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Evaluating the *in vitro* anti-diabetic activity of *Bryonia dioica* root extracts supported by molecular docking analysis

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ABSTRACT

Objective To evaluate the *in vitro* anti-diabetic effects of *Bryonia dioica* roots extracts, including water-acetone extracts and their ethyl acetate and butanol fractions, and chloroform-methanol extracts.

Methods The total phenolic, flavonoid, flavonol, and saponin contents in the *Bryonia dioica* root extracts (chloroform-methanol extracts, water-acetone extracts and their ethyl acetate and butanol fractions) were determined using colorimetric methods with Folin-Ciocalteu, aluminum trichloride, and vanillin reagents, respectively. The *in vitro* anti-diabetic activity was evaluated by measuring the half-maximal inhibitory concentration (IC_{50}) values of these root extracts against α-amylase and α-glucosidase activities, evaluating their effects on α-amylase kinetics, quantifying the inhibition of bovine serum albumin (BSA) glycation using fluorometry to assess advanced glycation end products (AGE) production, and determining glucose uptake by isolated rat hemidiaphragm. Additionally, molecular docking analysis was conducted to investigate the binding affinity and interaction types between *Bryonia dioica* ligands (cucurbitacin B, bryogénin, vitexin, and isovitexin) and target enzymes, and a phytochemical-targets interaction network was constructed.

Results For α-amylase inhibition, ethyl acetate fraction demonstrated the most potent activity (IC_{50} = 145.95 μg/mL), followed by chloroform-methanol extract (IC_{50} = 300.86 μg/mL). Water-acetone root extracts and their ethyl acetate and butanol fractions inhibited the α-glucosidase activity with IC_{50} values ranging from 562.88 to 583.90 μg/mL. Both ethyl

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acetate and butanol fractions strongly inhibited non-enzymatic BSA glycation (IC_{50} = 318.26 and 323.12 μg/mL, respectively). The incubation of isolated rat hemidiaphragms with the ethyl acetate fraction (5 mg/mL) significantly increased glucose uptake (35.16%; P < 0.000 1), exceeding the effects of insulin (29.27%), chloroform-methanol extract (24.07%), and catechin (15.27%). Molecular docking revealed that cucurbitacin B exhibited the strongest docking scores against α-amylase (– 16.4 kcal/mol), and α-glucosidase (– 14.2 kcal/mol). Compared with other ligands, isovitexin formed the maximum number of hydrogen bonds with the α-amylase active site residues (Asp300, Asp197, and Glu233), α-glucosidase residues (Ser13, Arg44, Met86, Gly10, Asp39, and Tyr131) and other residues (Arg195, Trp59, His299, and Tyr62). Network analysis identified 36 overlapping targets between Bryonia dioica phytochemicals and type 2 diabetes mellitus-associated genes, with cucurbitacins and polyphenols interacting with α-amylase, α-glucosidase, and Glut4 translocation pathway targets.

Conclusion *Bryonia dioica* root extracts demonstrated promising *in vitro* anti-diabetic activity through multiple mechanisms, including the inhibitory effect on digestive enzymes, protein antiglycation potential, and enhancement of glucose uptake, suggesting their potential as a source for anti-diabetic drugs development.

1 Introduction

Diabetes mellitus, the most common endocrine disorder globally, is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. As one of the most severe epidemics affecting both developed and developing countries, this disease exhibits alarming prevalence and severity. The International Diabetes Federation (IDF) reported that diabetes prevalence is escalating rapidly: in 2024, it affected 589 million adults aged 20 – 79 years, and the figure was projected to reach 853 million individuals by 2050 [2]. Chronic hyperglycemia, along with other risk factors, drives diabetic complications through oxidative stress pathways. Specifically, prolonged hyperglycemia induces glucose oxidation, non-enzymatic glycosylation of proteins and lipids, and subsequent free radical generation, thereby amplifying diabetes progression and its complications [3]. Pharmacological treatment of diabetes, mainly type 2 diabetes mellitus, primarily targets hyperglycemia through mechanisms, including stimulation of insulin secretion, insulin sensitivity, and the inhibition of intestinal glucose digestion and absorption [4]. However, synthetic antidiabetic agents not only impose significant economic burdens on patients in developing countries but are also linked to adverse gastrointestinal effects, including diarrhea, flatulence, abdominal spasms, nausea, lactic acidosis, and neurological symptoms like dizziness and hypoglycemia [5]. These limitations have spurred extensive research efforts to discover novel hypoglycemic agents, with natural products emerging as a predominant focus [1, 6].

In traditional medicine, several studies document the global use of medicinal plants in diabetes management. Natural resources exhibit well-documented anti-diabetic properties, including metformin, a frontline commercial anti-diabetic agent that was first discovered from *Galega officinalis*. Inhibition of α-amylase and α-glucosidase digestive enzymes is a critical therapeutic strategy for type 2 diabetes mellitus. Notably, various natural compounds including terpenoids, alkaloids, polysaccharides, phenolic derivatives, and flavonoids demonstrate enzyme-inhibitory activities, with flavonoids exhibiting superior inhibitory efficacy [7]. Algeria, renowned for its rich diversity of medicinal plants in traditional medicine, hosts several species recognized for the powerful anti-diabetic applications [8, 9]. *Bryonia dioica* (white bryony) occupies a prominent position among these ethnomedicinal resources, particularly its roots (the most utilized plant part), which are administered orally in minute doses or applied externally to manage infections (cough, bronchitis, influenza, and pneumonia), inflammatory conditions (rheumatism), ulcerative pathologies, hypertension, diabetes mellitus, and neoplastic disorders [10, 11].

Bryonia dioica is a herbaceous, perennial, and climbing plant in the Cucurbitaceae family, native to Western Asia, temperate Europe, and North Africa [12]. Phytochemical studies have been conducted on *Bryonia dioica*, and several components have been identified in different parts of this plant (stem, leaf, and fruit tissues), including bryonoside, brydiosides, saponarin, cucurbitacin, and kaempferol 3,7-di-O-rhamnoside [13]. Literature reviews revealed correlations between its phytochemical composition and diverse biological properties, notably antioxidant [14], anti-inflammatory [10], antimicrobial [15], anticancer [16], and hepatoprotective effects [11]. Few scientific studies have focused on the anti-diabetic properties of bryony. The sole *in vivo* investigation of anti-diabetic effects of *Bryonia dioica* demonstrated that the root aqueous extract (30 mg/kg body weight) significantly reduces hyperglycemia in streptozotocin-induced neonatal diabetic rats over the short term (3 h follow-up) and medium-term evaluations (3 weeks follow-up) [17].

This study aimed to investigate the *in vitro* anti-diabetic effects of *Bryonia dioica* extracts by measuring their

effects on the activity of the digestive enzymes α-amylase and α-glucosidase, on protein glycation, and on glucose uptake. A molecular dynamics simulation was also conducted to determine the structural stability of certain *Bryonia dioica* phytochemicals in complex with α-amylase and α-glucosidase. Additionally, an interaction network was established between selected *Bryonia dioica* compounds and type 2 diabetes mellitus-associated targets to systematically visualize molecular mechanisms.

2 Materials and methods

2.1 Plant materials and extraction

Botanical authentication of *Bryonia dioica* was carried out at the laboratory of Ecology and Natural Ecosystems Management, Tlemcen University (Algeria). Root specimens were collected from Tlemcen area (northern Algeria), followed by cleaning, air-drying in darkness, and homogenization to obtain root powder. A total of 50 g of the ground roots were decocted in acetone-water mixture (80 : 20, v/v) for 1 h to prepare water-acetone crude extract. Another extraction was carried out using acetone-water, and the aqueous phase was split with ethyl acetate (1 : 1, v/v) to recover the ethyl acetate fraction, then followed by butanol to recover the butanol fraction. Moreover, 100 g of ground roots were macerated for 48 h in a chloroform-methanol mixture (50 : 50, v/v) at room temperature, and then the filtered extract was evaporated to dryness to obtain the chloroform-methanol extract.

