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Research article

Antidiabetic Efficacy and Hemolytic Activity of the Polyphenolic Extract Derived from *Allium sativum* L.: An *In vitro* and *In silico* Evaluation

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Abstract

This study aimed to identify the main polyphenolic compounds of *Allium sativum* L. (*A. sativum*) extract and evaluate its antidiabetic and antihemolytic properties. *A. sativum* polyphenolic extract (ASPE) was subjected to UHPLC-MS/MS analysis to determine its polyphenolic composition. The antidiabetic activity was assessed through enzyme inhibition assays targeting α-amylase and α-glucosidase, comparing the extract's efficacy to the standard drug acarbose. Hemolytic activity was evaluated using rat erythrocytes exposed to varying concentrations of the extract (5–100 mg/mL) to determine its cytotoxicity on blood cells. Statistical analyses were carried out to assess the significance of the observed effects. An *in silico* study was conducted using Mastro 11.5 from the Schrödinger suite to evaluate antidiabetic activity against α-amylase and α-glucosidase. UHPLC-MS/MS analysis revealed mongophenoside B as the most abundant compound, alongside catechin and cyanidin 3-O-beta-D sambubioside. The extract demonstrated a strong inhibitory effect on α-amylase (82.31% inhibition at 1 mg/mL; IC_{50} = 0.047 ± 0.002 mg/mL) and α-glucosidase (IC_{50} = 0.055 ± 0.004 mg/mL), surpassing acarbose in potency. Hemolytic assays indicated a dose-dependent increase in hemolysis, with significant cytotoxicity observed at higher concentrations (up to 21.24 ± 1.54% at 100 mg/mL), suggesting safe application only at lower doses. The ASPE exhibits potent antidiabetic properties, attributable to the identified compounds catechin, cyanidin 3-O-beta-D sambubioside, and mongophenoside B, the latter reported here for the first time in garlic. These findings suggest that the synergistic action of these compounds contributes to effective enzyme inhibition. However, the extract's hemolytic activity at higher doses indicates a need for caution in therapeutic applications. The *in silico* study showed strong inhibitory activity of mongophenoside B, catechin, and cyanidin 3-O-beta-D sambubioside against α-amylase and α-glucosidase, confirming the *in vitro* results. Further studies are warranted to explore the antidiabetic potential of mongophenoside B and to assess the extract's safety profile for clinical use.

Keywords: *Allium sativum* L., Polyphenolic extract, UHPLC-MS/MS analysis, Mongophenoside B, Catechin, Cyanidin 3-O-beta-D sambubioside, Antidiabetic activity, Hemolytic activity

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1. Introduction

Garlic (*Allium sativum* L.), a plant species in the onion family (Amaryllidaceae), has been used as food and medicinal remedy for thousands of years. From ancient civilizations to modern medicine, garlic has been prominent in treating various ailments (Papu et al., 2014). The medicinal power of garlic lies primarily in its rich composition of bioactive compounds, particularly sulfur-

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containing compounds such as allicin, diallyl disulfide, and S-allyl cysteine (Chebaibi et al., 2022; Shang et al., 2019). These compounds have been reported to contribute to garlic's notable antimicrobial, antioxidant, anti-inflammatory, and cardioprotective properties (Bayan et al., 2014).

One of the most significant areas of recent research is garlic's role in managing diabetes. Diabetes mellitus is a chronic metabolic disorder characterized by high blood sugar levels due to the body's inability to produce or effectively utilize insulin. Type 2 diabetes is a growing public health issue affecting millions globally. Managing diabetes often involves lifestyle changes, medications, and dietary interventions to control blood glucose levels (Galaviz et al., 2018).

Garlic has demonstrated potential as a natural therapeutic agent for diabetes management. Studies have shown that garlic can lower blood sugar levels, improve insulin sensitivity, and reduce inflammation, which are beneficial in managing type 2 diabetes (Choudhary et al., 2015). Allicin, a key bioactive compound in garlic, has been found to inhibit α-glucosidase and α-amylase, enzymes involved in the digestion of carbohydrates, thus reducing glucose absorption into the bloodstream (Khan et al., 2003). Moreover, garlic's antioxidant properties help mitigate oxidative stress, which is critical in the pathogenesis of diabetes and its complications (Banerjee et al., 2003).

In animal studies, garlic supplementation has been associated with improvements in lipid profiles, reduced insulin resistance, and lower blood glucose levels (Jelodar Gholamali et al., 2005). Human trials have also shown promising results, with several studies reporting that garlic supplementation can significantly reduce fasting blood glucose and HbA1c levels, which are important markers for diabetes control (Ried et al., 2013). Although more clinical trials are needed to confirm these findings, garlic's potential as an adjunct treatment for diabetes management is undeniable.

Garlic's role in hemolytic activity is another area of significant research interest. Hemolysis, the destruction of red blood cells, can occur due to various factors, including infections, autoimmune diseases, and the consumption of certain substances (Fattizzo and Barcellini, 2022). Garlic's phytochemicals have been studied for their hemolytic effects, particularly their ability to modulate oxidative stress in red blood cells.

Garlic contains numerous sulfur compounds that have been found to enhance antioxidant defenses, protecting red blood cells from oxidative damage (Rahman and Lowe, 2006). This is particularly relevant in conditions where hemolysis is driven by oxidative stress, such as in cases of glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD is an enzyme that protects red blood cells from oxidative damage. In individuals with G6PD deficiency, the consumption of oxidative substances can lead to severe hemolysis. Some studies suggest that garlic may help reduce the risk of hemolysis by enhancing the body's antioxidant capacity and reducing oxidative stress (Amin and Hamza, 2006).

Most studies on garlic have primarily focused on its sulfur-containing compounds, which are well-known for their numerous health benefits. However, garlic also contains phenolic compounds and flavonoids, which possess significant pharmaceutical properties. These compounds are believed to contribute to garlic's antioxidant and therapeutic effects. Using ultrasound-assisted extraction, our study aims to identify garlic extracts' phenolic compounds and flavonoids and evaluate their potential antidiabetic and antihemolytic activities.

