



## RESEARCH ARTICLE OPEN ACCESS

# Potential Hypoglycemic, Hypolipidemic, and Hypouricemic Bioactive Components From *Ampelopsis grossedentata* Explored by Affinity Ultrafiltration With Three Enzyme Targets

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## ABSTRACT

Hyperuricemia demonstrates synergistic interactions in the progression of hyperlipidemia and hyperglycemia. There is a pressing demand to explore natural products with negligible adverse effects for the prevention and regulation of hyperuricemia, particularly when combined with hyperglycemia and hyperlipidemia. *Ampelopsis grossedentata* has been traditionally employed as a functional food throughout an extensive historical duration. Nevertheless, the bioactive compounds that contribute to its hypoglycemic, hypolipidemic, and hypouricemic effects, as well as the molecular mechanisms involved, have not yet been completely clarified. This study initially conducted in vitro assays to evaluate the inhibitory activity of *A. grossedentata* towards  $\alpha$ -glucosidase, pancreatic lipase, and xanthine oxidase. The results revealed that the *A. grossedentata* extract exhibited substantial inhibitory activities against these enzyme targets. Subsequently, the four most promising active compounds (quercetin, myricitrin, dihydromyricetin and taxifolin) were screened and characterized by a bioaffinity ultrafiltration method combined with LC-MS/MS analysis. Further validation of these bioactive compounds was achieved through molecular docking studies. Additionally, an interaction network was established to elucidate the mechanisms of action between the candidate constituents and their respective targets. The results indicate that *A. grossedentata* holds great promise as a potential natural therapeutic functional food for managing diabetes, overweight, and hyperuricemia in the foreseeable future.

## 1 | Introduction

Nowadays, the shift towards a diet high in carbohydrates, fats, and sugars have led to a gradual increase in the incidence of long-term health conditions including diabetes and

hyperlipidemia (Song et al. 2024). Hyperuricemia, often referred to as the fourth common chronic disease, has been listed as a significant health concern following hyperglycemia, hyperlipidemia and hypertension (Feng et al. 2022). It is on the rise

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worldwide especially in Japan (26.8%), the United States (20.1%), and China (17.4%), which has been well-documented in both adults and children (Xue et al. 2023). Accumulating evidence shows that hyperuricemia poses important role to the development of both hyperlipidemia and hyperglycemia, which aggravating cardiovascular and cerebrovascular diseases (Yang et al. 2020; Yang et al. 2024). The development of hyperuricemia involves multiple molecular regulatory targets, including purine metabolism-related enzymes such as xanthine oxidase (XOD) and adenosine deaminase, as well as urate transporters, reflecting the complexity of uric acid homeostasis (Lu et al. 2025). Purine metabolic disorders impair blood uric acid regulation. This chaos can cause pancreatic beta-cell dysfunction (Borghi et al. 2020). The compromised insulin sensitivity then results in hyperinsulinemia, along with hypertension and renal arteriosclerosis. The further diminished renal blood flow would reduce the excretion of uric acid in turn (Yang et al. 2025). On the other side, Fang et al. (2024) revealed that fatty acid oxidation stimulates the synthesis of hepatic uric acid and thereby contributes to the progression of hyperuricemia as well. The above results demonstrate that hyperglycemia and hyperlipidemia might be potential triggers for hyperuricemia. Functional enzymes regulate multiple key biological processes. Chen et al. (2025) also pointed out that XOD is a promising therapeutic target for managing hyperuricemia because of its regulatory capacity of purine metabolism. Besides,  $\alpha$ -glucosidase and lipase are the crucial digestive enzymes for the management of hyperglycemia and hyperlipidemia (Chen et al. 2025). Although allopurinol, acarbose and orlistat are extensively applied as XOD,  $\alpha$ -glucosidase and lipase inhibitors, respectively, they are linked to various adverse reactions such as cutaneous manifestations, gastrointestinal discomfort, and abnormal liver function (Hua et al. 2024; Saag et al. 2025). Additionally, current therapeutic drugs available are typically not advised for individuals with hyperuricemia and early-stage hyperglycemia in the absence of gout symptoms. Recent studies have shown that bioactive compounds derived from natural plants can modulate uric acid metabolism through multiple pathways and exhibit dose-dependent urate-lowering and metabolic homeostasis-improving effects in vivo models, further highlighting the potential advantages of natural products in the integrated intervention of complex metabolic diseases (Ooi et al. 2021; Wang, Chen, et al. 2025). Therefore, we must explore natural compounds with minimal side effects for the prevention and regulation of hyperuricemia combined with hyperglycemia and hyperlipidemia.

*Ampelopsis grossedentata* (Handel-Mazzetti) W. T. Wang, a perennial vine plant classified under the *Vitaceae* family, is commonly employed for the preparation of Vine Tea, also referred to as sweet tea or rattan grass. It possesses a long history of use and serves as a traditional functional food utilized by residents across more than 10 provinces for the management of pharyngitis, oral inflammation, hepatic agitation, constipation, and hypertension (Zeng et al. 2023). In 2013, the National Health and Family Planning Commission approved the leaves of *A. grossedentata* as a novel food resource, which showed huge promise in the development of functional and health-promoting products (Zhou et al. 2021). *A. grossedentata* has proved to have potential therapeutic use in diabetic control with correction of glycolipid metabolism disorder and gut microbiota modification in the Zucker diabetic fatty rats model (Hu

