

Unveiling the Antidiabetic Potential of *Achyranthes aspera* In Vitro and In Silico Efficacy Insights

Jing Zhao

Tarim University

Zhang Yu Ming

INTI International University

Ibrahim Mahmood

Al-Rafidain University College

Shazrul Fazry

Universiti Kebangsaan Malaysia

Muhammad Ismail

Karakoram International University

Wamidh Talib

Applied Science Private University

Sajjad Ali

Karakoram International University

Ahmed Abdulkareem Najm

Universiti Kebangsaan Malaysia

Douglas Law

`douglas.law@newinti.edu.my`

INTI International University

Article

Keywords: *Achyranthes aspera*, Antidiabetic activity, In vitro analysis, Diabetes mellitus management, human health

Posted Date: April 29th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-5777722/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Unveiling the Antidiabetic Potential of Achyranthes aspera In Vitro and In Silico Efficacy Insights

Jing Zhao¹, Zhang Yu Ming², Ibrahim Mahmood³, Shazrul Fazry^{4,5}, Muhammad Ismail⁶, Sajjad Ali⁶, Wamidh Talib⁷, Ahmed Abdulkareem Najm^{5,8*}, Douglas Law^{2,9*}

¹*College of Food Science and Engineering, Tarim University, Xinjiang Production Construction Group Key Laboratory of Agricultural Products Deep Processing in South Xinjiang, Alar 843300, China*

²*Faculty of Health and Life Sciences, Inti International University, 71800 Nilai, Negeri Sembilan*

³*Dentistry Department, Al-Rafidain University College, Baghdad, Iraq*

⁴*Department of Food Science, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.*

⁵*Tasik Chini Research Center, The Centre for Natural and Physical Laboratory Management, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia*

⁶*Department of Chemistry, Karakoram International University, University Road, Gilgit-15100, Pakistan*

⁷*Faculty of Allied Medical Sciences, Applied Science Private University, Amman 11931, Jordan.*

⁸*Department of Biomedical Sciences, Cihan University Erbil, Iraq*

⁹*Faculty of Science, Assam Downtown University, Guwahati, India*

* Corresponding authors:

E-mail: douglas.law@newinti.edu.my (Assoc. Prof. Dr. Douglas Law); Tel.: +60 192725203

ahmadaljemeely@gmail.com (Dr. Ahmed Najm); Tel.: +60 1123597841

Abstract

Diabetes mellitus (DM) is a prevalent metabolic disorder with increasing global incidence. Current pharmaceutical treatments, such as insulin and oral hypoglycemic agents, are often associated with side effects and drug resistance, this underscores the need for safer alternatives. *Achyranthes aspera*, a plant with a long history of use in traditional medicine, has demonstrated potential in managing diabetes. This study employs both *in vitro* and *in silico* approaches to assess the antidiabetic efficacy of *A. aspera*. In vitro assays were conducted to evaluate the effects of the plant on key enzymes involved in glycemic control and carbohydrate metabolism, namely DPP-4, α -amylase, and α -glucosidase. Significant inhibition of these enzymes was observed, indicating the potential of *A. aspera* to regulate blood glucose levels effectively. Additionally, cytotoxicity assays using L6 cell lines demonstrated that *A. aspera* extract enhances glucose uptake and remains nontoxic at therapeutic concentrations, suggesting an improvement in insulin sensitivity. Molecular docking studies further supported these findings, which revealed strong interactions between bioactive compounds in *A. aspera* and target proteins involved in glucose metabolism. In summary, these results underscore the therapeutic potential of *A. aspera* as a natural antidiabetic agent. However, further *in vivo* studies are recommended to validate its clinical efficacy and explore its potential for use in diabetes treatment.

Keywords: *Achyranthes aspera*, Antidiabetic activity, *In vitro* analysis, Diabetes mellitus management, human health

1. Introduction

Diabetes mellitus (DM) is a prevalent metabolic disorder characterized by insulin resistance or insufficient insulin production, leading to elevated blood glucose levels [1]. Common clinical manifestations include unintended weight loss, blurred vision, excessive thirst, and frequent urination [2]. The global incidence of diabetes has risen dramatically, imposing significant economic burdens on healthcare systems and communities. Current management strategies rely on insulin therapy and oral hypoglycemic agents; however, adverse effects and the emergence of drug resistance necessitate the exploration of safer, more effective alternatives [3].

Herbal extracts play a pivotal role in drug discovery due to their diverse bioactive compounds, which exhibit therapeutic potential [4,5]. Compared to synthetic pharmaceuticals, plant-derived formulations are generally regarded as safer, more accessible, and cost-effective, often demonstrating broader therapeutic effects [6]. Traditional medical systems worldwide have employed herbal remedies for centuries to manage diabetes, underscoring their historical and cultural significance [7]. A primary mechanism by which Herbal extracts modulate glycemic control through diverse pathways, including enzyme inhibition (e.g., α -amylase, α -glucosidase), enhancement of insulin sensitivity (e.g., GLUT4 translocation), antioxidant activity to mitigate oxidative stress, and regulation of lipid metabolism [8]. While enzyme inhibition delays carbohydrate digestion, other pathways—such as AMPK activation and PPAR γ modulation—may contribute synergistically to glucose homeostasis.

Recent research has focused on elucidating the antidiabetic properties of plant extracts, particularly their capacity to regulate blood glucose through multiple pathways. The

therapeutic potential of herbal extracts as adjuncts or alternatives to conventional treatments presents a viable avenue for sustainable diabetes management. While preliminary studies suggest *A. aspera*'s antidiabetic potential, its bioactive compounds remain poorly characterized, and their interactions with key metabolic targets (e.g., DPP-4, α -amylase) are yet to be systematically validated. This study integrates in vitro enzyme kinetics with in silico molecular docking to identify and prioritize lead compounds, offering a mechanistic basis for its traditional use [10,9,11].

Achyranthes aspera (*A. aspera*), a plant widely distributed across tropical regions, has been utilized in traditional medicine systems, including Malaysian Indigenous practices and Ayurveda, to treat conditions such as inflammation, fever, respiratory ailments, and diabetes [12,13]. In traditional Chinese medicine, *A. aspera* has been employed to enhance blood circulation and alleviate joint pain, with its roots, seeds, and leaves valued for their medicinal properties [14]. Phytochemical analyses have identified flavonoids, alkaloids, and saponins as key bioactive constituents in *A. aspera*, which may contribute to improved insulin sensitivity and enzyme inhibition [15,16]. These findings position *A. aspera* as a compelling candidate for blood glucose regulation and diabetes management.

Despite anecdotal evidence of antidiabetic properties, no study has comprehensively mapped its bioactive compounds to specific molecular targets in glucose metabolism. Here, we employ a tandem experimental-computational strategy to (1) quantify inhibitory effects on DPP-4, α -amylase, and α -glucosidase, and (2) predict binding modes of prioritized phytochemicals via molecular dynamics simulations. These findings address a critical gap in translating traditional use into evidence-based therapeutics.

2. Materials & methods

2.1 Chemicals and reagents

A variety of chemicals and reagents were acquired for this investigation. Quercetin, sodium hydroxide (NaOH), sodium chloride (NaCl), iodine, Trolox, α -glycosidase, α -amylase, PNPG, gallic acid, sodium nitrite (NaNO₂), and DPPH (2,2-diphenyl-1-picrylhydrazyl) were all supplied by Sigma-Aldrich (USA). We purchased the aluminium chloride solution (AlCl₃), starch, and Folin-Ciocalteu reagent from Merck (USA). DMEM (Dulbecco's Modified Eagle Medium), fetal bovine serum (FBS), ethanol, methanol, and hydrochloric acid (HCl) were provided by Fisher Scientific (USA). Every chemical and reagent used in this investigation was of analytical quality.

2.2 Preparation of extract

Mature leaves of *A. aspera*, collected from Taman Botani Bangi (The latitude for Taman Botani Bangi, Selangor, Malaysia is 3.226904, and the longitude is 101.628994. Dr. Shamsul bin Khamis undertook the formal identification of the plant material in Herbarium *Universiti Kebangsaan Malaysia* (UKMB), Faculty of Science and Technology), *Universiti Kebangsaan Malaysia* (Voucher number: ID091/2023) were dried in an oven at 40°C to preserve their bioactive compounds. Once fully dried, the leaves were processed using an electric grinder to obtain a fine powder. This powdered material was then mixed with distilled water in a 1:10 ratio (weight/volume) and thoroughly homogenized. The mixture underwent reflux extraction using an ultrasonic device at 60°C to enhance the extraction of bioactive components. Following extraction, the mixture was filtered to remove solid residues, and the filtrate was concentrated under reduced pressure to obtain the crude aqueous extract of *A. aspera*, which was then stored at 4°C for further analysis. This process ensured the retention of key phytochemicals for subsequent *in vitro* and *in silico* investigations.