2. Material and methods

2.1. Plant Material and Extract Preparation

A. sativum was purchased and dried in a shaded place in a well-ventilated room before being ground into powder. The extraction of polyphenols was carried out using the method described by Slighoua and co-workers (Slighoua et al., 2022) with some modifications. 100g of the garlic plant's bulb was extracted three times with 300 mL of methanol at 25°C in a sonicator for 45 min. The extract was recovered and dried in the oven after removing the solvent with the filter papers. The extract was dissolved in 500 mL of distilled water and extracted three times with 200 mL of hexane and three times with 200 mL of chloroform to remove caffeine and chlorophyll. The aqueous phase was extracted with 200 mL of ethyl acetate and evaporated under low pressure. The polyphenol extract were dried and stored in the laboratory until use (Amrati et al., 2023).

2.2. HPLC-MS-MS Analysis

The *ASPE* was analyzed using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The experiment was conducted on an Acquity (Waters, CA, USA) that has a column oven, an autosampler and a quaternary pump. The suggested method made use of a Kinetex C18 reversed phase column (250 × 4.6 mm, 2.6 µm particles) supplied by Thermo Fisher Scientific (CA, USA). Solvent A (0.1%) formic acid aqueous solution and solvent B (Methanol) were used to establish a gradient separation. The gradient was made in multiple steps. The flow rate in the mobile phase was 0.5 mL/min. The Xevo TQD, CA, USA manufactured the TQD triple quadrupole mass spectrometer, which was outfitted with a heated electrostatic spray (H-ESI) ionization source operating in negative mode. The temperature of the ion transfer tube and the H-ESI vaporizer were both set to 250°C. 3500 V was the electrospray voltage that was set. Complete scan MS capture mode in Q1 (m/z 50–900) (Metouekel et al., 2024).

2.3. Antidiabetic activity

2.3.1. In Vitro Inhibition of Pancreatic α-amylase

To investigate the inhibitory activity of pancreatic α-amylase *in vitro*, a modified protocol adapted from Elrherabi et al. was applied. Initially, 200 µL of 0.02 M phosphate buffer (pH 6.9) was mixed with 200 µL of the α-amylase enzyme solution. This mixture was incubated at 37°C for 10 minutes until use. Each reaction tube then received 200 µL of the test solutions (M1G, M2G, and AG at concentrations of 0.062, 0.125, 0.25, 0.5, and 1 mg/mL) along with an additional 200 µL of a 1% starch solution, followed by a 15-minute incubation period at 37°C (Elrherabi et al., 2024).

To halt enzyme activity, 600 µL of the 3,5-dinitrosalicylic acid (DNSA) reagent was introduced, after which the tubes were heated to 100°C for 8 minutes. Rapid cooling in an ice bath was then performed. Each reaction was diluted by adding 1 mL of distilled water, and absorbance readings were taken at 540 nm using a spectrophotometer.

A control reaction was conducted in parallel by substituting the plant extract with 200 µL of phosphate buffer to signify

100% enzyme activity. Additionally, blank reactions were prepared using each plant extract concentration but without the enzyme solution to account for any baseline absorbance from the extracts. The positive control used acarbose, processed identically to the plant extracts.
The inhibition percentage for each sample was determined using the following formula:

$$\textbf{\% of inhibition} = \left(\frac{\textbf{Asample} - \textbf{Acontrol}}{\textbf{Acontrol}}\right) \times \textbf{100}$$

$A_{Control}$: Absorbance of enzymatic effects in the absence of an inhibitor.

A_{Sample}: Absorbance of the enzymatic effect in the presence of the extract or acarbose.

The IC_{50} (concentration of samples that inhibit 50% of α-amylase enzyme activity) was determined graphically using the function: percentage of inhibition = f(log(sample concentration)).

2.3.2. In vitro Inhibition of Intestinal α-glucosidase

This study utilized a slightly modified Elrherabi et al. (2024) method to assess α-glucosidase inhibitory activity. The test mixtures were prepared with 1 mL of phosphate buffer (pH 7.5), 0.1 mL of an α-glucosidase enzyme solution (10 IU), and 200 µL of ASPE, tested at concentrations of 0.062, 0.125, 0.25, 0.5, and 1 mg/mL. Control samples included distilled water as the negative control and acarbose at matching concentrations as the positive control (Elrherabi et al., 2024).
The reaction mixtures underwent a preliminary incubation at 37°C for 20 minutes, after which 0.1 mL of sucrose solution was added to initiate the reaction. To terminate the reaction, the tubes were heated at 100°C for 5 minutes. After cooling, 1 mL of GOD-POD (Glucose Oxidase-Peroxidase) reagent was added to each tube, followed by a secondary incubation at 37°C for 10 minutes. Absorbance was measured at a wavelength of 500 nm using a spectrophotometer.
The inhibition rate was then calculated using the following formula:

$$\textbf{\% Inhibition} = \left(\frac{\textbf{Asample} - \textbf{Acontrol}}{\textbf{Acontrol}}\right) \times \textbf{100}$$

$A_{control}$: Absorbance of enzymatic effects in the absence of an inhibitor.

A_{Sample}: Absorbance of the enzymatic effect in the presence of extract or acarbose.

The IC_{50} (sample concentration that inhibits 50% of α-amylase enzyme activity) was determined graphically using the function: inhibition percentage = f(log sample concentration).

2.4. Antihemolytic activity

2.4.1. Red Blood Cell Preparation

Freshly obtained rat blood samples were mixed with a heparin-based anticoagulant solution. The blood sample underwent three consecutive washing cycles with sterile NaCl saline (0.9%) to obtain pure erythrocyte suspension. At each washing stage, cells were separated by centrifugation (3500 rpm for 10 minutes at 4°C), and the supernatant was carefully aspirated. Finally, the erythrocytes were resuspended in a physiological solution to form the 3% solution used in the hemolytic assay.