2.2 Quantitative determination of bioactive compounds in *Bryonia dioica* root extracts

Total polyphenol and flavonoid contents were determined in the set extract (water-acetone, ethyl acetate, butanol, and chloroform-methanol) using standardized colorimetric methods [18, 19]. For the determination of polyphenol components, a mixture was prepared by combining 2 000 μL of 2% sodium carbonate (Na_2CO_3) solution with 100 μL of each prepared extract (1 mg/mL). This mixture underwent 5-min incubation at room temperature followed by addition of 100 μL of Folin-Ciocalteu reagent (0.2 mol/L). After 30-min light-excluded incubation, absorbance at 700 nm was measured against a reagent blank. Results were reported as micrograms of gallic acid equivalents per milligram of dry extract (μg GAE/mg DE). For the quantification of flavonoid components, 500 μL extract (1 mg/mL) was mixed with 200 μL distilled water and 150 μL 15% sodium nitrite ($NaNO_2$). Following 6-min incubation at room temperature, 150 μL 10% solution of aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) was introduced to initiate oxidation. After subsequent 6 min incubation, 200 μL of 4% sodium hydroxide and 200 μL of distilled water were added. The mixture was incubated in darkness for 15 min before measuring the absorbance at 510 nm against a blank. Catechin served as the standard, with results reported in micrograms of catechin equivalents per milligram of dry extract (μg CE/mg DE).

However, total flavonol content was measured in all extracts using a modified protocol [20]: 2 000 μL of each extract was mixed with 2 000 μL of aluminum chloride (10%) and 3 000 μL of sodium acetate (5%). The mixture was incubated for 150 min at 20 °C prior to absorbance measurement at 440 nm. Quercetin served as the reference standard, with results expressed as micrograms of quercetin equivalents per milligram of dry extract (μg QE/mg DE). In parallel, the determination of total saponins in the tested extracts was performed according to colorimetric procedures [21, 22]. Briefly, 50 μL of each extract was mixed with 500 μL of 8% vanillin solution and 5 000 μL of 72% sulfuric acid. The reaction mixture was agitated and incubated in a water bath (60 °C) for 10 min, followed by 15 min cooling in an ice bath. Absorbance at 544 nm was recorded, and oleanolic acid was used as the reference standard (micrograms oleanolic acid per milligram of equivalents, μg EOA/mg DE).

2.3 Inhibition assay for α-amylase activity

The α-amylase inhibitory activity was evaluated in previously prepared extracts with protocol modifications [18, 23]. Briefly, 200 μL of porcine pancreatic α-amylase solution (3.9 U/mL in 20 mmol/L sodium phosphate buffer with 6.7 mmol/L NaCl; pH 6.9) was preincubated at 37 °C for 10 min with 200 μL of each extract [tested at different concentrations (33 – 1 000 μg/mL) to achieve an optimum effect] water acetone, ethyl acetate, butanol, and chloroform-methanol extract. The reaction was initiated by adding 200 μL soluble starch solution (0.5%, w/v), followed by incubation at 37 °C for 15 min. The reaction was subsequently stopped by the addition of 600 μL of 3,5-dinitrosalicylic acid (DNSA) reagent. The reaction mixture was boiled for 15 min in a boiling water bath, then diluted with 1 000 μL of distilled water in an ice bath. The absorbance was measured at 540 nm. Acarbose (7 – 33 μg/mL) was used as a standard inhibitor [18]. The inhibitory activity was measured in percent inhibition and the IC_{50} of extracts was determined by plotting the inhibition percentage against the concentration of the samples.

2.4 Kinetics of inhibition against α-amylase

The inhibition modes of *Bryonia dioica* extracts (ethyl acetate and chloroform-methanol) against α-amylase activity were measured by varying concentrations of starch (1, 5, and 7 mg/mL) as a substrate in the absence or presence of ethyl acetate fraction and chloroform-meth-anol extract at different concentrations (0.100 – 0.333 mg/mL) selected according to IC_{50} values

determined in the α-amylase activity inhibition assay. Likewise, acarbose, the standard inhibitor, was tested at the concentration interval of 0.002 to 0.015 mg/mL. Inhibition type was determined by Lineweaver-Burk plot analysis of the data, using a double reciprocal plot of the substrate concentration and velocity (1/V vs. 1/[S]). K_m and V_{max} values were calculated according to Michaelis-Menten kinetics using the double inverted curve equation: $V_{max} = 1/b$; $K_m = a \times V_{max}$.

2.5 Inhibition assay for α-glucosidase activity

This study involved animal experimentation on seven Wistar rats (170 – 250 g, both sexes) sourced from the animal facility of the University of Tlemcen's Nature and Life Sciences (SNV-STU) faculty, conducted in compliance with the Declaration of Helsinki and approved by the Tlemcen University Ethics Committee [Laboratory Authorization for Animal Experimentation-Biochemistry LAPSAB-759 (Laboratory of Antibiotic, Antifungal, Physical Chemistry, Synthesis and Biological Activity)]. Rats were housed under controlled conditions (12-h light/dark cycle, 25 °C, 50% humidity) with ad libitum access to standard chow and water.

After overnight starvation, animals were sacrificed under intraperitoneal pentobarbital anesthesia injection (30 mg/kg). According to previous protocols [18, 24, 25], α-glucosidase was isolated from the small intestines of the rats rinsed with ice-cold saline, and the intestinal brush border mucosa was carefully scraped and sonicated in 15 volumes (w/v) of phosphate buffer (67 mmol/L, pH 6.8). The homogenate was centrifuged at 6 000 rpm for 20 min at 4 °C and the supernatant was retained as a crude enzyme solution.

The inhibitory effect of *Bryonia dioica* extracts (water-acetone, ethyl acetate, butanol, and chloroform-methanol) on α-glucosidase activity was determined with the following method with slight modifications [18]: 200 μL of a crude enzyme solution of rat intestinal α-glucosidase (53.80 U/mL) was mixed with 200 μL of *Bryonia dioica* extract (235 – 588 μg/mL) or acarbose (92 – 735 μg/mL) solutions, and 1 mL of phosphate buffer (67 mmol/L, pH 6.8). These intervals of concentration have been selected to achieve maximum effect. The medium was incubated at 37 °C for 10 min, and then 300 μL of p-nitrophenyl-α-D-glucopyranoside (p-NPG) substrate solution (10 mmol/L) was added for reaction. The mixture was incubated at 37 °C for 40 min. Then 3 mL of sodium carbonate solution (100 mmol/L) was added to stop the enzymatic reaction. p-Nitrophenol, released from p-NPG, was measured at 400 nm to determine α-glucosidase activity. The inhibitory activity was expressed in percent inhibition, and the IC_{50} of extracts was determined by plotting the inhibition percentage against the concentration of the samples.

2.6 Assay for nonenzymatic BSA glycation

The antiglycation activity of bovine serum albumin (BSA; Sigma Aldrich A7906-10G) was performed using fluorometry to assess advanced glycation end products (AGE) production as previously reported with slight modifications [26, 27]. Under sterile conditions, 400 μL of BSA (10 mg/mL) prepared in phosphate buffer (100 mmol/L; pH 7.4) was mixed with 400 μL of various concentrations of extracts and 400 μL of glucose (22 mmol/L) and fructose (22 mmol/L), then 100 μL of 0.02% sodium azide were added to the medium and the reaction mixture was incubated at 37 °C for 3 weeks. The fluorescence intensity of the produced AGE was measured at an excitation wavelength of 335 nm and an emission wavelength of 440 nm. The effect of *Bryonia dioica* extracts (water-acetone, ethyl acetate, butanol, and chloroform-methanol) (77 – 3 077 μg/mL), aminoguanidine and ascorbic acid (15 – 1 000 μg/mL) as positive controls, was determined by comparing the percentage inhibition with the maximal glycation induced by glucose and fructose. IC_{50} was determined by plotting the inhibition percentage against the concentration of the samples.