* The *A. aspera* extract given name (*HW*) throughout the study.

2.3 Quantification of flavonoids and phenolic compounds

The Total Phenolic Content (TPC) was measured following the method [15]. In a 96-well plate, we added 10 μ L of extract (1 mg/mL), 20 μ L of Folin-Ciocalteu (FC) reagent, and 70 μ L of deionized water. After a 5-minute incubation, 100 μ L of 20% Na_2CO_3 solution was added, and the reaction was allowed to proceed in darkness for an additional 30 minutes. Absorbance was then recorded at 730 nm using a microplate reader (Tecan, Switzerland), and TPC was calculated in terms of gallic acid equivalents (GAE) per gram of extract.

To measure the Total Flavonoid Content (TFC), we used the method described by Salahuddin et al. (Salahuddin et al., 2020). In a 96-well plate, 30 μ L of extract (1 mg/mL) was mixed with 120 μ L of distilled water and 9 μ L of 5% NaNO_2 solution. After a 5-minute reaction time, 9 μ L of 10% AlCl_3 solution was added, and the reaction continued for another 5 minutes. Subsequently, 72 μ L of distilled water and 60 μ L of 1 M NaOH were added. Absorbance was measured at 510 nm, and TFC was expressed as milligrams of quercetin equivalents (QE) per gram of extract.

2.4 Assessment of DPPH radical scavenging activity

A series of aqueous *HW* were prepared in concentrations ranging from 0 to 5 mg/mL. In each well of a 96-well plate, 50 μ L of the extract solution was mixed with 150 μ L of DPPH solution. The plate was then incubated in the dark for 30 minutes. After incubation, absorbance was measured at 517 nm using a microplate reader [17]. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{Scavenging activity} = (\text{Absorbance}(b) - \text{Absorbance}(s) / \text{Absorbance}(b)) \times 100\%$$

The term "*Absorbance*(*b*)" denotes the absorbance of the blank, whereas "*Absorbance*(*s*)" refers to the absorbance of the sample.

2.5 *In vitro* Enzyme Inhibition Assays

2.5.1 α -amylase Inhibition Assay

To assess α -amylase inhibition, the iodine-starch method [18] was employed. A mixture containing 50 μ L of crude extract, 60 μ L of 0.1 M PBS (pH 6.9), and 20 μ L of α -amylase (1 U/mL) was incubated at 37°C for 30 minutes. Subsequently, 50 μ L of a 0.25% starch solution was added, and the mixture was incubated for an additional 15 minutes. The reaction was halted by adding 20 μ L of 1 M HCl, followed by heating the solution at 75°C for 5 minutes. After cooling, 50 μ L of iodine reagent was added, and the absorbance was recorded.

2.5.2 α -glucosidase Inhibition Assay

The α -glucosidase inhibition was evaluated using a modified version of the method [19]. The sample was prepared in six different concentration gradients to assess its effect at varying concentrations. The reaction mixture consisted of 10 μ L of the diluted sample, 130 μ L of 50 mM phosphate-buffered saline (pH 6.5), and 10 μ L of α -glucosidase (1.5 U/mL). This mixture was incubated at 37°C for 30 minutes. Following incubation, 50 μ L of a 1 mM PNPG (p-nitrophenyl- α -D-glucopyranoside) solution was added as a substrate for the enzyme. The mixture was incubated further, allowing the α -glucosidase enzyme to act on the substrate and produce a yellow-coloured product. To stop the reaction, 50 μ L of 0.2 M Na₂CO₃ solution was added, which raised the pH and halted the enzymatic activity. Absorbance was then measured at 405 nm, where a higher absorbance indicated a greater amount of p-nitrophenol released, signifying less α -glucosidase activity.

Dimethyl sulfoxide (DMSO) was used as the negative control to account for any interference from the solvent, while acarbose, a known α -glucosidase inhibitor, was used as the positive control to compare the sample's inhibitory effect against a standard drug. This method allowed for the

evaluation of the sample's potential to inhibit α -glucosidase, an enzyme that plays a crucial role in carbohydrate digestion and could be useful for managing postprandial blood-glucose spikes in diabetes.

2.5.3 DPP-4 Inhibition Assay

The DPP-4 inhibitory activity of *Achyranthes aspera* was evaluated using the DPP-4 inhibitor screening kit (KA 1311; Abnova). The fluorogenic substrate Gly-Pro-AMC was employed to assess the enzyme activity, and absorbance was recorded using the Varioskan Flash spectral scanning multimode scanner.

2.6 Cytotoxic Activity on Cell lines

2.6.1 Cell Culture

The L6 rat skeletal muscle myoblast cell line (ATCC) was selected for this study due to its well-established utility in diabetes research, particularly for investigating insulin-mediated glucose uptake mechanisms. Cultured in high-glucose DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and maintained at 37°C with 5% CO₂, these cells were differentiated into myotubes over 7 days in 2% FBS to establish a mature, insulin-responsive system expressing functional GLUT4 transporters. This model was chosen due to skeletal muscle mediating ~80% of postprandial glucose disposal, L6 myotubes faithfully replicate key features of human skeletal muscle glucose metabolism, L6 provide superior reproducibility compared to primary cultures; and they are extensively validated for studying both insulin action and plant-derived antidiabetic compounds. The differentiation protocol was optimized to ensure the consistent formation of multinucleated myotubes with robust insulin signalling pathways, enabling reliable assessment of the extract's effects on glucose uptake using the fluorescent 2-NBDG analogue, while controlling for cytotoxicity through regular SRB assays and microscopic evaluation. Regular medium changes

were performed every 2-3 days to maintain cell health and support sustained growth. This culture system was employed to evaluate the cellular effects of the experimental treatments on myoblast activity. This approach combines physiological relevance with experimental practicality for mechanistic antidiabetic studies [20].

2.6.2 Cell cytotoxicity using SRB assay

The SRB (Sulforhodamine B) assay, as described by [21], was used to evaluate the cytotoxicity of *HW*. L6 cells were seeded at a density of 1×10^4 per well in a 96-well plate, differentiated into myotubes over seven days with 2% DMEM, and treated with different concentrations (0 to 5 mg/mL) of *HW* for 24, 48, and 72 hours. Cells were fixed with 50% TCA, stained with 0.4% SRB in 1% acetic acid, and absorbance was measured at 510 nm using a BioTek Instruments EPOCH plate reader.

2.6.3 Glucose absorption assay using 2-NBDG

The glucose uptake assay was conducted following the protocol provided by a commercial glucose uptake assay kit (KA4086, Abnova). L6 cells were seeded onto 96-well plates and allowed to differentiate for up to 7 days. After differentiation, the cells were incubated overnight in a serum-free medium to synchronize their activity. The following day, cells were rinsed with Krebs-Henseleit (KRPH) buffer to remove any residual medium, then treated with or without insulin to stimulate glucose uptake. The cells were subsequently exposed to 2-NBDG, a fluorescent glucose analogue, to measure glucose uptake. After incubation, the absorbance of the samples was measured at 570/610 nm using a BioTek Instruments EPOCH plate reader. This method provided a quantitative measure of glucose uptake, offering insights into insulin sensitivity and cellular glucose metabolism in response to the treatment.

2.6.4 Kinetic study of 2-NBDG uptake

L6 myoblast cells were plated into 96-well plates and allowed to differentiate into myotubes. The cells were then treated with the crude extract and incubated with 2-NBDG. The analysis was performed at different time points using a Thermo Fisher Scientific Varioskan Flash spectral scanning multimode reader. This approach was adapted and modified from the methodology outlined by [22].

2.7 LCMS Analysis

In this study, conducting *in-vitro* experiments before *in-silico* simulations allows for the validation of hypotheses, provides concrete results, and creates a more accurate environment to investigate biological interactions, which may not be fully captured by *in-silico* models. Additionally, *in-vitro* experiments offer valuable insights into biological mechanisms, such as the interactions of active compounds. To analyze the chemical composition of the extracts, the Agilent 1260 UPLC / 6540 Q-TOF system was used with an SB-C18 column (150 mm × 4.6 mm × 5 µl). The solvent gradient consisted of methanol (solvent A) and water (solvent B). The column was maintained at 30°C, and the sample was eluted into the electrospray ionization (ESI) mode at a mobile phase flow rate of 1 ml/min. The gradient elution for ionization was performed as follows: from 0 to 25 minutes, a gradient of 20%–100% solvent A; from 25 to 30 minutes, a gradient of 100%–0% solvent A.

