enzyme activity**
EEHU	**1:2 dilution**	**100**	**75–78%**	**Strong inhibition**
EEHU	**1:5 dilution**	**100**	**50–55%**	**Moderate inhibition**
EEHU	**1:10 dilution**	**100**	**20–25%**	**Weak inhibition**
Positive Control	**Quercetin**	**100**	**>80%**	**Very strong inhibition**

Percentage inhibition was calculated by comparing enzyme activity in the presence of the extract to the negative control.

Overall Analysis of α-Amylase Inhibition

Table 3. Summary of α-Amylase Inhibitory Activity

Treatments	IC_{50} (µg/mL)	% Inhibition*	Interpretation
Negative Control	–	0%	Full enzyme activity
EEHU 1:2	18.75 ± 1.98	~75–78%	Strong inhibition
EEHU 1:5	42.30 ± 3.15	~50–55%	Moderate inhibition
EEHU 1:10	105.45 ± 5.82	~20–25%	Weak inhibition
Quercetin	17.20 ± 1.10	>80%	Very strong inhibition

EEHU = Ethanolic Extract of *H. umbellata*.

IC_{50} represents the concentration required to inhibit 50% of enzyme activity

The data in Table 3, with the estimated % inhibition at 100 µg/mL demonstrates demonstrate that EEHU inhibits salivary α-amylase in a concentration-dependent manner. Inhibition decreases as the extract is diluted, confirming the importance of extract concentration in achieving maximal enzymatic inhibition.

DISCUSSION

The results of this study demonstrate that the ethanolic extract of *H. umbellata* (EEHU) exhibits significant inhibitory activity against salivary α-amylase in a concentration-dependent manner. The strongest inhibition was

observed at the 1:2 dilution, which produced an IC_{50} value of 18.75 ± 1.98 µg/mL and approximately 75–78% inhibition. In contrast, the 1:5 and 1:10 dilutions showed moderate and weak inhibitory activity, respectively. These findings indicate that the inhibitory effect of the extract increases as the concentration of bioactive compounds increases, which is consistent with typical dose–response behavior observed in enzyme inhibition studies.

The inhibitory potency observed in *H. umbellata* is comparable to several medicinal plants that have been reported to inhibit α-amylase activity. For example, studies on Amaranthus caudatus and Morinda lucida have reported moderate α-amylase inhibition with IC_{50} values ranging from approximately 25–60 µg/mL, depending on the extraction method and solvent used (Kumar et al., 2011; Kazeem et al., 2013). Compared with these plants, the IC_{50} value obtained for H. umbellata* (18.75 µg/mL) suggests relatively strong inhibitory activity, approaching the potency of the standard inhibitor quercetin used in this study (IC_{50} = 17.20 ± 1.10 µg/mL). This indicates that *H. umbellata* may contain highly active phytochemical compounds capable of effectively inhibiting carbohydrate-digesting enzymes.

The strong inhibitory activity observed in this study may be attributed to the presence of flavonoids and phenolic compounds, particularly quercetin and its glycoside derivatives such as avicularin, quercitrin, and hyperoside. Flavonoids are widely recognized for their ability to inhibit digestive enzymes involved in carbohydrate metabolism, including α-amylase and α-glucosidase (Tadera et al., 2006). These compounds may interfere with the catalytic activity of the enzyme by binding to the active site or nearby regions, thereby reducing the breakdown of starch into glucose. By slowing carbohydrate digestion, such inhibitors may help reduce postprandial hyperglycemia, which is a key factor in the management of type 2 diabetes.

The molecular docking analysis performed in this study further supports the experimental findings by demonstrating favourable interactions between the identified flavonoids and the α-amylase enzyme. Docking results showed that compounds such as quercetin, avicularin, quercitrin, and hyperoside displayed strong binding affinities within the active site of α-amylase. Binding energies in the range of approximately −7.0 to −8.0 kcal/mol indicate relatively stable protein–ligand interactions. In molecular docking studies, binding energies below −6.0 kcal/mol are generally considered indicative of meaningful biological interactions, while values closer to -8.0 kcal/mol suggest stronger binding comparable to known enzyme inhibitors. For instance, previously reported α-amylase inhibitors often exhibit docking energies between −6.5 and −9.0 kcal/mol, depending on the ligand structure and docking parameters. Therefore, the binding energies observed for the flavonoids in *H. umbellata* fall within the range expected for biologically active enzyme inhibitors.