2.7 Glucose uptake by isolated rat hemidiaphragm assay

The effect of *Bryonia dioica* extracts (ethyl acetate and chloroform-methanol) on glucose uptake was evaluated using the rat hemidiaphragm methods with previously reported protocol modifications [28, 29]. The Wistar rats (n = 14) of either sex (180 – 250 g), obtained from the animal facility of the SNV-STU faculty at the University of Tlemcen and maintained under the same breeding and ethical conditions outlined in Section 2.5, were fasted overnight and sacrificed under intraperitoneal pentobarbital anesthesia injection (30 mg/kg). The diaphragms were dissected out and rinsed with Tyrode solution (without glucose) until obtaining a clear pink color, then divided into equal halves (hemidiaphragms). In plates of 12 wells, the hemidiaphragms were placed with the samples and incubated at 37 °C for 45 min in an atmosphere of 100% O_2, with shaking at 60 rpm. Two hemidiaphragms from the same animal were not used for the same sample. Plates were divided into nine groups (three hemidiaphragms for each group). Group 1 served as control medium and contained 4 mL of Tyrode solution with 2% glucose. Group 2 and 3 contained 3.5 mL of Tyrode solution with 2% glucose, and 0.5 mL of insulin at 0.2 and 0.4 U/mL, respectively. Group 4 and 5 contained 3.5 mL of Tyrode solution with 2% glucose, and 0.5 mL of *Bryonia dioica* ethyl acetate extract at 2.5 and 5 mg/mL, respectively. Group 6 and 7 contained 3.5 mL of Tyrode solution with 2% glucose, and 0.5 mL of *Bryonia dioica* chloroform-methanol extract at 2.5 and 5 mg/mL, respectively. These concentrations were chosen to obtain

maximum effect. Group 8 and 9 contained 3.5 mL of Tyrode solution with 2% glucose, and 0.5 mL of catechin at 2.5 and 5 mg/mL, respectively. Following incubation, the hemidiaphragms were taken out and glucose content of the incubated medium was measured by glucose oxidase (GOD)/peroxidase (POD) enzymatic method. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium at $T_{15\ min}$, $T_{30\ min}$, $T_{45\ min}$, respectively, and the results were expressed as percentages.

2.8 Network construction

2.8.1 Phenolic and saponin contents and tested proteins network The network between the phenolic and saponin contents amounted in *Bryonia dioica* extracts (water-acetone, ethyl acetate, butanol, and chloroform-methanol), and the different IC_{50} values experimentally determined for the following proteins, α-amylase, α-glucosidase, and BSA, was built using the Flourish online tool (https://flourish.studio/visualisations/network-charts/) accessed on 27 February 2025.

2.8.2 Phytochemical-targets interaction network The phytochemicals derived from *Bryonia dioica* were chosen based on existing literature. Their canonical simplified molecular input-line entry system (SMILES) were subsequently obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and entered into both the Comparative Toxicogenomics Database (CTD) (https://www.ctdbase.org/) and the BindingDB database (https://www.bindingdb.org/), utilizing "homo sapiens" mode to identify various targets associated with the compounds. The genetic names corresponding to these identified targets were sourced from the UniProtKB (https://www.uniprot.org/) and Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/) databases, specifically selecting the human organism option. Additionally, targets pertinent to diabetes mellitus were compiled from the GeneCards (https://www.genecards.org/) disease gene database by employing the search term "type 2 diabetes mellitus". The gathered compound targets and disease-related targets were then analyzed using Venny v2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) to identify and select overlapping targets. Ultimately, the resulting data were integrated into Cytoscape v3.10.3 software to visualize and construct a network interaction between phytochemical compounds and their targets.

2.9 Molecular docking investigation

2.9.1 Softwares and databases All software and databases used in this study were freely accessible for academic purposes. Protein three-dimensional (3D) structures were retrieved from the Protein Data Bank (https://www.rcsb.org/). Ligand structures, including cucurbitacin B, bryogenin, vitexin, isovitexin, and acarbose, were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Python v3.9.1 (https://www.python.org/downloads/) served as the programming environment. Molecular visualization and analysis were conducted using Discovery Studio Visualizer 2021 (https://discover.3ds.com/discovery-studio-visualizer-download) and MGLTools v1.5.7 (https://ccsb.scripps.edu/projects/visualization/). AutoDock Vina v4.2.6 (https://ccsb.scripps.edu/projects/docking/) was employed for protein-ligand docking simulations. The software programs were installed in a Windows-based computer system with the following features: installed memory: 32 GB; processor: AMD Ryzen5 3600XT 6-Core.

2.9.2 Preparation of receptor files The 3D structures of α-amylase [Protein Data Bank (PDB) Identifier (ID): 3BAJ] and α-glucosidase (PDB ID: 1OBB) proteins (receptors) were downloaded from the PDB. Load the protein structure file into Discovery Studio and execute molecular cleanup to retain only protein components. This involved removing non-protein entities such as water molecules and ligands through atomic selection filters. Finally, the processed structure was saved in the .pdb file format using the Save As function with standardized coordinate formatting.

2.9.3 Grid setting and file preparation The MGLTools v1.5.7 was used for setting the grid parameter on the receptors. The protein file with the .pdb extension was opened in the MGLTools, the polar hydrogen atoms were added to the protein molecules, and then the selected proteins were saved as macromolecules in the .pdbqt file. After that, the ligands were added, the torsion angle was set, and the ligands were saved in the .pdbqt file.

2.9.4 Preparation of conf .txt file The conf .txt file was prepared with all the center axes and size axes of set grid were written with the receptor, ligand, and output files extension. The grid size of α-amylase receptors was set at $x = 40$, $y = 40$, $z = 40$ points, and the grid center was designated at $x = 8.32$, $y = 28.602$, and $z = 50.437$ dimensions, and at $x = 40$, $y = 40$, and $z = 40$ points, and the grid center was designated at $x = 26.187$, $y = 38.705$, and $z = 22.132$ dimensions for α-glucosidase receptors.

2.9.5 Molecular docking Molecular docking was performed using AutoDock Vina, which attempts a set of different ligand conformers to obtain the best disposition of the molecule's atoms for maximizing the scoring function that quantifies ligand-receptor interaction. This software gave the best orientation of ligands with protein. The AutoDock Vina performed the molecular docking between receptor and ligand according to previous procedures [30, 31].

Prepared protein files in .pdbqt format were put in the Vina folder. The Vina configuration file was created with

the Notepad application and saved with the name config. txt. Molecular docking was carried out according to the validation results of molecular docking. Molecular docking commands were carried out using the "cmd" command prompt program. The programming command to run molecular docking was "C:\vina.exe. --config config. txt. -out output.pdbqt", and then press enter. The results of molecular docking could be seen in the output document with the .pdbqt format and log files, which can be opened using the Discovery Studio Visualizer software. The log file is a document that contains data on ΔG values in units of kcal/mol. The interaction of protein and ligands was visualized by Discovery Studio Visualizer 2021.

2.10 Statistical analysis

Data were represented as mean ± standard deviations (SD) of three replicated determinations. Statistical significance was evaluated using one-way analysis of variance (ANOVA), and the Tukey's test was carried out to measure significant differences between the groups at $P < 0.05$, which was considered statistically significant. Principal component analysis (PCA) was carried out using R software. ChiPlot (https://www.chiplot.online/) was used to produce the correlation heatmap (accessed on 20 January 2025).

3 Results

3.1 Determination of total polyphenol and saponin contents

The contents of total polyphenols and saponins in the various extracts, water-acetone, ethyl acetate, butanol, and chloroform-methanol, are reported in Table 1. According to the obtained results, the chloroform-methanol extract showed the highest levels of total polyphenols, flavonoids, flavonols, and saponins, followed by the ethyl acetate fraction ($P < 0.000\,1$). The water-acetone extract, which showed a low polyphenol content compared with the butanol fraction, had high level of flavonoids, flavonols, and saponins ($P < 0.000\,1$). However, the butanol fraction had the lowest levels of flavonoids, flavonols, and saponins.