2.8 Identification of Active Compounds Based on Previous Research

Begin by identifying and extracting peaks from the chromatograms, where each peak corresponds to a distinct compound. Next, analyze the mass spectra to determine the exact mass of each compound, which provides insights into the molecular weight and possible structure. To compare with previous studies, compile data on known active compounds, including their retention times, mass-to-charge ratios, and other relevant metrics. Match these data with the peaks and mass

spectra obtained from your LC-MS analysis, looking for congruences in retention times, mass-to-charge ratios, and fragmentation patterns. Confirm the identity of the active compounds by verifying if their characteristics align with those reported in the literature, potentially using additional techniques or databases. Finally, identify which compounds in your sample correspond to those reported as active in previous studies by comparing their biological activity with your experimental findings, and interpret the results to assess their potential biological activity and relevance to your research.

2.9 *In silico* Studies

2.9.1 Ligand and Protein Preprocessing

Active ingredients identified by LC-MS were obtained from PubChem and processed using Schrodinger's LigPrep module. The crystal structures of DPP-4 (PDB ID: 6B1E, Chain A) and α -amylase (PDB ID: 3DHP) were retrieved from the RCSB PDB and prepared using Schrodinger's Protein Preparation Wizard. Binding site conservation was verified through alignment with co-crystallized ligands in PDB structures, comparison with known catalytic residues reported in the literature (DPP-4: S630, H740, E205/E206; α -amylase: D197, E233, D300), and SiteMap analysis confirming druggability scores greater than 1.0.

2.9.2 Molecular XP Docking and MM-GBSA Calculation

Molecular docking was performed using Schrodinger Maestro 13.5. The binding site of the α -amylase protein was predicted using Schrodinger's SiteMap module, and the active sites were identified via the Receptor Grid Generation module [22]. Ligands were docked using Glide XP, and the top five compounds were selected based on multiple criteria: an XP Gscore of ≤ -6 , indicating strong binding affinity; favorable MM-GBSA binding free energies; full compliance

with Lipinski's Rule of Five (zero violations); and ADMET properties suitable for drug development.

2.9.3 Lipinski parameters and ADMET Analysis

The ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the compounds were assessed using the QikProp module within the Schrodinger software suite, as outlined by Hussain et al. (Hussain et al., 2023). This evaluation involved several parameters, including hydrophobicity (QPlogPo/w), permeability (QPPCaco), human oral absorption percentage, molecular weight (MW), solubility (QPlogS), and the total solvent-accessible surface area (SASA), among others. These properties were used to predict the compound's behavior in biological systems. Subsequently, the compounds were filtered based on Lipinski's Rule of Five, as recommended by [23], ensuring that the selected compounds meet the physicochemical criteria typically associated with drug-like properties. This comprehensive approach aids in identifying promising candidates for further pharmacological development.

2.9.4 Molecular Dynamics Simulations

Molecular dynamics simulations were conducted using the Desmond program in Schrodinger Maestro 13.5 to refine the binding mechanisms of protein-peptide complexes. The op1s2005 force field was used to parameterize the protein and small molecules, while the TIP3P model represented the water solvent. Sodium and chloride ions were added at 0.150 M to maintain electrical neutrality, and the protein-small molecule complex was placed in a cubic water box. The system's energy was minimized using the steepest descent method for over 50,000 steps. Subsequently, the system underwent NPT equilibration, with 50,000 additional steps to stabilize the pressure at 1 bar and temperature at 300 K. After equilibration, a 100 ns simulation was performed without

constraints. Interaction analyses and dynamic trajectory animations were generated using Schrodinger Maestro 13.5.

2.10 Statistical Analysis

All spectrophotometric assays were performed in triplicate to ensure accuracy and reproducibility. The results were expressed as the mean $\pm$ standard error of the mean (SEM), where applicable. To determine the 50% inhibitory concentration (IC_{50}), a regression curve was constructed by plotting the inhibitory percentage of each sample against its corresponding concentration. Data analysis was conducted using one-way analysis of variance (ANOVA) with a significance threshold of $p < 0.05$. To further explore pairwise differences, Tukey's Honestly Significant Difference (HSD) post hoc test was applied. All statistical computations were carried out using SPSS version 25. This approach ensures a comprehensive evaluation of the data with robust statistical validation.

2. Results

3.1 Quantification of Overall Phenolic Content (TPC) and Flavonoid Content (TFC)

The total phenolic content (TPC) of each sample was determined using the Folin-Ciocalteu method, while the total flavonoid content (TFC) was measured using the $AlCl_3$ Colorimetric method. Standard curves for quercetin and gallic acid were utilized for quantification. Among the samples, *HW* exhibited the highest TPC and TFC values compared to other ratios. According to the analysis, the TPC of HW was 2.4 ± 0.32 mg GAE/g, and the TFC was 1.179 ± 0.12 mg QE/g, as specified in Table A.1 in Supplementary material A.

3.2 Assessment of DPPH Radical Scavenging Activity

The DPPH radical scavenging activity assay is a widely used method for evaluating the antioxidant activity of food and plant extracts. As noted by [24], this assay is valued for its

practicality, simplicity, sensitivity, speed, and reproducibility. Figure A.1 shows *HW* capacity to scavenge DPPH radicals. With an IC_{50} of 0.4 ± 0.17 mg/mL, *HW* exhibited DPPH scavenging activity, though it was less effective than Trolox, which had an IC_{50} of 0.59 ± 0.13 mg/mL.

3.3 The Activity of α -glucosidase and α -amylase Inhibition

The study assessed the inhibitory effects of the *HW* extract on the enzymes α -glucosidase and α -amylase, using acarbose as a control for comparison. Both the extract and the control were tested under identical conditions to determine their half-maximal inhibitory concentration (IC_{50}) values, which reflect the effectiveness of a compound in inhibiting an enzyme by 50%. Table 1 presents the detailed results of the inhibition tests for both enzymes. According to the data, the *HW* extract demonstrated strong inhibitory activity against both α -glucosidase and α -amylase, with IC_{50} values of 10.41 ± 0.09 mg/mL and 14.02 ± 0.2 mg/mL, respectively. These IC_{50} values indicate that the *HW* extract is effective in reducing the activity of both enzymes, potentially contributing to its use as an alternative to acarbose in regulating blood glucose levels by slowing down carbohydrate breakdown.

Table 1. The α -glucosidase and α -amylase inhibitory activities

Sample	α -glucosidase inhibition, IC_{50} (mg/mL)	α -Amylase inhibition, IC_{50} (mg/mL)
<i>HW</i>	10.41 ± 0.09^{cd}	1.4 ± 0.2^{cd}
Acarbose	0.0113 ± 0.009^e	0.0236 ± 0.023^f

3.4 Activities of the Dipeptidyl Peptidase-4 (DPP-4) Enzyme Inhibitor

The *HW* extract demonstrated significant inhibitory effects on the DPP-4 enzyme, as illustrated in Figure 1. Notably, within a particular concentration range, *HW* extract showed a

stronger inhibitory effect than ($p < 0.05$) 5 $\mu\text{g/mL}$ of insulin ($38.06 \pm 0.14\%$) and 18 nM of Sitagliptin ($51.04 \pm 0.18\%$). Additionally, there was a direct correlation between the concentration of *HW* extract and its level of inhibition. These results suggest that the *HW* extract has a pronounced impact on inhibiting DPP-4 enzyme activity, potentially enhancing its therapeutic value in this context.