2.4.2. Hemolytic Assay

Evaluating the plant's hemolytic activity followed the previously described modified protocol (Saleh et al., 2021). In each hemolysis tube, 100 µL of extract at varying initial concentrations (5, 10, 50, and 100 mg/mL) was added to 1.9 mL of a prepared erythrocyte suspension. These mixtures were incubated in a water bath at 37 °C for one hour. Every 15 minutes throughout this period, a 500 µL sample was drawn from each tube and diluted with 1.5 mL of phosphate-buffered saline (PBS) solution. After this, the tubes were subjected to centrifugation at 3000 rpm for a duration of 10 minutes to separate the components. The absorbance of the resulting supernatants was then recorded at a wavelength of 540 nm to detect hemoglobin release due to erythrocyte lysis. This measurement was taken using a UV-visible spectrophotometer, with PBS serving as a reference blank. Additionally, a negative control was prepared under the same conditions, using only PBS buffer in place of the extract to establish a baseline (Zejli et al., 2024).
The percentage of hemolysis was evaluated using a total hemolysis tube that included distilled water instead of extract under similar conditions. The rate of hemolysis of extract samples was calculated as a percentage (%) of total hemolysis after 60 minutes of incubation using the following equation:

$$\textbf{Hemolysis rate (\%)} = [(A - A_0)/(A_t - A_0)] \times 100$$

A, $\mathbf{A_0}$, and $\mathbf{A_t}$ represent the absorbance of the sample, the absorbance of the negative control, and the positive control (total hemolysis), respectively.

2.5. In silico evaluation of antidiabetic activity

2.5.1. Protein Preparation

The structures of α-amylase (PDB: 1B2Y) and α-glucosidase (PDB: 5NN8) were sourced from the Protein Data Bank (PDB) using the RCSB portal (http://www.rcsb.org/). Once transferred into the Maestro 11.5 interface, each protein structure underwent thorough preparation to ensure computational readiness. This included defining bond orders, incorporating hydrogen atoms, establishing disulfide bonds where applicable, and filling any gaps inside chains or rings with Prime tools. To avoid interference, termini were capped, and any water molecules located beyond 5 Å from hetero groups were excluded. Additional refinements involved removing any remaining crystallization water and extraneous heteroatoms. Using the PROPKA tool, protonation states for both residues and ligands were adjusted to approximate experimental pH conditions (Kumar et al., 2022; Tourabi et al., 2023).

2.5.2. Ligand Preparation

Structures of catechin and cyanidin 3-O-beta-D sambubioside were obtained from the PubChem database, identified by PubChem CID: 9064 and 6602304, respectively. Mongophenoside B was drawn manually in ChemDraw, downloaded, and imported into the Maestro interface in .SDF format. The structures of these compounds were then optimized using the LigPrep tool. To ensure the ligands were prepared at a pH of 7.0 ± 2.0, the Epik module was employed to maintain chirality, perform desalting, and generate required tautomers. The 2D structures were converted into 3D models, followed by geometric minimization using the OPLS3e force field to

achieve the most stable conformation with necessary structural adjustments. The resulting optimized ligands were subsequently utilized in docking simulations (Amrati et al., 2023; Chebaibi et al., 2024).

2.5.3. Binding site identification and molecular docking

The Glide module's receptor lattice generation tool is employed to map potential docking sites for ligands within the protein structure. The grid must cover the region around the co-crystallized ligand, which is intentionally left out of the minimized protein structure to avoid any unintended effects on the docking process. The acceptor lattice is constructed using default settings, which include a van der Waals radius scaling factor of 1 Å and a partial charge cutoff of 0.25 Å. Flexible docking is then performed using the standard precision (SP) mode of the Glide module, enabling ligands to be positioned within the receptor lattice. Ligands are ranked based on their interactions with the target protein and their docking scores, with those demonstrating the highest affinity for the active site of the receptor being prioritized (El Abdali et al., 2023).

3. Results and Discussion

3.1. HPLC-MS-MS Analysis

The extraction yield obtained for polyphenols was 7%. The UHPLC-MS/MS analysis of the SAPE identified three major compounds: mongophenoside B, cyanidin 3-O-beta-D sambubioside, and catechin. Mongophenoside B is the most abundant compound. Cyanidin 3-O-beta-D sambubioside, an anthocyanin, was identified. Finally, catechin, a flavonoid known for its antioxidant properties, was also detected (Table 1, Figure 1).

Table 1*:* Chromatographic analysis of polyphenolic compounds detected *A. sativum* by HPLC-MS-MS.

No	RT	m/z (M-H)-	Proposed compounds	Concentration (µg/mg)
1	1.66	517.89	Mongophenoside B	59.19
2	24.24	580.22	Cyanidin 3-O-beta-D- sambubioside	24.782
5	29.34	289.9	catechin	16.017

The identification of these compounds is in agreement with scientific research on polyphenols in *A. sativum* extracts. The presence of catechin was confirmed by (Yang et al., 2020). In addition, *A. sativum* is common for the presence of anthocyanins (Alam et al., 2023; Fossen and Andersen, 1997). In addition, *A. sativum* is common for the presence of anthocyanins. Our study showed the presence of cyanidin 3-O-beta-D-sambubioside, while another study showed the presence of several anthocyanins, such as cyanidin-3-(6'-malonyl)- from Australian garlic cultivars (Phan et al., 2019).

The identification of mongophenoside B has never been reported in *A. sativum*. The only study that reported this phytocompound in plants was by (Dong et al., 2020), where it was identified in another species of allium, *Allium mongolicum* Regel.

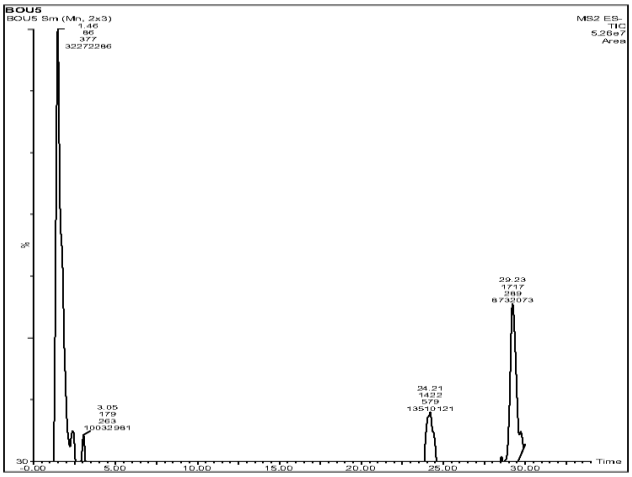

Figure 1: HPLC-MS-MS chromatogram of ASPE

3.2. Antidiabetic activity

The antidiabetic results indicate that *ASPE* demonstrates notable inhibitory activity against the enzymes α-amylase and α-glucosidase, compared to acarbose, a standard inhibitor.