3.2 Inhibition of α-amylase, α-glucosidase, and non-enzymatic BSA glycation activities

All tested extracts (water-acetone, ethyl acetate, butanol, and chloroform-methanol), exhibited concentration-dependent inhibitory activity. According to the results shown in Table 2, the ethyl acetate fraction showed the most potent inhibition, with a statistically significant lowest α-amylase IC_{50} compared with other extracts ($P < 0.000\,1$), followed by the chloroform-methanol extract. However, the butanol fraction and the water-acetone extract displayed comparatively weaker activity. The extracts

Table 1 Total polyphenol and saponin contents in *Bryonia dioica* root extracts

Extract	Total polyphenol (μg GAE/mg DE)	Flavonoid (μg CE/mg DE)	Flavonol (μg QE/mg DE)	Saponin (μg OAE/mg DE)
Water-acetone	87.56 ± 0.02^{d}	6.01 ± 0.01^{c}	6.33 ± 0.02^{c}	0.40 ± 0.01^{c}
Ethyl acetate	157.01 ± 0.01^{b}	8.64 ± 0.001^{b}	24.67 ± 0.03^{b}	0.90 ± 0.01^{a}
Butanol	134.86 ± 0.02^{c}	2.93 ± 0.004^{d}	2.35 ± 0.01^{d}	0.18 ± 0.01^{d}
Chloroform-methanol	299.51 ± 0.01^{a}	49.09 ± 0.00^{a}	26.27 ± 0.02^{a}	0.58 ± 0.01^{b}

Data were represented as mean ± SD ($n = 3$). $^{a\text{-}d}$ Values with different superscript letters within the same column are significantly different at $P < 0.000\,1$ as determined by one-way ANOVA followed by Tukey's test, and represent a classification of groups according to experimental values from the highest to the lowest.

Table 2 *In vitro* anti-diabetic activity of *Bryonia dioica* roots extracts

Extract	α-Amylase IC_{50} (μg/mL)	α-Glucosidase IC_{50} (μg/mL)	BSA antiglycation IC_{50} (μg/mL)
Water-acetone	963.06 ± 0.05^{a}	562.88 ± 0.01^{c}	550.78 ± 4.10^{b}
Ethyl acetate	145.95 ± 0.01^{d}	583.90 ± 0.01^{a}	318.26 ± 0.81^{c}
Butanol	566.08 ± 0.02^{b}	564.22 ± 0.02^{b}	323.12 ± 1.37^{c}
Chloroform-methanol	300.86 ± 0.04^{c}	nd	608.69 ± 2.45^{a}
Acarbose	13.46 ± 0.03^{e}	115.05 ± 0.02^{d}	nt
Ascorbic acid	nt	nt	143.01 ± 1.12^{d}
Aminoguanidine	nt	nt	66.36 ± 3.96^{e}

Data were represented as mean ± SD ($n = 3$). $^{a\text{-}e}$ Values with different superscript letters within the same column are significantly different at $P < 0.000\,1$ as determined by one-way ANOVA followed by Tukey's test, and represent a classification of groups according to experimental values from the highest to the lowest. nd, not determined. nt, not tested.

effect was significantly less than the effect of the α-amylase inhibitor acarbose ($P < 0.000\,1$).

Based on these findings, the ethyl acetate fraction and the chloroform-methanol extract were selected for kinetic studies of α-amylase inhibition. As shown in Figure 1, both extracts showed mixed competitive and uncompetitive inhibition of porcine pancreatic α-amylase; also, the kinetic parameters of the ethyl acetate fraction V_{max} and K_m decreased, while in the presence of chloroform-methanol extract, V_{max} decreased whereas K_m increased (Table 3).

Regarding α-glucosidase activity, the water-acetone extract and both its ethyl acetate and butanol fractions provided an IC_{50} value varying between 562.88 and 583.90 μg/mL, which remained significantly ($P < 0.000\,1$) higher than that of acarbose (Table 2).

The effect of *Bryonia dioica* extracts (water-acetone, ethyl acetate, butanol, and chloroform-methanol) on BSA glycation, tested by their ability to inhibit AGE formation in the reactive medium, exhibited a concentration-dependent inhibition, of which the ethyl acetate and butanol fractions showed the lowest IC_{50} values of 318.26 and 323.12 μg/mL, respectively (Table 2). The crude water-acetone and chloroform-methanol extracts showed higher IC_{50} values ranging from 550.78 to 608.69 μg/mL.

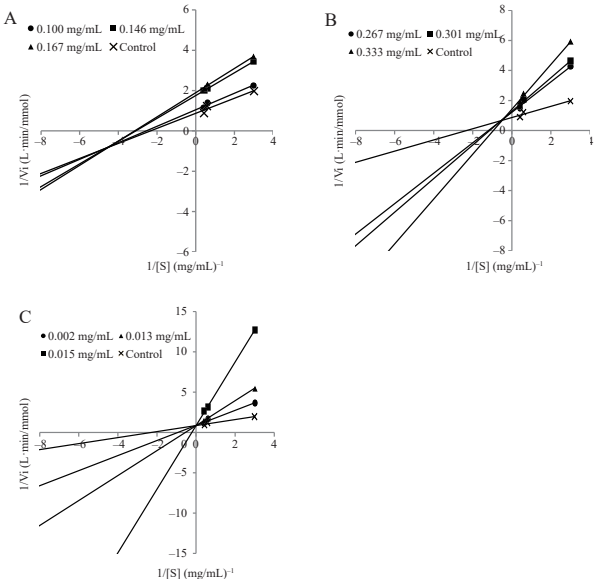

Figure 1 Lineweaver-Burk plots of α-amylase inhibition by various samples

A, *Bryonia dioica* ethyl acetate fraction. B, chloroforme-methanol extract. C, acarbose.

Table 3 Effects of *Bryonia dioica* root extracts on pancreatic α-amylase inhibition kinetics

Extract	Concentration (mg/mL)	V_{max} [mmol/(L·min)]	K_m (mg/mL)	Inhibitory mode
Ethyl acetate	0.100	0.96	0.39	Mixed inhibition
	0.146	0.57	0.32	
	0.167	0.53	0.32	
Chloroform-methanol	0.267	0.83	0.84	Mixed inhibition
	0.301	0.78	0.87	
	0.333	0.70	1.05	
Acarbose	0.002	1.14	1.06	Competitive inhibition
	0.013	1.16	1.80	
	0.015	1.15	4.53	
Control (no inhibitor)	0	1.15	0.43	No inhibition

3.3 Heatmap and principal component analysis

An alternative method for assessing the obtained results involved the application of principal PCA heatmap analysis. The PCA provided a clear visualization of the data (Figure 2). The graphical interpretation of the PCA results was mainly displayed with the two-axis plane. These axes reached 55.69% (Dim1) and 26.44% (Dim2) of variance. Therefore, they represented 82.13% of the total variance. The scores plot showed clearly defined clusters, which may signify the presence of subgroups within the data presented. On one hand, these findings imply that the variables exhibit independent correlations and may vary across each subgroup. The variables "flavonoids" and "total polyphenols" were strongly correlated, as were the variables "α-amylase IC_{50}" and "saponins". In contrast, a negative correlation between "saponins" and "total polyphenols" was revealed. Besides, the group corresponding to the chloroform-methanol extract showed high values for the variables "flavonoids" and "total polyphenols", as well as the variable "BSA antiglycation IC_{50}". On the other hand, butanol, water-acetone, and

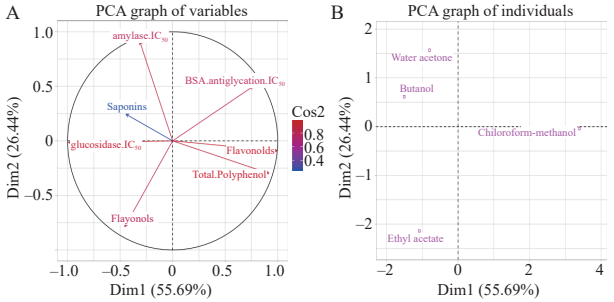

Figure 2 Graphical representation of variables and extracts using PCA

A, variables. B, extracts.