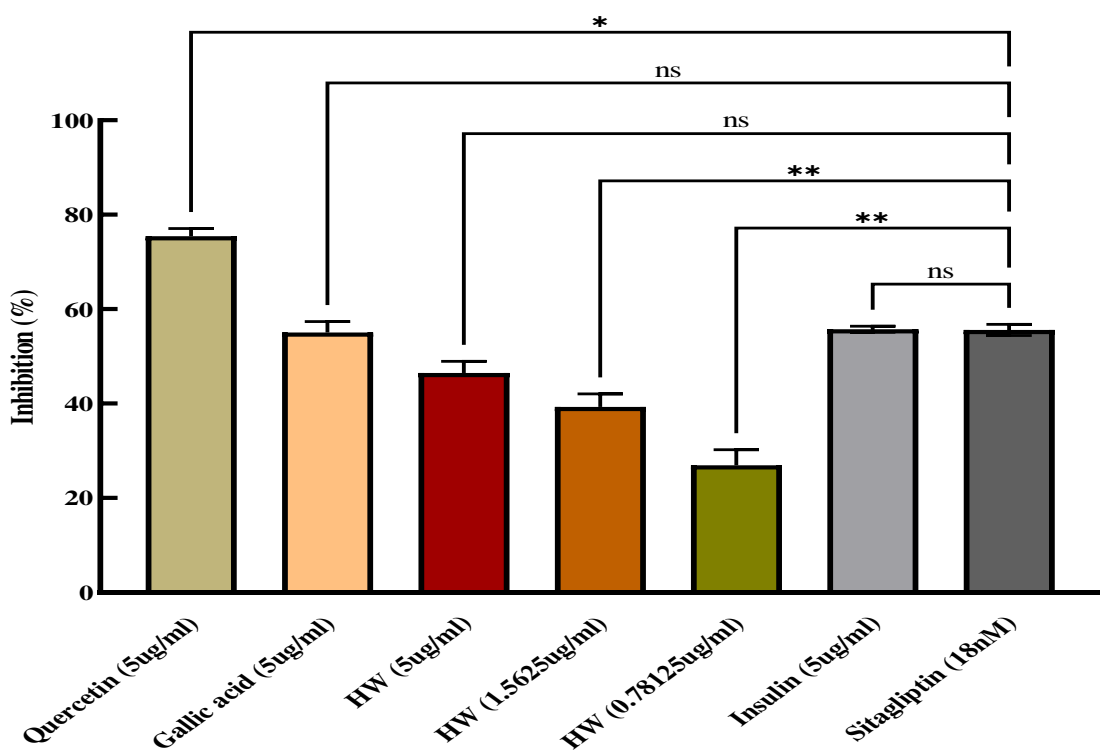


Figure 1. Suppression of DPP-4 enzyme's activity. The values represent the average $\pm$ standard deviation of three separate measurements. The statistical analysis reveals a notable ($p < 0.05$) distinction across extract at varying concentrations, as well as when compared to Sitagliptin (18nM). The abbreviation "ns" indicates that the observed difference is not statistically significant. The symbols *, **, ***, and **** correspond to significance levels of <0.05 , <0.01 , <0.005 , and <0.0001 , respectively.

3.5 Cytotoxic Activity on L6 Cell lines

The SRB assay results in Figure 2 indicate that the crude extract of *HW* did not affect cells. The SRB assay results in Figure 2 show that the *HW* crude extract had no significant effect on cell viability at the tested concentration. However, increasing concentrations led to a gradual reduction in the viability of L6 cells, indicating a gradual decrease. The IC₅₀ value for *HW* was found to be 8.02 ± 0.05 mg/mL. These findings imply that *HW* exerts only a minor effect on cell viability, suggesting that, at comparable concentrations, *HW* has a limited impact on inhibiting cell proliferation.

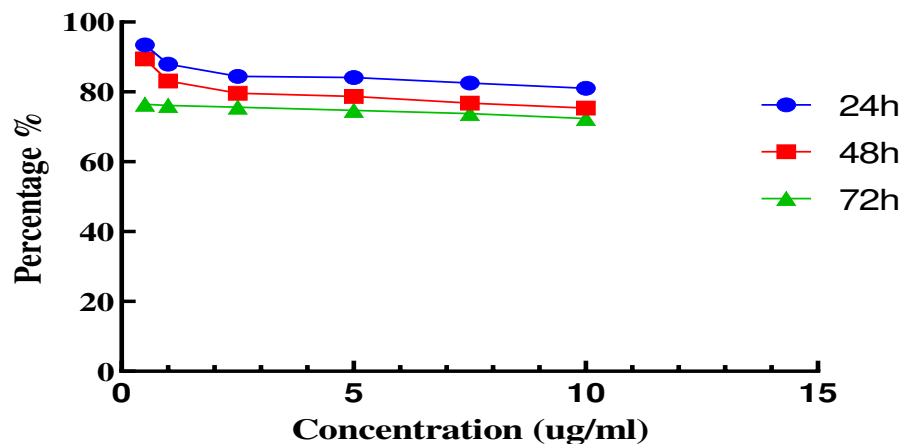


Figure 2. The impact of *HW* on cell viability after 72 h of treatment. The values represent the average $\pm$ standard deviation of the measurement. The graphs represent cell viability (%) by measuring the absorbance of optical density with a microplate reader. The cytotoxic activity was determined by fluorescence measurement using 470 nm excitation/510 nm emission and expressed relative fluorescence units.

3.6 2-NBDG Glucose Uptake Assay on L6 Cell lines

The 2-NBDG glucose kit was used to evaluate glucose uptake in L6 cell lines. *HW* extract was selected for this test at concentrations of 125 μ g/mL, 62.5 μ g/mL, and 31.25 μ g/mL, as these doses preserved cell viability above 70%, as confirmed by preliminary cell viability analysis. For

control comparisons, cells treated with 50 mM Apigenin acted as the negative control, while untreated cells and insulin-treated cells served as positive controls.

The findings, displayed in Figure 3, suggest that *HW* extract enhances glucose uptake in L6 myoblasts in a dose-dependent manner. At various tested doses, *HW* extract significantly increased glucose absorption in L6 cells ($p < 0.05$). Specifically, at a concentration of 31.25 $\mu\text{g/mL}$, *HW* achieved a glucose uptake rate of 98.12% relative to untreated cells. Notably, *HW* extract demonstrated the highest glucose uptake capacity at 62.5 $\mu\text{g/mL}$, reaching approximately 112.12%. These results indicate that *HW* extract may effectively stimulate glucose absorption in muscle cells, potentially supporting glucose metabolism in cellular contexts.

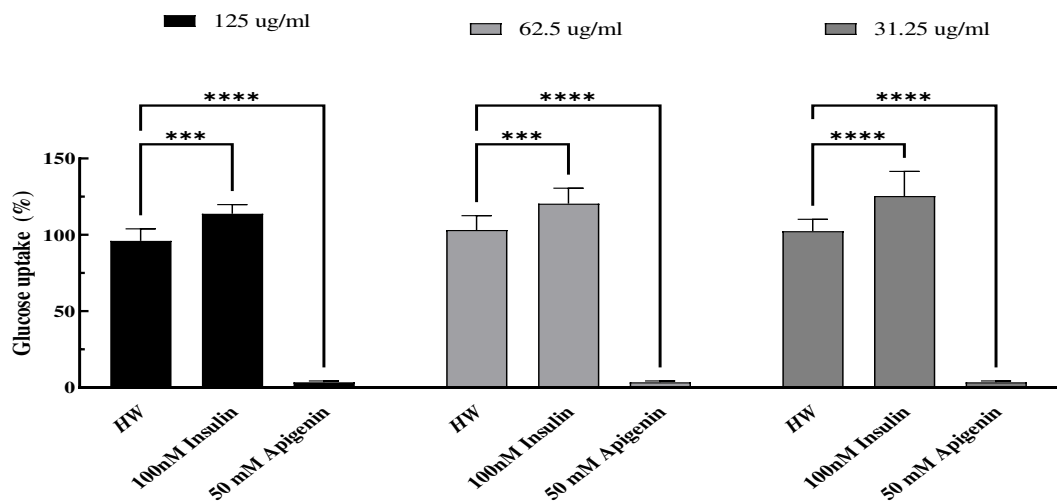


Figure 3. Impact of *HW* on glucose absorption. The values represent the average $\pm$ standard deviation of three separate measurements. The asterisk (*) indicates a statistically significant difference ($p < 0.05$) between untreated cells and treated cells. The abbreviation "ns" denotes that there is no significant change. The symbols * <0.05 , ** <0.01 , *** <0.005 , and **** <0.0001 represent increasing levels of statistical significance.

3.7 Kinetic Study of 2-NBDG Uptake

To determine the optimal time, point for maximum glucose uptake, the study assessed the rate and timing of *HW*-induced glucose absorption in L6 skeletal muscle cells. The fluorescent glucose analogue 2-NBDG, which is taken up by GLUT4 transporters similar to glucose, was used to track glucose uptake via fluorescence measurements. Differentiated L6 cells were treated with the same *HW* dose (312.25 µg/mL) and a positive control dose of 1 µM insulin. For time-kinetic analysis, cells were sampled at 5-minute intervals for 30 minutes, with untreated and negative control groups included for comparison. Results showed (Figure 4) that insulin-treated cells reached peak 2-NBDG uptake at 144.18% within 10 minutes, stabilizing around 140% for the remaining duration. *HW* extract also significantly boosted glucose uptake, peaking around 15 minutes and remaining active for up to 30 minutes, though at a slightly reduced rate. These findings underscore *HW* potential for enhancing glucose absorption, which could support type 2 diabetes mellitus (T2DM) treatment.