In the inhibition of α-amylase, the results in figure 2 and table 2 showed that all tested doses significantly inhibited the enzymatic activity of α-amylase. The 1 mg/mL concentration had the most active effect, exhibiting inhibitory activities of 82.31% for *A. sativum*. This suggests that ASPE is an effective inhibitor of α-amylase, with an IC_{50} of 0.047 ± 0.002 mg/mL, which is stronger than even that of acarbose (0.071 ± 0.008 mg/mL), indicating better efficacy (Figure 2A, Table 2). Furthermore, in α-glucosidase inhibition, *A. sativum* showed an inhibitory concentration (0.055 ± 0.004 mg/ml) comparable to that of the positive control (0.059 ± 0.009) (Figure 2B, Table 2).

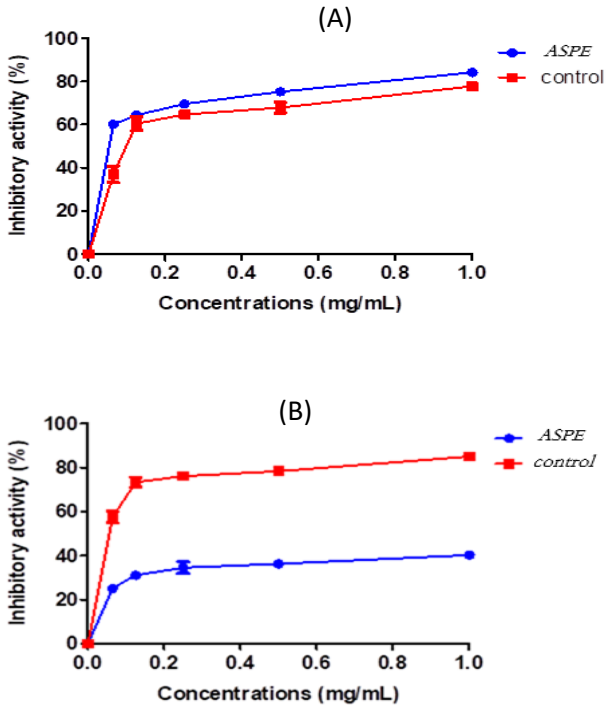

Figure 2: *A. sativum* polyphenols *in vitro* effect compared to the control on α-amylase enzyme inhibition (**A**) and α-glucosidase enzyme inhibition (**B**).

Table 2: *A. sativum* polyphenols inhibition of pancreatic α-amylase and intestinal α-glycosidase

	IC_{50} mg/mL	
	α-amylase	α-glucosidase
A. sativum polyphenols extract	0,047 ± 0,002	0,055 ± 0,004
Acarbose (control)	0,071 ± 0,008	0,059 ± 0,009

The potential of *A. sativum* as an antidiabetic agent has been extensively studied, and its efficacy as a natural approach to diabetes management is widely acknowledged. Both *in vivo* and *in vitro* research have demonstrated its ability to regulate blood glucose levels, improve insulin sensitivity, and alleviate diabetes-related complications, making it a promising addition to diabetes care strategies (Ashraf et al., 2011; Eidi et al., 2006; Younas and Hussain, 2014).

The sulfur compounds found in *A. sativum*, such as allicin, cysteine sulfoxide, and related substances, contribute significantly to lowering blood glucose levels. This is achieved through the inhibition of insulin activation in the liver, stimulation of insulin secretion from pancreatic beta cells, release of insulin from its bound forms, and improved cellular responsiveness to insulin (Faroughi et al., 2018; Zhai et al., 2018). Beyond these sulfur compounds, the essential oils, saponins, and steroids in *A. sativum* act as dose-dependent inhibitors of both α-glucosidase and aldose reductase (Wu et al., 2015).

Polyphenols and flavonoids exhibit powerful antidiabetic effects by engaging in multiple mechanisms. These compounds help preserve pancreatic β-cell function, reduce cell death, encourage β-cell regeneration, alleviate oxidative stress, enhance insulin signaling, and stimulate insulin secretion from the pancreas. Additionally, they act through insulin-independent mechanisms, such as inhibiting glucose absorption, blocking digestive enzymes, modifying gut microbiota, regulating inflammatory responses, and preventing the formation of advanced glycation end products (Marella, 2017; Sun et al., 2020; Umeno et al., 2016). Hydrocinnamic acids, including p-coumaric, ferulic, caffeic, and rosmarinic acids, alongside other polyphenolic compounds, are key contributors to diabetes management. These acids improve insulin resistance and glucose intolerance in streptozotocin (STZ)-induced diabetic rats, lower blood glucose, raise insulin levels across various diabetic models, improve glucose tolerance, reduce carbohydrate absorption in the intestines, regulate enzymes involved in glucose metabolism, and enhance insulin sensitivity (Jung et al., 2007; Kasetti et al., 2012).

The antidiabetic properties of catechins and anthocyanins/anthocyanidins emphasize their complementary roles in managing diabetes and associated metabolic complications. Catechins, derived from some vegetal sources, demonstrate their effectiveness by enhancing insulin sensitivity and activating AMP-activated protein kinase (AMPK), a key regulator of cellular energy balance. Through AMPK activation, catechins promote glucose uptake and reduce blood glucose levels (Park et al., 2014; Thielecke and Boschmann, 2009). Furthermore, their role in increasing the fecal excretion of bile acids and cholesterol contributes to improved lipid profiles, which is crucial for individuals with type 2 diabetes who often struggle with dyslipidemia (Thielecke and Boschmann, 2009). Beyond glucose regulation, catechins impact a wide array of biological functions. They inhibit pancreatic enzymes like α-glucosidase, α-amylase, and maltase, slowing carbohydrate breakdown and reducing postprandial blood sugar spikes (Li et al., 2018; Matsui et al., 2007). Catechins also interact with Na+-dependent glucose transporters and suppress reactive oxygen species (ROS) production, helping to mitigate oxidative stress, a contributor to diabetic complications (Kobayashi et al., 2000; Solinas and Becattini, 2017). By reducing white fat depots and maintaining a healthy lipid profile, catechins support metabolic health and may reduce the risk of cardiovascular issues commonly linked to diabetes.