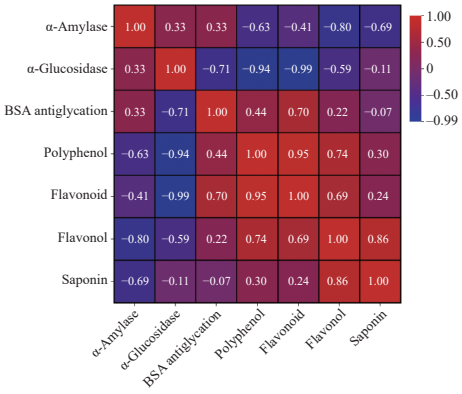

Figure 3 The correlation heatmap among the measured biological activities and phytochemical contents of *Bryonia dioica* extracts

ethyl acetate extracts were grouped based on the variables saponins, α-amylase IC_{50}, and flavonols, respectively. Likewise, the correlation heatmap (Figure 3) revealed a strong positive correlation between polyphenols and flavonoids (Pearson's $r = 0.95$) and a negative correlation between α-amylase and saponins (Pearson's $r = -0.63$). Flavonoids correlated strongly with α-glucosidase and BSA antiglycation but weakly with α-amylase. However, polyphenols showed a strong negative correlation with α-glucosidase and α-amylase. Flavonols were found to have a high correlation with α-amylase and saponins and a moderate correlation with α-glucosidase.

3.4 Stimulation of glucose uptake by isolated rat hemidiaphragm

The study investigated the *in vitro* glucose uptake capacity of the rat hemidiaphragm in the presence or absence of ethyl acetate fraction and chloroform-methanol extract from *Bryonia dioica*. Hemidiaphragms incubation with the extracts (Figure 4 and 5) showed a concentration- and time-dependent increase in glucose uptake compared with the untreated control hemi-diaphragms and the hemi-diaphragms treated with insulin (0.4 U/mL). Insulin-induced glucose uptake increased from 13.99% after 15 min incubation to 29.27% after 45 min incubation, while glucose uptake by the control hemidiaphragm ranged from 2.19% to 4.97%. The ethyl acetate fraction and chloroform-methanol extract significantly ($P < 0.000\ 1$) increased glucose uptake. At 2.5 mg/mL, chloroform-methanol extract enhanced glucose uptake (19.91% – 23.08%) compared with the ethyl acetate fraction (11.33% – 20.35%) and catechin (8.40% – 15.27%) used as a control molecule but was lower than the effect of insulin (13.99% – 29.27%). At high concentrations of 5 mg/mL (Figure 5), *Bryonia dioica* extracts, particularly the ethyl acetate fraction (35.16%) significantly increased glucose uptake ($P < 0.000\ 1$) and were found to be more effective than insulin (29.27%) and catechin (15.27%). These results suggested that the ethyl acetate fraction and

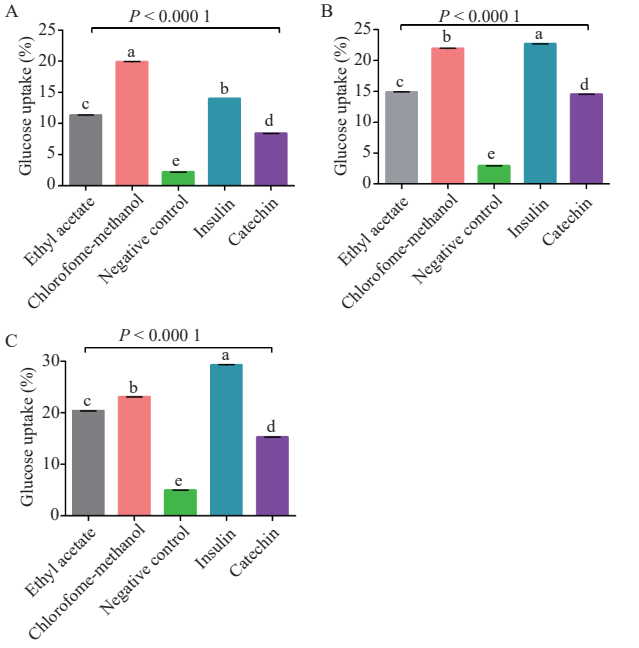

Figure 4 Effects of *Bryonia dioica* root extracts on glucose uptake by isolated rat hemidiaphragm

A, uptake at 15 min. B, uptake at 30 min. C, uptake at 45 min. Data were represented as mean ± SD ($n = 3$). [a-e] Samples not connected by the same letter are significantly different at $P < 0.0001$ as determined by Tukey's test, and represent a classification of groups according to experimental values from the highest to the lowest.

chloroform-methanol from *Bryonia dioica* may have a direct insulin-like activity that improves peripheral glucose utilization and expresses an extra-pancreatic effect.

3.5 Phytochemical-target interaction network construction

The network integrated phenolic and saponin compounds with *in vitro* IC_{50} values, visualized through nodes (colored by entity type) and links (representing interactions). As illustrated in Figure 6, source nodes included polyphenol, flavonoid, flavonol, and saponin, while target nodes consisted of α-amylase, α-glucosidase, and BSA. Node size correlated with phytochemical abundance and IC_{50} values: larger nodes indicated higher IC_{50} values ranging from 550.78 to 608.69 μg/mL. Conversely, the smaller nodes represented the lowest values, which include saponins, flavonol, and flavonoid contents from the tested extracts, water-acetone, ethyl acetate, butanol, and chloroform-methanol.

In parallel, the construction of phytochemicals-targets interaction network using various bioinformatics tools revealed that compounds under investigation interacted with 67 distinct targets, while type 2 diabetes mellitus was associated with 595 gene targets. The overlap between these two sets of targets identified 36 genes that may be targeted by *Bryonia dioica*-selected compounds (Venn map of overlapped targets). The network, shown in

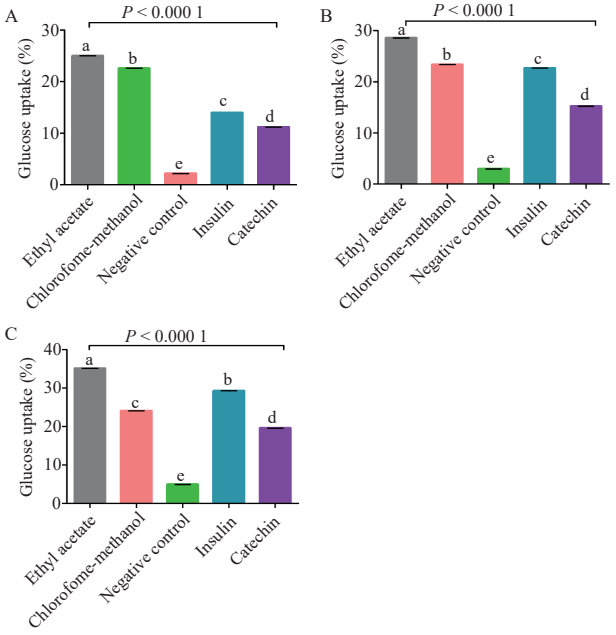

Figure 5 Effects of *Bryonia dioica* ethyl acetate and chloroform-methanol root extracts (5 mg/mL) on glucose uptake by isolated rat hemidiaphragm

A, uptake at 15 min. B, uptake at 30 min. C, uptake at 45 min. Data were represented as mean ± SD (n = 3). [a-e] Samples not connected by the same letter are significantly different at P < 0.000 1 as determined by Tukey's test, and represent a classification of groups according to experimental values from the highest to the lowest.