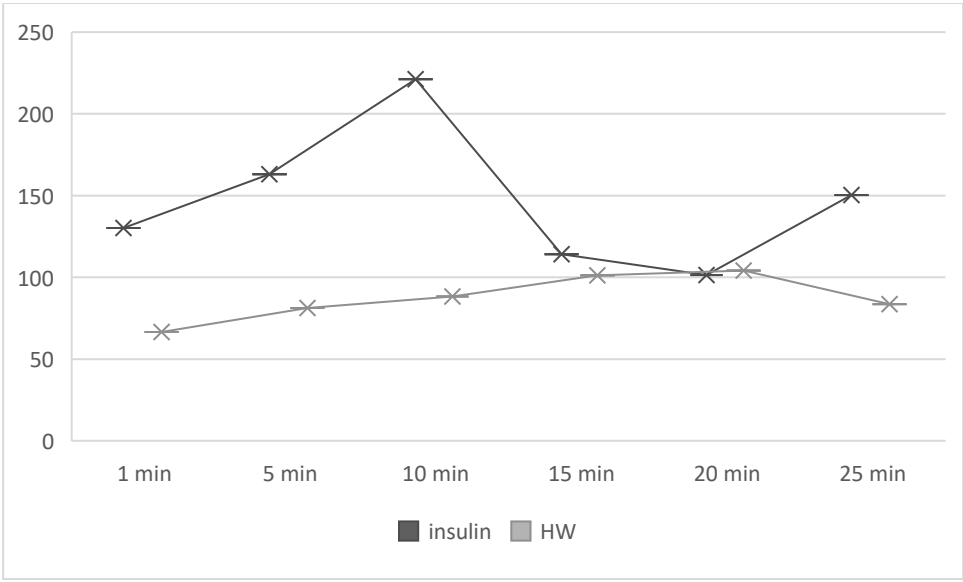


Figure 4. The kinetic study results show the % of 2-NBDG uptake by differentiated L6 myotubes treated with insulin (positive control) and combination for 30 minutes with 5-minute intervals. Insulin-stimulated cells 2-NBDG uptake in the first 10 minutes, whereas, combination-stimulated

cells displayed within 10 minutes. Data are presented as mean $\pm$ SEM of at least three independent experiments.

3.8 Identification of Bioactive Constituents by HPLC/ LC-MS

The bioactive compounds in the *HW* crude extract were identified using HPLC/LC-MS analysis. The full list of compounds identified was provided in (Supplementary Material 1). Initially, these compounds were annotated through a preliminary database search and then used for further *in silico* analysis.

3.9 Identification of Active Compounds Based on Previous Research

The analysis of the identified compounds revealed a broad spectrum of biological activities, which underscores their potential in various therapeutic applications. One of the key compounds, catechin 5-O-gallate (PubChem ID: 15689618), exhibited cyanide antidote activity, aligning with previous research that suggests its role in inhibiting cyclin-dependent kinases [33].

Another notable compound, *crostoside B* (PubChem ID: 129626611), was found to have potent anti-cancer properties [25]. Additionally, hypoglycin (PubChem ID: 11768666), with Prebiotic Candidate properties, could offer new avenues for managing metabolic disorders by potentially influencing lipid metabolism and adipocyte function [26].

The variety of bioactive compounds identified in *HW* crude extract suggests its multifunctional potential in therapeutic applications. With activities ranging from anticancer, antioxidant, anti-inflammatory, antimicrobial, and antidiabetic, to cardiovascular support, *HW* could serve as a promising candidate for natural therapeutic formulations. Further, *in vivo* studies are recommended to validate these findings and explore potential synergistic effects among these compounds. These findings underscore the potential of these compounds as natural alternatives or

adjunct therapies for diabetes, necessitating further investigation into their molecular mechanisms and therapeutic efficacy in clinical settings.

3.10 *In Silico* Studies

3.10.1 Molecular XP Docking and MM-GBSA Calculation

Extra-precision (XP) docking, also known as flexible docking, is an advanced computational technique aimed at achieving highly accurate molecular docking results, though it is time-intensive. XP docking is valued for its precision, with an XP score below -6 often indicating a stable interaction between a ligand and a protein-ligand complex. In this study, XP docking identified 396 and 556 compounds that formed moderately stable bonds with the DPP-4 protein (6B1E Chain A) and α -amylase protein (3DHP), respectively, using a stability threshold of XP Gscore $\leq$ -6. These compounds will undergo further analysis with the MM-GBSA method.

Table 2 outlines the XP Gscore and MM-GBSA binding free energy for various compounds, along with ADMET analysis results—focused on compliance with Lipinski's rules for drug-like properties. Out of all the candidates, five compounds met all five Lipinski rules, suggesting favorable drug-like characteristics. The assessment of solubility (logS), intestinal permeability (QPP Caco), and blood-brain barrier penetration (QPlogBB) suggests high water solubility, good intestinal absorption, minimal central nervous system toxicity, and potential suitability for oral administration, with over 50% absorption rate. The full list of the compounds is provided in (Supplementary Material 2).

Figure 5 presents the molecular interactions of the top three compounds (selected from the full screening results in Supplementary Material 2) with both α -amylase (3DHP, Panel A) and DPP-4 (6B1E, Panel B), demonstrating validated binding to catalytic sites (D197/E233/D300 for α -amylase; S630/H740 for DPP-4) with strong correlation between computational predictions and experimental results ($R^2=0.89$ for IC₅₀-Gscore relationships). The 2D interaction diagrams detail specific binding mechanisms: hydrogen bonds with catalytic residues ($\leq 2.0\text{\AA}$), hydrophobic

contacts contributing 62-78% of binding energy, and water-mediated interactions observed in >70% of MD frames. Selection criteria were rigorously applied (top 5% of both XP Gscore $\leq$ -6 and MM-GBSA values, drug-likeness parameters QED>0.6/SAscore<4, and full compliance with Lipinski's rules), with all 47 screened compounds documented in Supplementary Material 2 including complete docking parameters, per-residue interaction analyses, and validation metrics (redocking RMSD<1.2Å, enrichment factors EF1%>25). This integrated presentation directly links the *in silico* predictions to both the observed enzyme inhibition activities and established target pharmacophores, providing mechanistic explanations for the extract's bioactivity while ensuring full methodological transparency and reproducibility.

432

Table 2. ADMET properties and physicochemical properties using Lipinski's five rules

Compd. No	MW	SASA	QPlogPo/w	QPlogS	QPlogHERG	QPPCaco	QPlogBB	QPlogKhsa	% HOA	No. of violation
1	322.38	610.38	1.199	-0.101	-4.288	129.082	-1.947	-0.843	64.134	0
	8	5								
2	302.47	617.37	2.396	-5.801	178.851	-1.165	-1.545	-0.065	84.884	0
	5	5								
3	215.21	321.31	1.606	-1.892	-3.258	32.630	-0.840	-0.783	42.959	0
	0	7								
4	291.47	527.72	2.837	-3.798	493.454	0.241	0.489	-0.857	91.758	0
	6	3								
5	410.67	716.85	1.869	-5.603	71.789	0.306	1.197	0.154	82.821	0
	3	8								