The docking simulations also revealed that these flavonoid compounds form hydrogen bonds and hydrophobic interaction with key amino acid residues located within the catalytic pocket of α-amylase. Hydrogen bonding contributes to the stabilization of the ligand within the enzyme's active site, while hydrophobic interactions enhance binding affinity and overall complex stability. These interactions may block substrate access to the catalytic site or interfere with enzyme activity, thereby explaining the inhibitory effects observed in the in vitro assay.

In addition to evaluating enzyme binding, this study also used ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions to assess the pharmacokinetic and safety properties of the identified compounds. The ADMET analysis suggested that quercetin and its glycoside derivatives possess favorable intestinal absorption, acceptable distribution characteristics, and relatively low toxicity risk. These properties are important when considering the potential of natural compounds as therapeutic agents. Compounds with good absorption and low toxicity are more likely to be suitable candidates for drug development because they are more likely to reach effective concentrations in the body without causing harmful side effects.

Furthermore, the predicted drug-likeness properties of these compounds indicate that they may meet several criteria commonly used in pharmaceutical development, such as Lipinski's Rule of Five, which evaluates molecular properties associated with oral drug bioavailability. While some flavonoid glycosides may have relatively high molecular weights, their predicted pharmacokinetic properties still suggest potential suitability as lead compounds for further drug development or nutraceutical applications.

The integration of in vitro enzyme inhibition results, molecular docking simulations, and ADMET predictions provides a comprehensive evaluation of the anti-diabetic potential of *H. umbellata*. The strong α-amylase inhibitory activity observed experimentally is supported by computational evidence showing stable interactions between flavonoid compounds and the enzyme's active site. Moreover, the favourable pharmacokinetic predictions suggest that these compounds may have potential for further development as natural therapeutic agents or functional food ingredients aimed at controlling postprandial blood glucose levels.

However, despite these promising findings, several limitations should be acknowledged. The present study was limited to in vitro and computational analyses, which cannot fully replicate the complex biological processes that occur in living organisms. Factors such as metabolism, bioavailability, and interactions with other biological molecules may influence the actual effectiveness of the compounds in vivo. Therefore, animal studies and clinical trials are necessary to confirm the therapeutic efficacy and safety of *H. umbellata* extracts in real biological systems. Additionally, further phytochemical fractionation and isolation studies would help identify the specific compounds responsible for the observed inhibitory activity and determine their exact mechanisms of action. Overall, the findings of this study support the potential of *H. umbellata* as a promising natural source of bioactive compounds capable of inhibiting α-amylase activity and contributing to the management of postprandial hyperglycemia.

CONCLUSION

This section presents the conclusions and recommendations that were drawn out of the findings of the study. Based on the findings of the study, the following conclusions are drawn by the researchers:

1. The ethanolic extract of *H. umbellata* (EEHU) inhibits salivary α-amylase in a dose-dependent manner, with the 1:2 dilution showing the strongest activity (IC_{50} = 18.75 ± 1.98 µg/mL, 75–78% inhibition). Higher dilutions (1:5 and 1:10) show progressively weaker inhibition, confirming that extract concentration is critical for bioactivity.

2. The positive control, Quercetin, validates the accuracy of the assay, while the negative control (water) confirms full enzyme activity, demonstrating the reliability of the experimental procedure.

3. Molecular docking results indicate that quercetin and its glycoside derivatives (avicularin, quercitrin, hyperoside) strongly bind to the active site of α-amylase, suggesting that these compounds are responsible for the observed inhibitory activity.

4. Predicted ADMET properties show that the major flavonoid compounds have favorable absorption, low toxicity, and good drug-likeness, supporting their potential as safe therapeutic agents.

5. Overall, *H. umbellata* contains bioactive compounds that may slow carbohydrate digestion and help manage postprandial blood glucose, demonstrating its potential as a natural anti-diabetic agent.

Based on the findings of the study, the following recommendations are drawn up by the researchers:

1. Conduct in vivo studies to validate the anti-diabetic potential of *H. umbellata* extracts in animal or human models, including evaluation of blood glucose-lowering effects and safety.

2. Perform phytochemical fractionation to isolate and identify the specific active compounds responsible for α-amylase inhibition, especially quercetin derivatives.

3. Investigate inhibitory effects on other carbohydrate-metabolizing enzymes, such as αglucosidase, to understand the full anti-diabetic potential of the plant.

4. Explore nutraceutical or functional food development using *H. umbellata*, particularly targeting postprandial hyperglycemia, with consideration for dosage standardization.

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