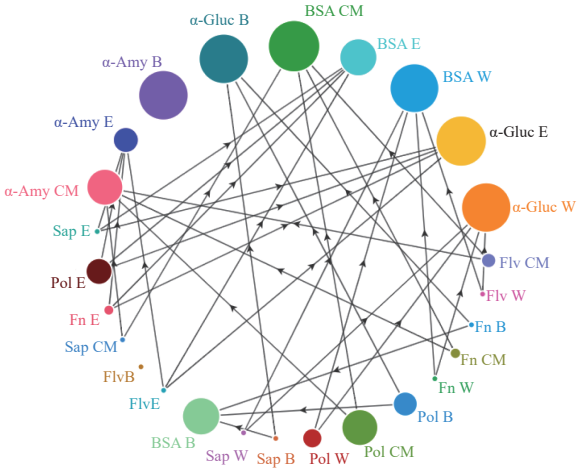

Figure 6 Interaction network of polyphenols and saponins in *Bryonia dioica* extracts with α-amylase, α-glucosidase, and BSA

W, water-acetone. E, ethyl acetate. B, butanol. CM, chloroform-methanol. Pol, polyphenol. Flv, flavonoid. Fn, flavonol. Sap, saponin. α-Amy, α-amylase. α-Gluc, α-glucosidase.

Figure 7, formed by twelve chosen compounds and diverse targets associated with type 2 diabetes mellitus, comprised 48 nodes and 79 edges. The network illustrated varying interactions and notable identified targets, including α-amylase 1A (AMY1A), α-glucosidase (GAA), signal transducer and activator of transcription 3

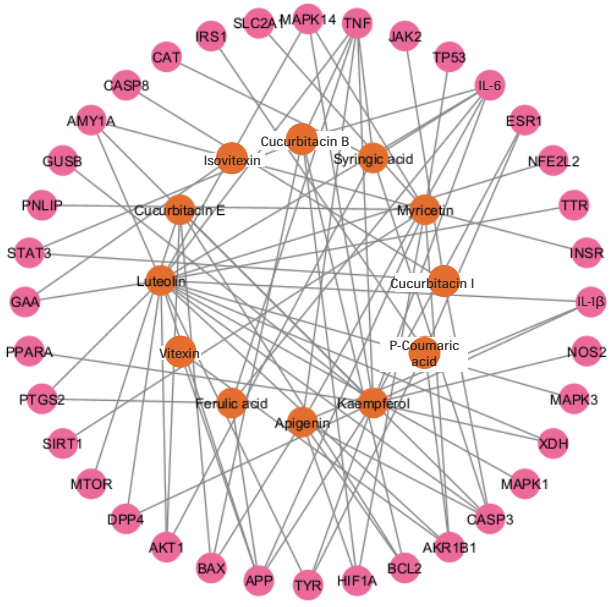

Figure 7 *Bryonia dioica* selected phytochemical compounds and targets interaction network

SLC2Al, solute carrier family 2 member 1. TNF, tumor necrosis factor, JAK2, janus kinase 2. TP53, tumor protein P53. IL-6, interleukin-6. ESRl, estrogen receptor. NFE2L2, NFE2 like bZIP transcription factor 2. TTR, transthyretin. INSR, insulin receptor. IL-lβ, interleukin-1β. NOS2, nitric oxide synthase 2. XDH, xanthine dehydrogenase. CASP3, caspase 3. AKRlBl, aldo-keto reductase family I member B1. BCL2, BCL2 apoptosis regulator. HIF1A, hypoxia inducible factor 1 subunit α. TYR, tyrosinase. APP, amyloid-β precursor protein. BAX, BCL2 associated X, apoptosisregulator. AKTl, AKT serine/threonine kinase 1. DPP4, dipeptidyl peptidase 4. MTOR, mechanistic target of rapamycin kinase. SIRTl, sirtuin 1. PTGS2, prostaglandin G/H synthase2. PPARA, peroxisome proliferator activated receptor α. PNLIP, pancreatic triacylglycerol lipase. GUSB, glucuronidase β. CASP8, caspase 8. CAT, catalase. IRSl, insulin receptor substrate 1.

(STAT3), mitogen-activated protein kinase 1 (MAPK1), mitogen-activated protein kinase 3 (MAPK3), mitogen-activated protein kinase 14 (MAPK14), insulin receptor (INSR), and others, of which AMYlA was specifically targeted by luteolin, kaempferol, and myricetin, whereas α-glucosidase was the target of luteolin and isovitexin compounds. Luteolin, kaempferol, and myricetin represent the hub nodes with the highest degrees, 18, 13, and 10 edges, respectively. However, apigenin and cucurbitacin B, respectively, interacted with 7 and 6 targets, followed by cucurbitacin E and syringic acid, which were associated with 5 targets. Likewise, cucurbitacin I, p-coumaric acid, and ferulic acid were connected with 4 targets, while isovitexin and vitexin demonstrated the least interactions with only 1 and 2 connections, respectively.

3.6 Molecular docking study with α-amylase and α-glucosidase

All tested molecules gave a higher binding energy score to α-amylase and α-glucosidase than acarbose

(Supplementary Table S1 and S2). The binding energies of the selected molecules with α-amylase ranged from – 16.4 to – 11.6 kcal/mol, the binding energies being in the following order: acarbose (– 11.4 kcal/mol) < vitexin (– 11.6 kcal/mol) < isovitexin (– 12.1 kcal/mol) < bryogenin (– 14.5 kcal/mol) < cucurbitacin B (– 16.4 kcal/mol). Whereas, the binding energies with α-glucosidase varied between – 14.2 and – 10.5 kcal/mol, in the following bin-ding energies obtained being as follows: vitexin (– 10.5 kcal/mol) < acarbose (– 10.8 kcal/mol) < isovitexin (– 11.5 kcal/mol) < bryogenin (– 12.8 kcal/mol) < cucurbitacin B (– 14.2 kcal/mol). Details of the molecular docking analysis in this study are illustrated in Supplementary Table S1 and S2, and Figure 8 and 9. Cucurbitacin B showed an electrostatic interaction (positive charge attraction) with Asp300 at the α-amylase catalytic site, and two hydrogen bonds with Ile235 and Ala198. For α-glucosidase, cucurbitacin B exhibited three hydrogen bonds with Asn153, Asp119, and Tyr296, and an electrostatic bond (pi-cation) with Phe238. The molecular docking analysis revealed that bryogenin interacted more with α-amylase than with α-glucosidase. It provided one electrostatic bond (pi-anion) with Asp300 and five hydrogen bonds with Arg195, Trp59 (pi-donor hydrogen bond; pi-alkyl), and Tyr62 (pi-pi stacked). Whereas with α-glucosidase bryogenin formed hydrogen bond with Phe238 and with Asp260. Compared with other ligands, isovitexin provided the maximum interactions with the enzymes, creating eleven hydrogen bonds, three bonds with the active site residues of α-amylase Asp300, Asp197, and Glu233, others with residues Arg195, Trp59, His299, and Tyr62, and one hydrophobic interaction (Alkyl) with Leu165. However, the hydrogen bonds of isovitexin were established with Ser13, Arg44, Met86, Gly10, Asp39, and Tyr131 of α-glucosidase. The interactions of vitexin were mainly hydrogen bonds with residues outside the active site of α-amylase (Asp353, Thr314, Arg303, Ile312, and Arg346) and of α-glucosidase (Gly176, Gln241, Asp26, Asp 240, and Phe238). Acarbose, used as a control, established hydrogen bonds with residues Gln63, Glu233, and Asp300, and electrostatic bonds with α-amylase active site residues Asp300, Asp197, and Glu233, while its interaction with α-glucosidase showed only hydrogen bonds with residues Arg44, His90, Gly301, Asp39, Gln297, and Gly10.