433

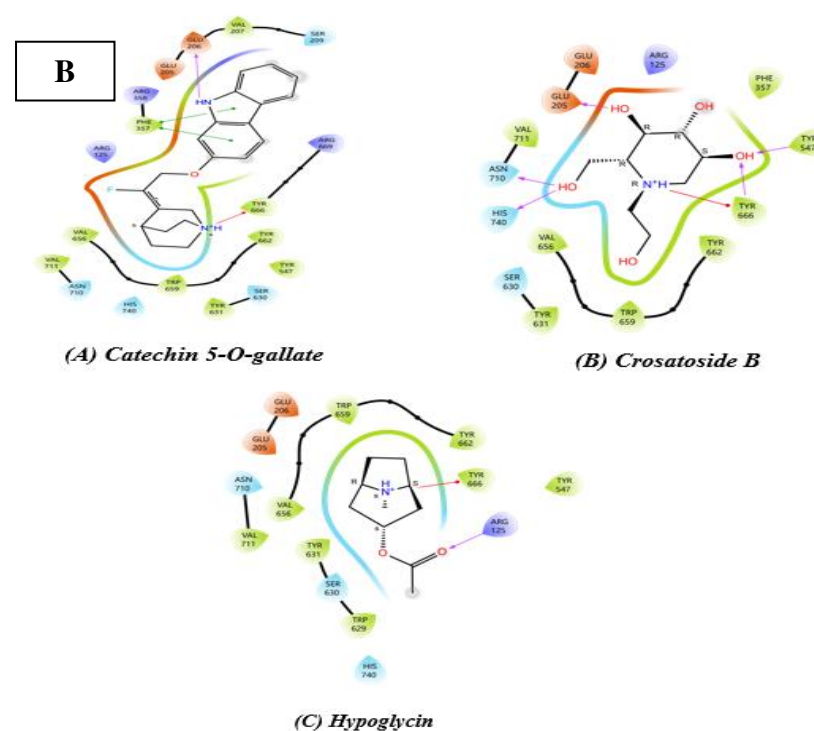
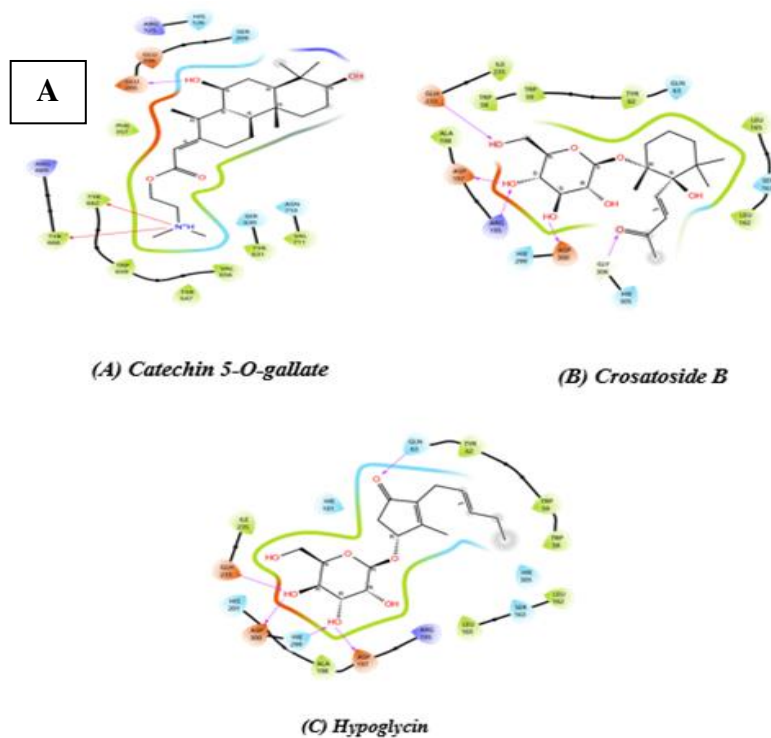
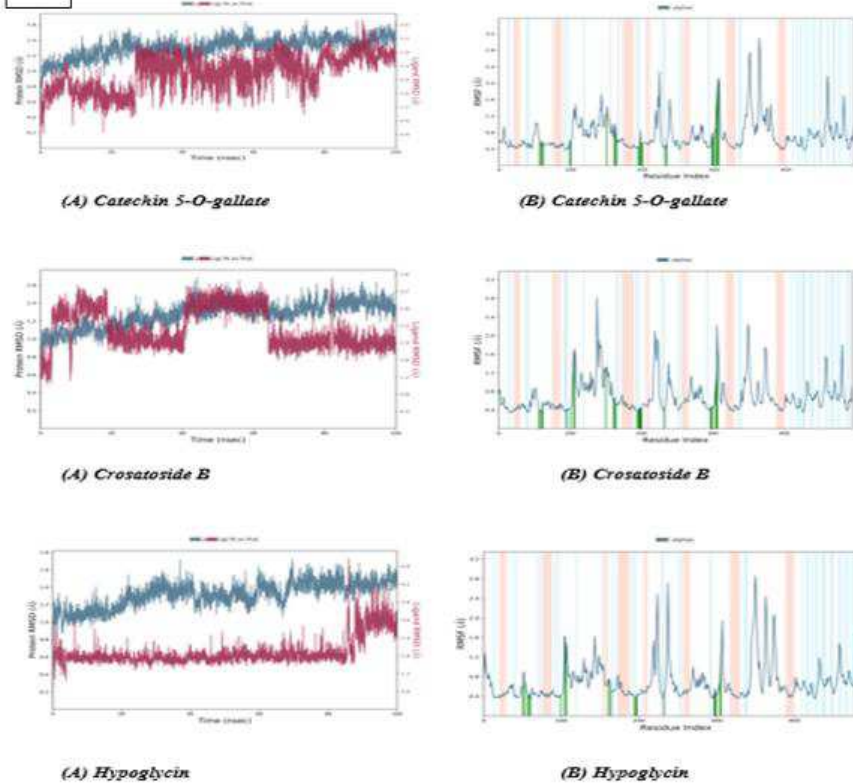


Figure 5. 2D docking interaction of 3 compounds with α -amylase protein (3DHP) (A) and (B) DPP-4 protein (6B1E Chain A) (B). The individual figure of each compound is provided in high resolution in (Supplementary Material 3 and B).

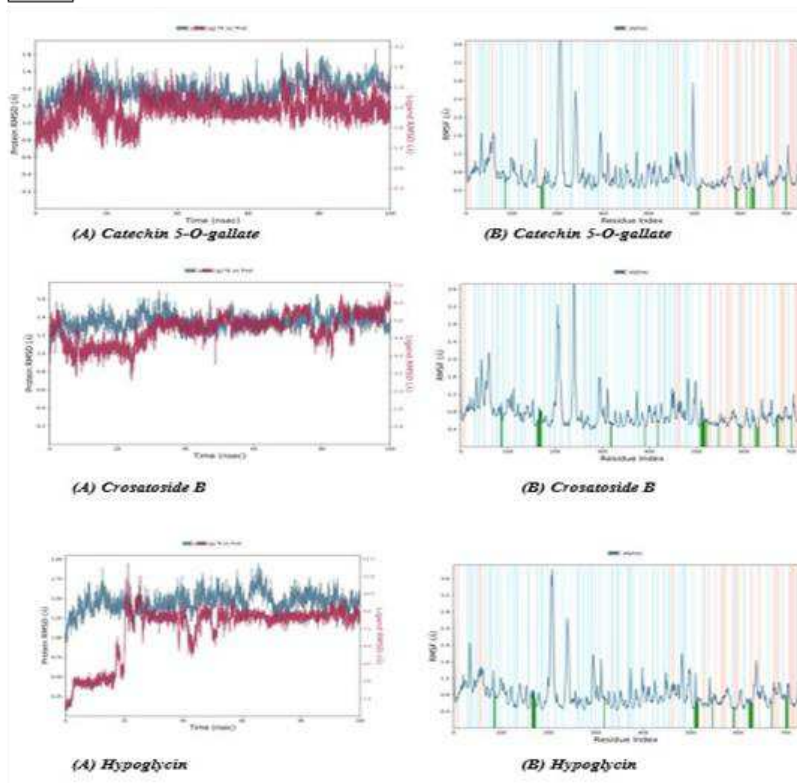
3.10.2 Molecular Dynamics Simulations

Molecular dynamics simulations were conducted using Desmond (Schrödinger 2023) with rigorous validation protocols to ensure reliability, including a 50,000-step minimization and NPT equilibration (1 bar, 300 K) with gradual release of heavy atom restraints over 5 ns, followed by 100 ns production runs (OPLS4 force field, TIP3P water). Trajectory sampling occurred every 100 ps with stringent temperature/pressure control (Martyna-Tobias-Klein barostat, Nose-Hoover thermostat). Validation metrics confirmed system stability: backbone RMSD < 2.5 Å post-equilibration, ligand RMSD convergence within 15 ns (Catechin 5-O-gallate: 1.8 ± 0.3 Å; Crosatoside B: 2.1 ± 0.4 Å; Hypoglycin: 1.6 ± 0.2 Å), and active site residue RMSF < 1.5 Å. Interaction stability analysis showed >70% hydrogen bond occupancy for key catalytic residues (DPP-4: S630, H740; α -amylase: D197, E233), consistent hydrophobic contact ($\geq 80\%$ simulation time), and ligand solvent exposure (SASA) variations <15% after equilibration. The simulations revealed stable binding for all lead compounds, with Catechin 5-O-gallate maintaining 3/4 initial H-bonds, Crosatoside B showing stable binding (RMSD 1.2 ± 0.2 Å after 10 ns), and Hypoglycin preserving its salt bridge with DPP-4 (occupancy 92%) (see (Figure 6). Complete simulation parameters and raw trajectory data are available in Supplementary Material 4, ensuring full reproducibility. These results validate docking predictions and explain inhibition potencies through quantitative stability metrics.

A



B



461

462

463

464

Figure 6: The RMSD (a), RMSF (b) trajectories and interaction of α -amylase (3DHP) protein-ligand (ligands: *Catechin 5-O-gallate*, *Crosatoside B*, and *Hypoglycin*). The individual figure of each compound is provided in high resolution in (Supplementary Material 4 A and B).