Present in a variety of colorful fruits and vegetables, anthocyanins and anthocyanidins enhance the effects of catechins with their strong antioxidant and anti-inflammatory properties. These compounds play a key role in managing blood glucose levels and reducing oxidative stress, both of which are crucial for effective diabetes control (Zhu et al., 2012). Additionally, anthocyanins contribute to better cardiovascular health by lowering cholesterol, triglycerides, and LDL cholesterol, factors often compromised in individuals with diabetes (Shi et al., 2017). By reducing oxidative stress and inflammation, they protect tissues from damage, potentially improving insulin sensitivity and the function of pancreatic β-cells.

Our study indicates that the antidiabetic effects of *ASPE*, demonstrated by its potent inhibition of α-amylase and α-glucosidase, may be attributed to the presence of bioactive compounds such as catechins, anthocyanidins, and potentially Mongophenoside B. Catechins and anthocyanidins, like cyanidin 3-O-beta-D sambubioside, are known for enhancing insulin sensitivity, activating AMPK, and inhibiting digestive enzymes, which aligns with our observed results. Additionally, mongophenoside B, a compound not previously studied for its antidiabetic properties, may contribute uniquely to these effects, offering a new potential mechanism for blood glucose regulation. Thus, the synergistic action of these compounds underpins the superior enzyme inhibition of *A. sativum*, highlighting its promise as a natural antidiabetic agent and paving the way for further exploration of mongophenoside B in diabetes management.

3.3. Antihemolytic activity

The hemolytic test was performed on *ASPE* to assess its biocompatibility or potential safety for blood cells. This *in vitro* cytotoxic test examines the extract's compounds for their ability to damage erythrocyte membranes, releasing hemoglobin during cell lysis. The evaluation was conducted on rat blood cells exposed to different concentrations of *ASPE*, ranging from 5 to 100 mg/mL. The results obtained (Figure 3) show a dose-dependent increase in absorbance levels observed at intervals of 0, 15, 30, and 60 minutes compared with the negative control (PBS). Phosphate-buffered saline (PBS) serves as a negative control in hemolysis assays due to its isotonic nature, which helps maintain the integrity of red blood cells (RBCs) during the experiment. Its primary role is to provide a baseline for comparison with other test conditions, ensuring that

observed hemolysis results are due to the test compound and not experimental artifacts.

This result correlates directly with an escalation in the hemolysis rate, indicating a proportional increase in hemolytic activity corresponding to higher extract concentrations.

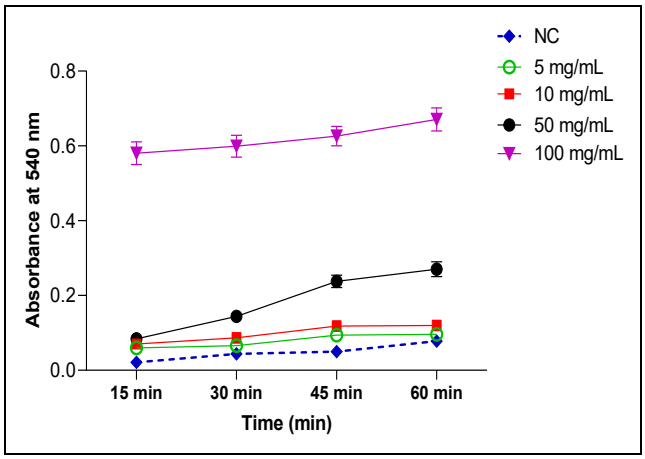

Figure 3: Evolution of absorbance at 540 nm of various concentrations of *ASPE* and negative control (NC) in the hemolysis test during 60 min**.**

The hemolysis rate (%) produced by *ASPE* was determined relative to the total hemolysis observed. The results indicate that concentrations ranging from 5 to 100 mg/mL led to hemolysis percentages ranging from 1.53 ± 0.25% to 21.24 ± 1.54% (Figure 4). Statistical analysis revealed a significant difference (p<0.05) in the different extract concentrations compared to the positive control. Consequently, the findings highlight the relative toxicity of *ASPE* to erythrocytes at higher concentrations and suggest its biocompatibility with normal tissues only at lower concentrations.

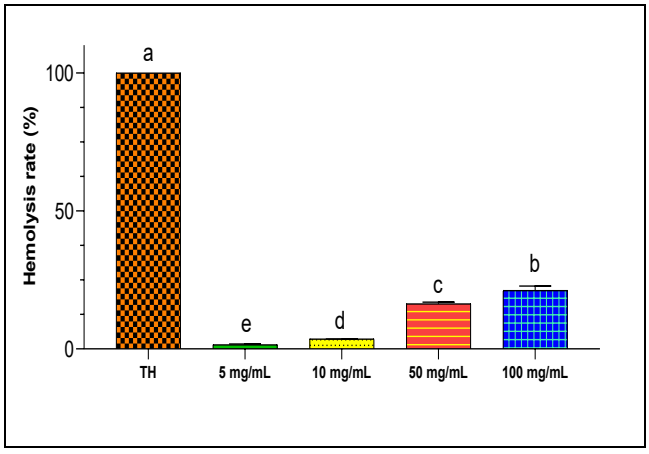

Figure 4: Hemolytic rate (%) of various concentrations of *ASPE* after 60 min of incubation compared to total hemolysis (TH). Bars with different letters represent statistically different values (p<0.05).

Erythrocytes, the most abundant cells in the human body, possess distinctive biological and morphological characteristics, including their ability to replicate. Hemoglobin and polyunsaturated fatty acids interact primarily with erythrocytes, given their role in redox-active oxygen transport. Consequently, oxidation of erythrocyte membrane lipids and proteins leads to hemolysis. This process is influenced by a variety of factors, including deficiencies in the coordination of erythrocyte antioxidants, radiation exposure, high levels of transition metals, oxidizing drugs, and hemoglobinopathies (Hamidi and Tajerzadeh, 2003; Karim et al., 2020).