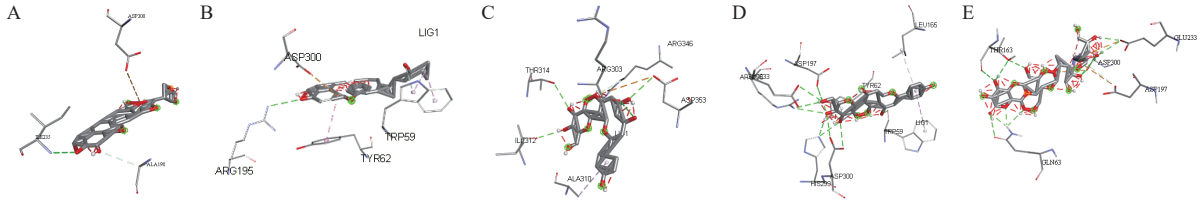

Figure 8 3D predicted binding modes of cucurbitacin (A), bryogenin (B), vitexin (C), and isovitexin (D) from *Bryonia dioica*, and acarbose (E) with α-amylase (3BAJ)

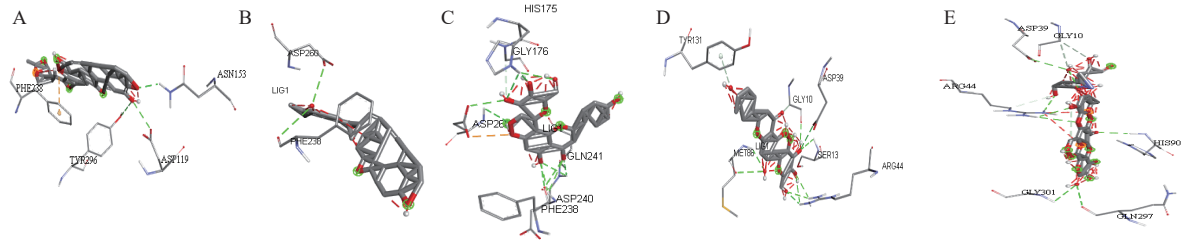

Figure 9 3D predicted binding modes of cucurbitacin (A), bryogenin (B), vitexin (C), and isovitexin (D) from *Bryonia dioica*, and acarbose (E) with α-glucosidase (1OBB)

4 Discussion

Bryony's antidiabetic potential remains underexplored, with limited scientific investigations. Phytochemical analyses confirm *Bryonia dioica* is rich in C-glycosylated flavonoids (vitexin, isovitexin, apigenin 6-C-glucoside-7-O-glucoside, luteolin 6-C-glucoside-7-O-glucoside) and triterpenoid saponins (bryoniosides A-G, cabenoside, bryoamaride) [32, 33], which may mechanistically underpin its observed bioactivity.

Certain phytocompounds such as terpenes, alkaloids, polyphenols, and mainly flavonoids and phenolic acids, have been identified as potent inhibitors of α-amylase and α-glucosidase [34, 35]. Momordicosides (0.67 – 0.87 mmol/L), cucurbitacin triterpenes isolated from fresh fruit of *Momordica charantia*, significantly inhibited the activity of α-amylase (61% – 70%) and α-glucosidase (35% – 56%) [36, 37]. In addition, vitexin and isovitexin isolated from *Ficus deltoidea* leaves showed significant inhibition of α-amylase [38].

Bryonia dioica extracts displayed a mixed inhibition mode in the kinetics of α-amylase. This type of inhibition may arise from simultaneous action of multiple phytoconstituents with both catalytic and allosteric sites of the

enzyme. Previous findings reported that acarbose and vitexin exhibit synergistic inhibition of α-glucosidase, while vitexin alone provides non-competitive inhibition of this enzyme and leads to changes in its conformation [39]. In addition, gallic acid competitively inhibited α-amylase and α-glucosidase by interacting with their active site residues [40].

Concerning BSA antiglycation activity, the significant effect registered is mainly related to phenolic compounds and saponin, suggesting their preventive role against hyperglycemia-induced protein glycation. The inhibitory effects of polyphenols on the formation of AGEs have garnered considerable scientific attention. Polyphenols' antiglycation capacity is structurally dependent, with hydroxylation at A/B rings enhancing inhibition, while C-ring hydroxylation and flavonoid glycosylation reduce efficacy. Notably, vitexin and isovitexin (100 μmol/L) suppressed glucose-induced BSA glycation by more than 85% [41]. Similarly, other flavonoids demonstrated an inhibitory capacity on AGEs production, such as rutin, quercetin, kaempferol, epigallocatechin, and luteolin [42].

This study provides the first report on *Bryonia dioica* root extracts effects on glucose uptake in rat hemidiaphragm, precluding direct comparison with existing literature. Previous studies have documented phytochemicals and plant extracts enhancing glucose uptake, though typically at higher concentrations [e.g., Hybanthus enneaspermus (25 mg/mL), Pedalium murex (25 mg/mL), Maerua pseudopetalosa (100 mg/mL)] [43, 44]. In contrast, our extracts demonstrated significant glucose uptake enhancement at low concentrations (2.5 and 5 mg/mL). According to the literature, cucurbitacins and flavonoids, such as quercetin, catechin, epicatechin, kaempferol, rutin, naringenin, anthocyanidins, and vitexin enhance glucose uptake *in vitro* by stimulating the glucose transporter Glut4 translocation via phosphatidyl inositol 3-kinase (PI3K)/AKT serine/threonine kinase (AKT), Cb1-associated protein (CAP)/Cb1 proto-oncogene (Cb1)/lipid-raft-associated protein TC10 (TC10), and AMP-activated protein kinase (AMPK) pathways in adipocyte and muscle cell lines [45-47].

Bryonia dioica is reported to be rich in flavonoids and cucurbitacins, which were prioritized for molecular docking analysis to investigate their interactions with α-amylase and α-glucosidase enzymes. Specifically, cucurbitacin B, bryogenin, vitexin, and isovitexin were examined in our study. The complexes formed by cucurbitacin B/α-amylase and bryogenin/α-amylase have the lowest energy scores compared with the control complex acarbose/α-amylase. Similarly, these compounds demonstrated superior binding affinities to α-glucosidase versus other tested molecules and acarbose. Cucurbitacin B and bryogenin showed an electrostatic interaction with Asp300 of the α-amylase catalytic site. These compounds exhibited, respectively, three (Asn153, Asp119, and Tyr296) and two (Phe238 and Asp260) hydrogen bonds with α-glucosidase. Isovitexin provided the maximum interactions with both enzymes, creating eleven hydrogen bonds. The molecular docking findings are in agreement with PCA and correlation heatmap analysis.

Previous study has shown that inhibitors can bind directly to Asp197, Glu233, and Asp300 amino acid side chains of α-amylase active site [48]. Other residues, through their side chains, can also interact with the ligand Trp59, Tyr62, Gln63, Val98, His101, Leu162, Thr163, Leu165, Asp197, Ala198, and Glu233, whereas the residues for α-glucosidase are Asp203, Thr204, Thr205, Typ299, Asp327, Ile328, Ile364, Asp366, Trp406, Trp441, Asp443, Met444, Ser448, Phe450, Arg526, Trp539, Asp571, Phe575, Arg598, and His600 [49]. According to an earlier investigation, momordicosides, cucurbitacin compounds from fresh *Momordica charantia* fruit, provided hydrogen and Van Der Waals bonds with crucial residues of the domain A of α-amylase, such as Asp300, Glu233, Glu240, Asp197, Gly308, Gly306, Gly238, Trp58, Trp59, His201, His305, Val163, Leu162, Lys200, and Ile235 [37]. Also, the triterpenoid cucurbitacins from the roots of *Siraitia grosvenorii*, have shown a high binding affinity for α-glucosidase, with the formation of hydrogen bonds with the following residues: Gln279, Arg315, Asp352, Gly309, and Arg442, and hydrophobic interactions with Val319, Phe321, Asp325, Asp307, and Val308 [50]. Recent molecular docking studies have highlighted anti-diabetic potential of many natural compounds on protein targets. Cucurbitacin B, cucurbitacin E, and oleanolic acid compounds from *Luffa acutangula* (Cucurbitaceae family) were reported as potential anti-diabetic with high binding energy to insulin receptor, aldose reductase, and protein tyrosine phosphatase [51]. Flavonoids including diosmin, biochanin A, hesperidin, quercetin, and hesperetin, revealed significant affinities with phosphatidylinositol 3-kinase (PI3K), with some noncovalent interactions of Lys833, Asp964, Asp841, Val882, Met953, and Ile879 residues [52]. Theaflavin 3,3'-O-digallate, naringin 4'-O-glucoside, and naringin 6'-malonate exhibit a significant number of dynamic features, such as stability, flexibility, and binding energy with protein tyrosine phosphatases PTP1B, which they may potentially be used in type 2 diabetes management [53].