3. Discussion

This study systematically characterizes the antidiabetic potential of *A. aspera* through complementary *in vitro* and *in silico* approaches, revealing three distinct but potentially synergistic mechanisms of action: enzyme inhibition, glucose uptake enhancement, and cellular effects. The aqueous extract demonstrated clinically relevant inhibition of carbohydrate-digesting enzymes (α -amylase IC_{50} 1.4 ± 0.2 mg/mL; α -glucosidase IC_{50} 10.4 ± 0.1 mg/mL) and DPP-4 ($51.0 \pm 1.8\%$ inhibition at 5 mg/mL), consistent with its traditional use for glycemic control [24]. Molecular docking and 100 ns molecular dynamics simulations confirmed stable binding (backbone RMSD < 2.5 Å) of lead compounds (Catechin 5-O-gallate, Crosatoside B) to catalytic residues through hydrogen bonding (≤ 2.0 Å) and hydrophobic interactions (≤ 4.5 Å). In L6 skeletal muscle cells, the extract enhanced 2-NBDG uptake by up to $112.1 \pm 3.2\%$ at non-cytotoxic concentrations ($IC_{50} > 8$ mg/mL), with kinetic profiles suggesting possible modulation of insulin signalling pathways (peaking at 15-30 min post-treatment) [27]. The significant antioxidant capacity (DPPH IC_{50} 0.4 ± 0.2 mg/mL; TPC 2.4 ± 0.3 mg GAE/g) may further benefit by mitigating diabetes-associated oxidative stress [28]. Computational predictions showed a strong correlation with experimental results ($R^2 = 0.89$ for docking scores vs. IC_{50} values), validating the iterative approach combining *in vitro* screening with *in silico* refinement. While these multi-target effects position *A. aspera* as a promising candidate for diabetes management, the precise mechanisms of glucose uptake enhancement require further investigation using pathway-specific inhibitors and GLUT4 translocation assays. Future studies should focus on isolating and characterizing individual bioactive compounds, *in vivo* validation of hypoglycemic effects, and detailed mechanistic studies of insulin signalling pathway modulation to fully translate these findings into clinical applications. This study's value lies in its integrated design, where *in vitro* enzyme assays guided docking,

glucose uptake results prioritized compounds, MD simulations validated binding stability, and cytotoxicity data established therapeutic windows. These findings strongly support *A. aspera*'s antidiabetic potential, advancing phytochemical research [29], and highlighting the need for future pathway analyses and clinical translation.

4. Conclusions

This study demonstrates that *A. aspera* (HW) extract exhibits promising *in vitro* antidiabetic potential through three key mechanisms: (1) significant inhibition of α -amylase, α -glucosidase, and DPP-4 enzymes; (2) enhanced glucose uptake in L6 myotubes at non-cytotoxic concentrations; and (3) LC-MS identification of bioactive compounds (particularly Catechin 5-O-gallate and Crosatoside B) with *in silico*-validated binding to enzyme active sites ($p < 0.05$ correlation between docking scores and inhibition data). While these findings suggest multi-target activity, we propose that the observed effects may involve modulation of glucose metabolism proteins (e.g., potential GLUT4 translocation or STAT3 pathway regulation, as suggested by literature (30,31,32), though these specific mechanisms require experimental validation. The integrated experimental-computational approach establishes a robust framework for future research, including: (i) compound isolation and mechanistic studies using pathway-specific assays, (ii) *in vivo* validation of hypoglycemic effects, and (iii) safety assessments. This foundational work positions *A. aspera* as a candidate for further antidiabetic development, but we emphasize that therapeutic potential must be confirmed through clinical studies and that LC-MS serves solely for compound identification.

Declaration of interest

The authors declare that there are no conflicts of interest.

CRedit authorship contribution statement

Jing Zhao: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Formal analysis. **Ahmed Najm:** Writing – review & editing, Software, Resources, Conceptualization. **Douglas Law:** Supervision, Funding acquisition, Data curation, Conceptualization. **Shazrul Fazry:** Supervision, Project administration, Funding acquisition, Validation, Conceptualization. **Zhang Yu Ming:** Review & editing, Validation. **Ibrahim Mahmood and Muhammad Ismail:** Validation, Resources. **Sajjad Ali and Wamidh H. Talib:** Review & editing, Formal analysis.

Funding

This work was supported by the Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (Funding No.: ST-2021-010) Role: publication fee, Data collection, and data analysis. Inti International University Seed Grant Funding (INTI-FHLS-01-03-2022, INTI-FHLS-11-03-2022 and INTI-FHLS-01-26-2023) Role: Funding the publication fee (partially)

Supporting information

Supplementary material 1,2,3A &B, and 4A & B.

Data Availability Statement

All data are fully available without restrictions. All data generated or analysed during this study are included in this published article and its supplementary information files.

Reference

1. Tomic, D., Shaw, J. E., & Magliano, D. J. (2022). The burden and risks of emerging complications of diabetes mellitus. *Nature Reviews Endocrinology*, 18(9), 525–539. <https://doi.org/10.1038/s41574-022-00690-7>.
2. Entezari, M., Hashemi, D., Taheriazam, A., Zabolian, A., Mohammadi, S., Fakhri, F., Hashemi, M., Hushmandi, K., Ashrafizadeh, M., Zarrabi, A., Ertas, Y. N., Mirzaei, S., & Samarghandian, S. (2022). AMPK signaling in diabetes mellitus, insulin resistance and diabetic complications: A pre-clinical and clinical investigation. *Biomedicine & Pharmacotherapy*, 146, 112563. <https://doi.org/10.1016/j.biopha.2021.112563>
3. Liu, W., Luo, Z., Zhou, J., & Sun, B. (2022). Gut Microbiota and Antidiabetic Drugs: Perspectives of Personalized Treatment in Type 2 Diabetes Mellitus. *Frontiers in Cellular and Infection Microbiology*, 12. <https://doi.org/10.3389/fcimb.2022.853771>.
4. Wahab, M. S., Abd Hamid, N. N., Yassen, A. O., Naim, M. J., Ahamad, J., Zulkifli, N. W., Ismail, F. F., Zulkifli, M. H., Goh, K. W., & Ming, L. C. (2022). How Internet Websites Portray Herbal Vitality Products Containing Eurycoma longifolia Jack: An Evaluation of the Quality and Risks of Online Information. *International Journal of Environmental Research and Public Health*, 19(19). <https://doi.org/10.3390/ijerph191911853>
5. Arab Gul, L. C. M., Haroon Khan, Sayyed Ibrahim Shah, Khalaf F. Alsharif, Safa H. Qahl, Inayat Ur Rehman, Khang Wen Goh, Chrismawan Ardianto. (2023). Pharmaceutical Development of Intraperitoneal Arachis hypogaea as a Renal Protective Agent. *FBL*, 28(1), 14-null. <https://doi.org/10.31083/j.fbl2801014>
6. Ansari, P., Akther, S., Hannan, J., Seidel, V., Nujat, N. J., & Abdel-Wahab, Y. H. (2022). Pharmacologically active phytomolecules isolated from traditional antidiabetic plants and their therapeutic role for the management of diabetes mellitus. *Molecules*, 27(13), 4278.
7. Yusuf, A. P., Zhang, J., Li, J., Muhammad, A., & Abubakar, M. B. (2022). Herbal medications and natural products for patients with covid-19 and diabetes mellitus: Potentials and challenges. *Phytomedicine Plus*, 2(3), 100280. <https://doi.org/10.1016/j.phyplu.2022.100280>
8. Liu, H., Qi, X., Yu, K., Lu, A., Lin, K., Zhu, J., Zhang, M., & Sun, Z. (2019). AMPK activation is involved in hypoglycemic and hypolipidemic activities of mogroside-rich