The hemolysis test is a key method for assessing cytotoxicity, as it examines the degradation of red blood cells exposed to varying concentrations of a natural extract. This technique plays a vital role in evaluating the toxicity of extracts, contributing significantly to both phytotherapy and pharmaceutical formulation. Typically, hemolysis can occur through different mechanisms, such as the dissolution of the cell membrane, increased membrane permeability, or complete cell lysis (Sayes et al., 2007).

A recent study revealed that a concentration of 30 mg/mL of aqueous extract of *A. sativum* was found to be toxic against red blood cells and resulted in a 50% hemolytic rate (Andleeb et al., 2020). In contrast, another previous study proved that red blood cells were strongly protected (97.87%) by the *ASPE* at 1 mg/mL, thus demonstrating the protective effect of low concentrations of garlic extracts on red blood cells, in accordance with our results (Azantsa et al., 2019). The hemolytic effect of high concentrations of *A. sativum* can be attributed to the presence of specific metabolites or reagents with a hemolytic impact, such as saponins. These substances interact with cholesterol in the membranes of blood cells, thereby increasing their permeability and ultimately causing hemolysis (Andleeb et al., 2020). In addition, this hemolytic action of garlic can be related to its sulfur-containing compounds, such as allicin. These compounds can penetrate and disrupt red blood cell membranes, leading to hemolysis. This process involves oxidative stress and the modification of thiol groups in membrane proteins (El-Saber Batiha et al., 2020). Generally, it has been observed that the hemolytic effect of any compound or molecule depends on several factors, such as temperature, incubation time, the presence of side chains such as saponins, and the specific composition of the membrane (Andleeb et al., 2020; Urbańska et al., 2009). On the other hand, the protective effect of the *ASPE*, especially at low concentrations, can be attributed mainly to the presence of various phenolic metabolites in the plant extract. Previous studies suggest that the presence of phenolic compounds plays a crucial role in protecting the erythrocyte membrane from oxidation, thus conferring resistance to hemolytic activity (Ali et al., 2018).

3.4. In silico antidiabetic evaluation

In our study, we evaluated the antidiabetic potential of three major polyphenolic compounds identified in *A. sativum*: mongophenoside B, cyanidin 3-O-beta-D-sambubioside, and catechin. The inhibitory effects of these compounds were assessed through molecular docking against α-amylase and α-glucosidase enzymes, central targets in managing hyperglycemia (Taslimi and Gulçin, 2017). Mongophenoside B, an unstudied compound in the context of diabetes, showed the highest docking affinity for α-amylase with a glide gscore of -6.546 kcal/mol and also demonstrated the most potent inhibition for α-glucosidase with a gscore of -7.584 kcal/mol. Cyanidin 3-O-beta-D-sambubioside and catechin also exhibited potent inhibition of α-amylase and α-glucosidase, with gscores of -5.931 and -4.908 kcal/mol for α-amylase, and -5.975 and -6.283 kcal/mol for α-glucosidase, respectively (Table 3).

These *in silico* findings align well with our *in vitro* results, where *ASPE* extract demonstrated notable inhibition of both enzymes, outperforming acarbose in terms of IC_{50} values. The results suggest that the potent inhibitory activity observed in the extract may be attributed to these three polyphenols, particularly mongophenoside B, which warrants further investigation, given its unique efficacy. Additionally, the activities of cyanidin 3-O-beta-D-sambubioside and catechin resonate with existing literature on the antidiabetic potential of anthocyanins and catechins, as they are recognized for their insulin-sensitizing and digestive enzyme-inhibiting properties. These findings underscore the importance of polyphenolic components in *A. sativum* and highlight mongophenoside B as a promising novel compound for future diabetes research and therapeutic applications.

Table 3: Molecular docking results of compounds in active receptor sites.

	α-amylase (PDB: 5NN8)			**α-glucosidase (PDB: 1B2Y)**		
	Glide gscore (Kcal/mol)	Glide emodel (Kcal/mol)	Glide energy (Kcal/mol)	Glide gscore (Kcal/mol)	Glide emodel (Kcal/mol)	Glide energy (Kcal/mol)
Catechin	-4.908	-49.562	-35.815	-6.283	-61.12	-42.611
Cyanidin 3-O-beta-D-sambubioside	-5.931	-77.939	-57.028	-5.975	-80.838	-60.267
Mongophenoside B	-6.546	-81.517	-61.644	-7.584	-84.286	-58.177