While biochemical and molecular analysis may fail to identify the active compound responsible for observed effects of a raw plant extract. However, targeted screening method through various databases facilitates more rapid and straightforward identification of protein effects and pathways compared to conventional single-component analysis [54]. Multi-component drugs are increasingly prevalent for complex diseases like diabetes mellitus, as these drugs exert their effects across various systemic, tissue, and cellular levels. Network pharmacology has proven to be highly effective in elucidating the signaling

pathways by integrating experimental data to map compound-target interactions and therapeutic pathways. This approach is based on experimental data sourced from existing research literature. It may offer a comprehensive view of structural relationships between compounds and their therapeutic targets, which can be instrumental in natural compounds screening and understanding their mechanisms of action [54]. In the current study, a network investigation was carried out to visualize interactions between *Bryonia dioica* phytochemicals and targets related to diabetes mellitus. The limited number of phytochemical compounds from *Bryonia dioica* and their targets associated with diabetes mellitus are supported by limited scientific work published on *in vivo* and *in vitro* molecular studies of anti-diabetic effect of this plant. The main published work focuses on the anti-inflammatory and anti-cancer properties of *Bryonia dioica* extracts, which were revealed in our network analysis with high interactions of cucurbitacins and polyphenols with the following targets: TNF, IL-6, IL-1β, JAK2, STAT3, CASP3, and CASP8. Meanwhile, the phytochemical-targets network findings revealed an interaction of cucurbitacin and phenolic compounds with α-amylase, α-glucosidase, and Glut4 translocation signaling pathway, which confirms our promising experimental results. This investigation highlighted cucurbitacins, polyphenols, and flavonoids as promising anti-diabetic phytoconstituents, which corroborate previous reports [55, 56]. Comparative target analysis highlighted luteolin and kaempferol as top interactors with type 2 diabetes mellitus targets (vs. minimal vitexin interactions) [54].

In summary, our findings indicate that *Bryonia dioica* root extracts can effectively mitigate hyperglycemia and its complications through multifaceted mechanisms. *Bryonia dioica* root extracts can inhibit digestive enzyme α-amylase and α-glucosidase, inhibit AGE production, as well as enhance glucose uptake by peripheral tissues. Molecular docking and network analysis demonstrated that *Bryonia dioica* phytocompounds exert their effects by bonding with enzyme active sites through hydrogen bonding and modulating the Glut4 translocation signaling pathway. This study, considered to be the first report on the *in vitro* evaluation of anti-diabetic effects, provides experimental information on the effectiveness of *Bryonia dioica* in treating type 2 diabetes mellitus. Our focus on enzymatic inhibitory activity, protein antiglycation, and glucose utilization, offers important perspectives on the anti-diabetic properties of *Bryonia dioica in vitro*. However, it is essential to recognize certain limitations. For instance, mechanisms such as insulin secretagogue activity and glucose utilization in 3T3-L1 adipocytes remain unexplored. Further investigations into additional molecular targets, signaling pathways, and cell models are warranted to fully elucidate the therapeutic scope of *Bryonia dioica*.

5 Conclusion

This study reported the *in vitro* anti-diabetic effect of *Bryonia dioica* root extracts. *In vitro* results revealed an impact on glucose uptake by the isolated rat hemidiaphragm, a significant inhibition of BSA glycation, as well as a promising inhibitory capacity against α-amylase and α-glucosidase activities with a mixed inhibition mode of α-amylase's kinetics. Molecular docking analysis revealed that major constituents of *Bryonia dioica* roots, such as cucurbitacins and flavonoids, interact within and outside the active sites of α-amylase and α-glucosidase. The results are further substantiated by a phytochemical-targets interaction network, which indicated interactions between cucurbitacin and phenolic compounds with α-amylase, α-glucosidase, and the signaling pathway targets involved in Glut4 translocation. Therefore, *Bryonia dioica* may provide a potential source for new anti-diabetic agent development and may offer a better therapeutic approach for treating hyperglycemia and its complications.

Competing interests

The authors declare no conflict of interest.

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基于分子对接的泻根提取物体外抗糖尿病活性研究

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【摘要】目的 评价泻根根部水-丙酮提取物及其乙酸乙酯组分和丁醇组分，以及氯仿-甲醇提取物的体外抗糖尿病作用。**方法** 分别用福-乔试剂、三氯化铝和香草醛试剂采用比色法测定泻根提取物（氯仿-甲醇提取物、水-丙酮提取物及其乙酸乙酯和丁醇组分）中的总酚、黄酮、黄酮醇和皂苷含量。通过测定这些提取物对 α-淀粉酶和 α-葡萄糖苷酶活性的半抑制浓度（IC_{50}）值，评价其对 α-淀粉酶动力学的影响，评价其体外抗糖尿病活性。采用荧光测定法定量测定对牛血清白蛋白（BSA）糖基化的抑制作用，以评估晚期糖基化终末产物的生成，并通过离体大鼠半隔膜测定葡萄糖摄取。此外，进行了分子对接分析，以研究泻根配体（葫芦素 B、泻根苦素、牡荆素和异牡荆素）与 α-淀粉酶和 α-葡萄糖苷酶之间的结合亲和力和相互作用类型。同时构建了植物化学-靶标相互作用网络。**结果** 对于 α-淀粉酶抑制作用，乙酸乙酯部分表现出最强的活性（IC_{50} = 145.95 μg/mL），其次是氯仿-甲醇提取物（IC_{50} = 300.86 μg/mL）。水-丙酮根提取物及其组分对 α-葡萄糖苷酶活性有抑制作用，IC_{50} 值为 562.88 – 583.90 μg/mL。乙酸乙酯和丁醇组分强烈抑制非酶 BSA 糖基化，IC_{50} 分别为 318.26 和 323.12 μg/mL。离体大鼠膈肌与乙酸乙酯组分（5 mg/mL）孵育显著增加葡萄糖摄取（35.16%），超过胰岛素（0.4 IU/mL；29.27%）、氯仿-甲醇提取物（24.07%）和儿茶素（15.27%）的作用。葫芦素 B 与 α-淀粉酶（– 16.4 kcal/mol）和 α-葡萄糖苷酶（– 14.2 kcal/mol）的对接评分最强。与其他配体相比，异牡荆素与 α-淀粉酶活性位点残基（Asp300、Asp197 和 Glu233）和 α-葡萄糖苷酶残基（Ser13、Arg44、Met86、Gly10、Asp39 和 Tyr131）以及其他残基（Arg195、Trp59、His299 和 Tyr62）形成最大数量的氢键。网络分析发现泻根植物化学成分与 2 型糖尿病相关基因之间存在 36 个重叠靶点，其中葫芦素类和多酚类化合物与 α-淀粉酶、α-葡萄糖苷酶以及 Glut4 转位通路靶点发生相互作用。**结论** 体外研究表明，泻根提取物具有较好抗糖尿病活性。对消化酶的抑制作用、蛋白抗糖基化潜能和葡萄糖摄取的增强提示了泻根提取物可能通过调节高血糖及其并发症的机制。该种属可能成为管理 2 型糖尿病的新型抗糖尿病药物的潜在来源。

【关键词】泻根；葡萄糖摄取；蛋白质糖基化；α-淀粉酶；α-葡萄糖苷酶；分子对接；相互作用网络