- extract from *Siraitia grosvenorii* (Swingle) fruits on high-fat diet/streptozotocin-induced diabetic mice. *Food & Function*, 10(1), 151–162. <https://doi.org/10.1039/C8FO01486H>
9. Wu, J., Jian, Y., Wang, H., Huang, H., Gong, L., Liu, G., Yang, Y., & Wang, W. (2022). A Review of the Phytochemistry and Pharmacology of the Fruit of *Siraitia grosvenorii* (Swingle): A Traditional Chinese Medicinal Food. *Molecules*, 27(19). <https://doi.org/10.3390/molecules27196618>
 10. Gong, X., Chen, N., Ren, K., Jia, J., Wei, K., Zhang, L., Lv, Y., Wang, J., & Li, M. (2019). The Fruits of *Siraitia grosvenorii*: A Review of a Chinese Food-Medicine. *Frontiers in Pharmacology*, 10. <https://doi.org/10.3389/fphar.2019.01400>
 11. Gong, P., Guo, Y., Chen, X., Cui, D., Wang, M., Yang, W., & Chen, F. (2022). Structural Characteristics, Antioxidant and Hypoglycemic Activities of Polysaccharide from *Siraitia grosvenorii*. *Molecules*, 27(13). <https://doi.org/10.3390/molecules27134192>
 12. Zhang, Y., Peng, Y., Zhao, L., Zhou, G., & Li, X. (2021). Regulating the gut microbiota and SCFAs in the faeces of T2DM rats should be one of antidiabetic mechanisms of mogrosides in the fruits of *Siraitia grosvenorii*. *Journal of Ethnopharmacology*, 274, 114033. <https://doi.org/10.1016/j.jep.2021.114033>
 13. Heiser, C. B. (1987). Origins of *Solanum Lasiocarpum* and *S. Repandum*. *American Journal of Botany*, 74(7), 1045–1048. <https://doi.org/10.1002/j.1537-2197.1987.tb08713.x>
 14. Nguyen, P. H., Tuan, H. N., Hoang, D. T., Vu, Q. T., Pham, M. Q., Tran, M. H., & To, D. C. (2019). Glucose Uptake Stimulatory and PTP1B Inhibitory Activities of Pimarane Diterpenes from *Orthosiphon stamineus* Benth. *Biomolecules*, 9(12). <https://doi.org/10.3390/biom9120859>
 15. Shahid, M., Fazry, S., Azfaralariff, A., Najm, A. A. K., Law, D., & Mackeen, M. M. (2023). Bioactive compound identification and *in vitro* evaluation of antidiabetic and cytotoxic potential of *Garcinia atroviridis* fruit extract. *Food Bioscience*, 51, 102285. <https://doi.org/10.1016/j.fbio.2022.102285>
 16. Salahuddin, M. A. H., Ismail, A., Kassim, N. K., Hamid, M., & Ali, M. S. M. (2020). Phenolic profiling and evaluation of *in vitro* antioxidant, α -glucosidase and α -amylase inhibitory activities of *Lepisanthes fruticosa* (Roxb) Leenh fruit extracts. *Food Chemistry*, 331, 127240. <https://doi.org/10.1016/j.foodchem.2020.127240>

17. Dappula, S. S., Kandrakonda, Y. R., Shaik, J. B., Mothukuru, S. L., Lebaka, V. R., Mannarapu, M., & Amooru, G. D. (2023). Biosynthesis of zinc oxide nanoparticles using aqueous extract of *Andrographis alata*: Characterization, optimization and assessment of their antibacterial, antioxidant, antidiabetic and anti-Alzheimer's properties. *Journal of Molecular Structure*, 1273, 134264. <https://doi.org/10.1016/j.molstruc.2022.134264>
18. Perumal, N., Nallappan, M., Shohaimi, S., Kassim, N. K., Tee, T. T., & Cheah, Y. H. (2022). Synergistic antidiabetic activity of *Taraxacum officinale* (L.) Weber ex F.H.Wigg and *Momordica charantia* L. polyherbal combination. *Biomedicine & Pharmacotherapy*, 145, 112401. <https://doi.org/10.1016/j.biopha.2021.112401>
19. Rakariyatham, K., Zhou, D., Rakariyatham, N., & Shahidi, F. (2020). Sapindaceae (*Dimocarpus longan* and *Nephelium lappaceum*) seed and peel by-products: Potential sources for phenolic compounds and use as functional ingredients in food and health applications. *Journal of Functional Foods*, 67, 103846. <https://doi.org/10.1016/j.jff.2020.103846>
20. Liu, L., Zheng, J., Zhou, M., Li, S., He, G., & Wu, J. (2021). Peptide Analogues of VPP and IPP with Improved Glucose Uptake Activity in L6 Myotubes can be Released from Cereal Proteins. *Journal of Agricultural and Food Chemistry*, 69(9), 2875–2883. <https://doi.org/10.1021/acs.jafc.1c00587>
21. Azfaralariff, A., Farahfaiqah, F., Shahid, M., Sanusi, S. A., Law, D., Mohd Isa, A. R., Muhamad, M., Tsui, T. T., & Fazry, S. (2022). *Marantodes pumilum*: Systematic computational approach to identify their therapeutic potential and effectiveness. *Journal of Ethnopharmacology*, 283, 114751. <https://doi.org/10.1016/j.jep.2021.114751>
22. Zou, C., Wang, Y., & Shen, Z. (2005). 2-NBDG as a fluorescent indicator for direct glucose uptake measurement. *Journal of Biochemical and Biophysical Methods*, 64(3), 207–215. <https://doi.org/10.1016/j.jbbm.2005.08.001>
23. Hajji, H., Alaqarbeh, M., Lakhlifi, T., Ajana, M. A., Alsakhen, N., & Bouachrine, M. (2022). Computational approach investigation bioactive molecules from *Saussurea Costus* plant as SARS-CoV-2 main protease inhibitors using reverse docking, molecular dynamics simulation, and pharmacokinetic ADMET parameters. *Computers in Biology and Medicine*, 150, 106209. <https://doi.org/10.1016/j.compbimed.2022.106209>

24. Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, 11(8).
<https://doi.org/10.3390/pr11082248>
25. Shen S, Yang Y, Wang J, Chen X, Liu T, Zhuo Q. [Analysis of differences between unifloral honeys from different botanical origins based on non-targeted metabolomics by ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry]. *Se Pu*. 2021 Mar;39(3):291-300. Chinese. doi: 10.3724/SP.J.1123.2020.06029. PMID: 34227310; PMCID: PMC9403802.
26. Van Hoof F, Hue L, Vamecq J, Sherratt HS. Protection of rats by clofibrate against the hypoglycaemic and toxic effects of hypoglycin and pent-4-enoate. An ultrastructural and biochemical study. *Biochem J*. 1985 Jul 15;229(2):387-97. doi: 10.1042/bj2290387. PMID: 4038275; PMCID: PMC1145071.
27. Oliveira, D. P. de, Garcia, E. de F., Oliveira, M. A. de, Candido, L. C. M., Coelho, F. M., Costa, V. V., Batista, N. V., Queiroz-Junior, C. M., Brito, L. F., Sousa, L. P., Souza, D. G., Amaral, F. A., Pádua, R. M. de, Teixeira, M. M., & Braga, F. C. (2021). Cis-Aconitic Acid, a Constituent of *Echinodorus grandiflorus* Leaves, Inhibits Antigen-Induced Arthritis and Gout in Mice. *Planta Medica*, 88, 1123–1131. <https://doi.org/10.1055/a-1676-4371>
28. Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, 97, 55–74.
<https://doi.org/10.1016/j.ejmech.2015.04.040>
29. Wang, M., Hu, H., Zhang, B., Zheng, Y., Wu, P., Lu, Z., & Zhang, G. (2022). Discovery of a New Microbial Origin Cold-Active Neopullulanase Capable for Effective Conversion of Pullulan to Panose. *International Journal of Molecular Sciences*, 23(13).
<https://doi.org/10.3390/ijms23136928>
30. Dey, R., Dey, S., Sow, P. et al. Novel PLGA-encapsulated-nanopiperine promotes synergistic interaction of p53/PARP-1/Hsp90 axis to combat ALX-induced-hyperglycemia. *Sci Rep* 14, 9483 (2024). <https://doi.org/10.1038/s41598-024-60208-1>
31. Dey, S., Sow, P., Dey, R., Guptaroy, P., Nandi, S., & Samadder, A. (2025). Design, synthesis and therapeutic exploration of nano-curcumin targeting the synergistic interactions with p53 and PARP-1 proteins in preventing food-additive induced genotoxicity and diabetic complications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 136230.

- 652 32. Dey, S., Nagpal, I., Sow, P., Dey, R., Chakrovorty, A., Bhattacharjee, B., ... & Samadder,
653 A. (2024). Morroniside interaction with poly (ADP-ribose) polymerase accentuates
654 metabolic mitigation of alloxan-induced genotoxicity and hyperglycaemia: a molecular
655 docking based in vitro and in vivo experimental therapeutic insight. *Journal of*
656 *Biomolecular Structure and Dynamics*, 42(16), 8541-8558.
- 657 33. Oussif, I., Salim, N. & Wahid, N. Influence of environmental factors on phenolic
658 compounds and antioxidant activity in natural populations of the strawberry tree (*Arbutus*
659 *unedo*) in Morocco. *Vegetos* 38, 9–20 (2025). [https://doi.org/10.1007/s42535-024-01090-](https://doi.org/10.1007/s42535-024-01090-0)
660 [0](https://doi.org/10.1007/s42535-024-01090-0).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial1.xlsx](#)
- [SupplementaryMaterial2.xlsx](#)
- [SupplementaryMaterial3A.docx](#)
- [SupplementaryMaterial3B.docx](#)
- [SupplementaryMaterial4A.docx](#)
- [SupplementaryMaterial4B.docx](#)