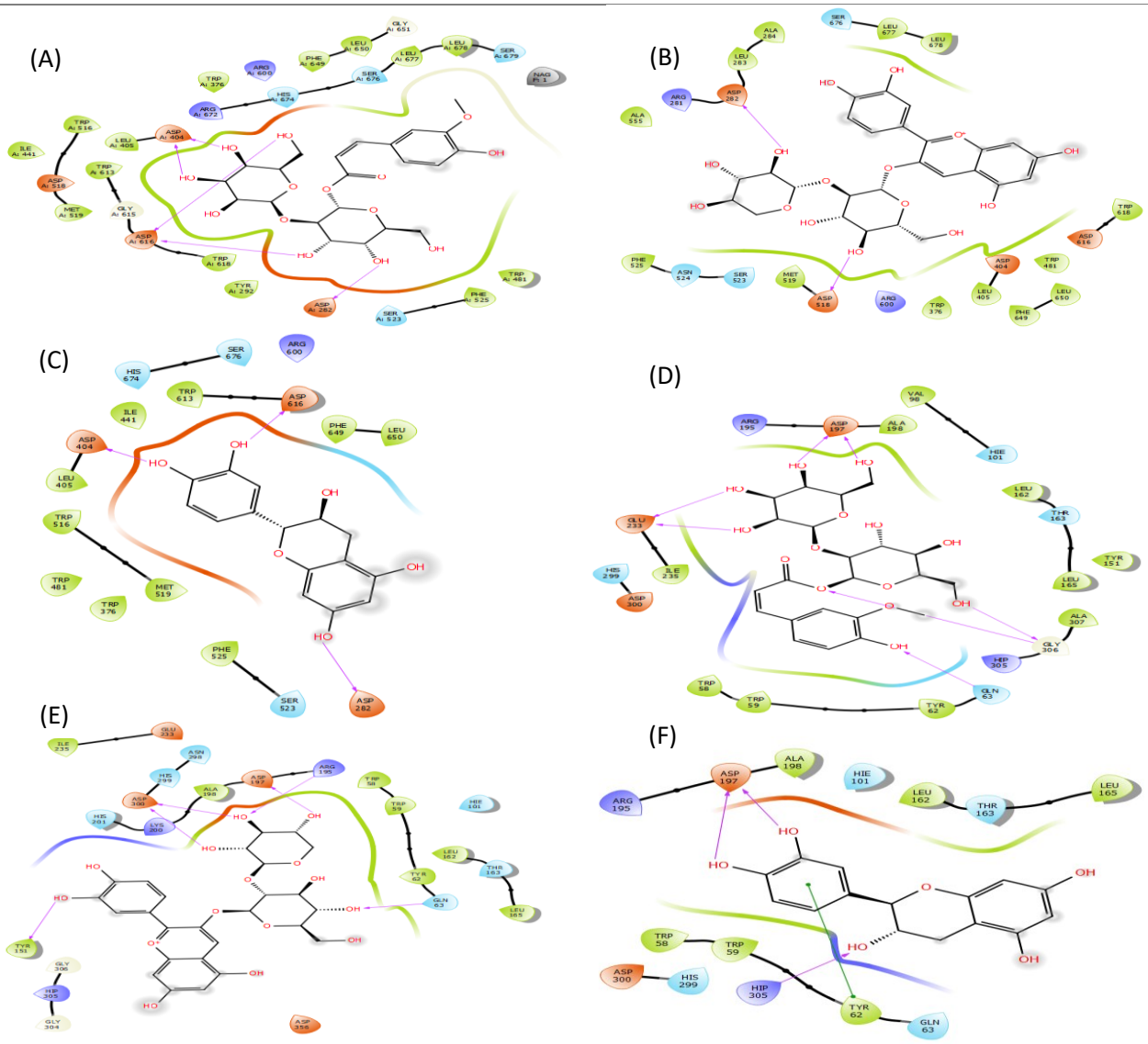

Figure 5: The 2D viewer of ligands interactions with the active sites. **A and D:** Mongophenoside B interactions with α-amylase and α-glucosidase active sites, **B and E:** Cyanidin 3-O-beta-D-sambubioside interactions with α-amylase and α-glucosidase active sites, **C and F:** catechin interactions with α-amylase and α-glucosidase active sites.

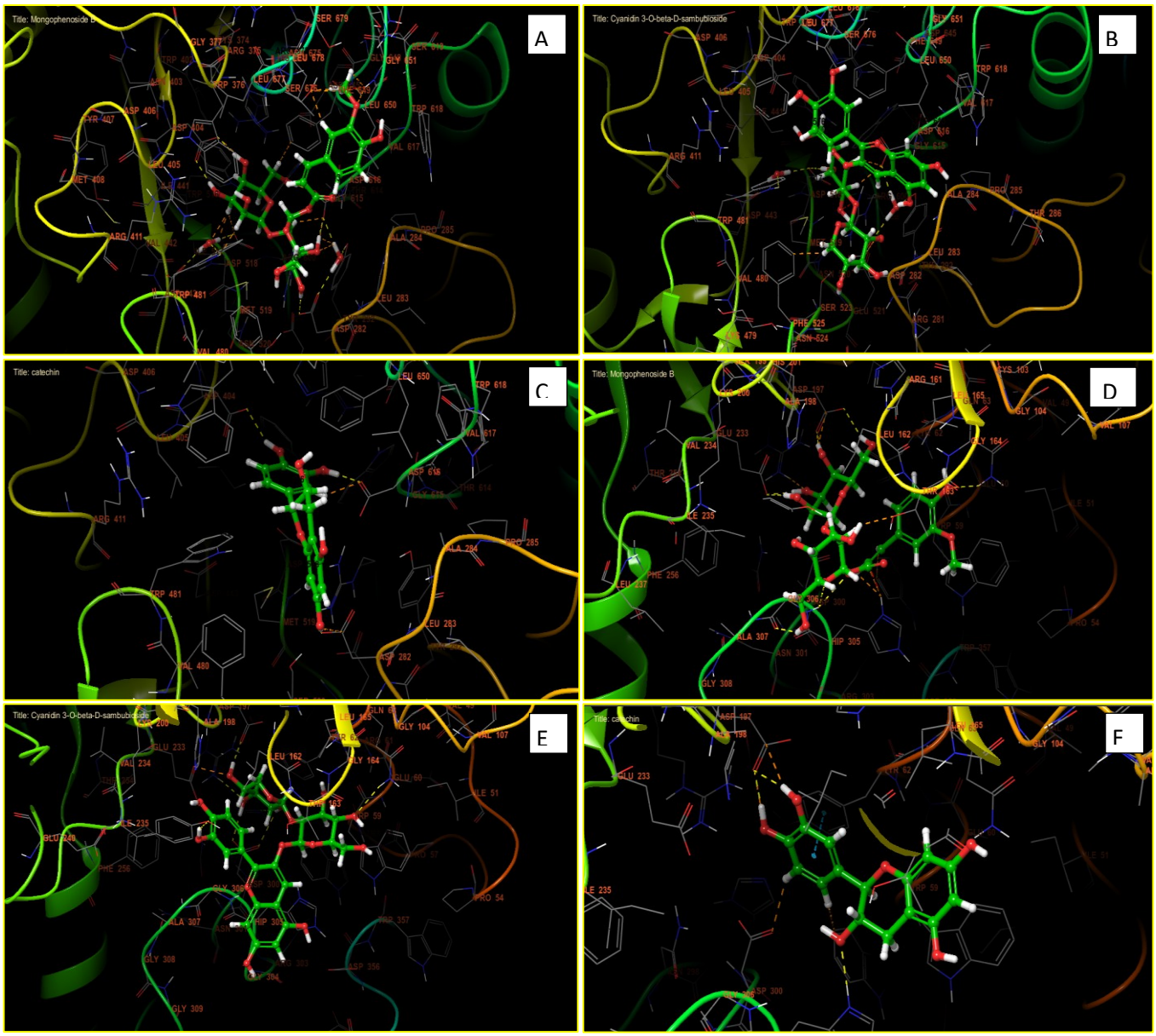

Figure 6: The 3D viewer of ligands interactions with the active sites. **A and D:** Mongophenoside B interactions with α-amylase and α-glucosidase active sites, **B and E:** Cyanidin 3-O-beta-D-sambubioside interactions with α-amylase and α-glucosidase active sites, **C and F:** catechin interactions with α-amylase and α-glucosidase active sites.

The molecular docking results provide insights into the specific interactions of mongophenoside B, cyanidin 3-O-beta-D-sambubioside, and catechin with the active sites of α-amylase and α-glucosidase, further confirming their potential roles in inhibiting these enzymes. Mongophenoside B, with the strongest binding affinity observed among the three compounds, formed five hydrogen bonds in the active site of α-amylase, specifically with residues ASP A: 404, ASP A: 616, and ASP A: 282 (Figure 5A and 6A). This extensive hydrogen bonding suggests strong stabilization within the enzyme's active site, which may contribute to the observed high binding affinity. Similarly, Mongophenoside B formed seven hydrogen bonds within the active site of α-glucosidase, interacting with key residues ASP 197, GLU 233, GLN 63, and GLY 306 (Figure 5D and 6D), indicating a robust interaction that aligns with its high docking score.

For cyanidin 3-O-beta-D-sambubioside, interactions in the α-amylase active site were established through two hydrogen bonds with residues ASP 282 and ASP 518 (Figure 5B and 6B). Its interactions were more complex in α-glucosidase, where it formed six hydrogen bonds with ASP 300, ASP 197, ARG 195, TYR 151, and GLN 63 (Figure 5E and 6E). The presence of multiple hydrogen bonds further supports the potential of cyanidin 3-O-beta-D-sambubioside to inhibit these enzymes.

Catechin demonstrated effective interactions with both enzymes as well. In α-amylase, it established three hydrogen bonds with residues ASP 616, ASP 404, and ASP 282, suggesting a stable interaction within the active site (Figure 5C and 6C). In α-glucosidase, catechin formed three hydrogen bonds with ASP 197 and HIP 305 and additionally engaged in a Pi-Pi stacking interaction with TYR 62 (Figure 5F and 6F). This combination of hydrogen bonding and Pi-Pi stacking suggests that catechin can stabilize and attract interactions within the enzyme's active site, contributing to its moderate inhibitory activity. The molecular docking results provide insights into the specific interactions of mongophenoside B, cyanidin 3-O-beta-D-sambubioside, and catechin with the active sites of α-

amylase and α-glucosidase, further confirming their potential roles in inhibiting these enzymes. Mongophenoside B, with the strongest binding affinity observed among the three compounds, formed five hydrogen bonds in the active site of α-amylase, specifically with residues ASP A: 404, ASP A: 616, and ASP A: 282 (Figure 5A and 6A). This extensive hydrogen bonding suggests strong stabilization within the enzyme's active site, which may contribute to the observed high binding affinity. Similarly, Mongophenoside B formed seven hydrogen bonds within the active site of α-glucosidase, interacting with key residues ASP 197, GLU 233, GLN 63, and GLY 306 (Figure 5D and 6D), indicating a robust interaction that aligns with its high docking score.

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These detailed molecular interactions support the inhibitory activities observed *in silico* and *in vitro* and help elucidate the mechanisms by which these compounds inhibit enzymes. The ability of Mongophenoside B to form multiple hydrogen bonds in both enzymes highlights its promise as a potent natural inhibitor with potential antidiabetic properties. Cyanidin 3-O-beta-D-sambubioside and catechin also demonstrate complementary interactions, underscoring the role of polyphenolic compounds in enzyme inhibition and supporting the observed *in vitro* effects of *ASPE*. The distinct interactions of these compounds with key catalytic residues in α-amylase and α-glucosidase suggest a multi-targeted approach to enzyme inhibition, reinforcing the therapeutic potential of *A. sativum* for diabetes management and further validating Mongophenoside B's novel bioactivity in this context.

4. Conclusion

This study highlights the significant antidiabetic potential of *A. sativum* polyphenolic extract, demonstrated through its potent inhibition of α-amylase and α-glucosidase enzymes, which are key to managing blood glucose levels in type 2 diabetes. The UHPLC-MS/MS analysis revealed the presence of bioactive compounds such as mongophenoside B, catechin, and cyanidin 3-O-beta-D sambubioside, with Mongophenoside B identified for the first time in *A. sativum*. Each of these compounds are known for beneficial effects related to diabetes management, and their combined action may account for the observed enzyme inhibition and their antioxidant and anti-inflammatory properties. The cytotoxicity assessment showed a dose-dependent hemolytic effect, indicating that the extract is biocompatible at lower concentrations but potentially cytotoxic at higher doses. This underscores the importance of concentration in therapeutic applications and suggests that low concentrations of *ASPE* could be safely utilized for antidiabetic purposes without significant harm to blood cells. Our *in silico* docking analysis highlighted Mongophenoside B, Cyanidin 3-O-beta-D-sambubioside, and catechin as potent inhibitors of both α-amylase and α-glucosidase, with Mongophenoside B demonstrating the strongest binding affinity among them. Future studies should investigate the pharmacokinetics and bioavailability of Mongophenoside B, Cyanidin 3-O-beta-D-sambubioside, and catechin in animal models to understand its absorption, distribution, metabolism, and excretion (ADME) profile. Such studies would provide critical data on its systemic exposure, half-life, and potential metabolic transformations. Overall, the study supports the therapeutic potential of *A. sativum* as a natural antidiabetic agent and provides new insights into the biochemical makeup of its polyphenolic extract, particularly with the discovery of Mongophenoside B. These findings not only confirm the traditional use of *A. sativum* in diabetes management but also open new avenues for exploring Mongophenoside B's role in glycemic control, contributing valuable information for future research and development of plant-based antidiabetic therapies.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data availability statement

Data will be available upon request from the corresponding author.

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