et al. 2023). Crude *A. grossedentata* extract also demonstrated good protection against liver diseases such as alcohol-induced or nonalcoholic steatohepatitis (NAFLD) by enhancing liver functions, reducing inflammatory response, managing lipid accumulation and relieving oxidative stress (Luo et al. 2025). In addition, the hypouricemic effect of *A. grossedentata* was verified by an in vivo experiment, and its material basis as well as mechanism was preliminary explored via multi-level interaction network (Zhou et al. 2024). *A. grossedentata*'s various benefits on people health are mainly attributed to its chemically complex and multi-functional ingredients. There are high contents of flavonoid, and the main chemical ingredients include dihydromyricetin, myricetin, auranthoflavin and myricitrin. In particular, the contents of dihydromyricetin are as high as 28% (Feng et al. 2018). In addition, there are abundant phenolic substances such as gallic acid, gallic acid esters, catechins and epicatechins in *A. grossedentata* (Zhang et al. 2025b). However, the involved mechanisms and interrelated actions of glucose-lowering, lipid-reducing, and urate-lowering of *A. grossedentata* with the corresponding responsible active components are still elusive.

The traditional dynamic component tracking mechanism of active substance is usually completed by extracting, purifying, separating, and isolating individual compound, and finally quantifying the activity of each compound. This technique is arduous, time-consuming, prone to the loss of trace substance or activity of components in the process of tracking (Hua et al. 2024). As a result, the formulation of a quick method for the screening of active components operationally simple, highly selective, and whose outcome should provide a reliable result, has emerged as a subject of investigation. Ultrafiltration affinity combined with high-performance liquid chromatography mass spectrum (UF-LC-MS/MS) is an effective and high-throughput screening method based on ultrafiltration technology and mass spectrum, which can be effectively used in the discovery and characterization of bioactive ingredients based on complex systems in enzymes (Hua et al. 2024). Our group previously used UF-LC-MS/MS method to characterize and isolate the  $\alpha$ -glucosidase and pancreatic lipase inhibitors from *Cyclocarya paliurus* leaves and fished out bioactive compounds for glucose-lowering and lipid-reducing effects (Xu et al. 2023). Zhang et al. (2024) found the bioactive activities of mulberry leaves extract and suggested that polyphenols might play a key role in its hypoglycemic and hypolipidemic activities.

Given the proposed therapeutic properties and health-promoting status of *A. grossedentata*, we consider that it is necessary to perform a thorough and systematic research on its hypoglycemic, hypolipidemic, and hypouricemic activities, as well to determine natural modulators of  $\alpha$ -glucosidase, pancreatic lipase and XOD, and also clarify underlying regulatory mechanisms. In this present work, in vitro enzyme inhibition assays of *A. grossedentata* with three enzyme targets were firstly estimated and then the bioactivity components were screened and characterized by UF-LC-MS. And finally, the efficacy of determined components was confirmed by molecular docking prediction. The UF-LC-MS strategy is applicable for the acquisition of fingerprint information about the main active ingredient of *A. grossedentata* for inhibiting various biological enzymes, and exposed the functional network of various compounds for their targets. This study demonstrated the synergistic hypoglycemic, hypolipidemic, and hypouricemic effects of

*A. grossedentata* and establishes a robust theoretical groundwork for the forthcoming research and advancement of *A. grossedentata* as a potential therapeutic functional food candidate for managing chronic metabolic dysfunctions.

## 2 | Materials and Methods

### 2.1 | Materials and Reagents

Porcine pancreatic lipase, XOD, xanthine, allopurinol, and acarbose were obtained from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China).  $\alpha$ -Glucosidase was obtained from Sigma-Aldrich Corp. (Shanghai, China). *p*-Nitrophenyl  $\alpha$ -D-glucopyranoside (*p*-NPG) and 4-nitrophenyl butyrate (*p*-NPB) were obtained from Aladdin Biochemical Technology Co. Ltd (Shanghai, China). Orlistat was obtained from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China), and Zhimai-kang capsule was purchased from Liaoyuanyulongyadong Pharmaceutical Co. Ltd.

### 2.2 | Preparation of the *A. grossedentata* Extracts

The air-dried botanical material of *A. grossedentata* was obtained from Shaanxi Snow Lotus Biotechnology Co. Ltd. The branches and leaves of *A. grossedentata* were pulverized, extracted twice via reflux using a solid-to-solvent ratio of 1:10 (ultrapure water), and thereafter spray-dried to produce a dry powder. The samples were stored under well-ventilated, dry, and light-protected conditions.

### 2.3 | In Vitro Inhibitory Assays of Three Enzyme Targets

#### 2.3.1 | $\alpha$ -Glucosidase Inhibitory Assay

Suppressive influence of *A. grossedentata* extracts on  $\alpha$ -glucosidase functionality was analyzed according to our previous work with minor modification (Zhang et al. 2024). Specifically, 20  $\mu$ L of diluted mixtures of different concentrations and 20  $\mu$ L of  $\alpha$ -glucosidase solution (0.5 U/mL) were initially maintained at 37°C for 15 min. Thereafter, 40  $\mu$ L of substrate *p*-NPG solution (0.4 mM) was introduced to initiate the reaction. Following an additional 10 min (linear phase) incubation period at 37°C, the reaction was stopped by the addition of 40  $\mu$ L of sodium carbonate solution (0.2 M). Ultimately, the absorption intensity at 405 nm was recorded on a microplate reader (Tecan Spark, Tecan). Reaction linearity with time and enzyme concentration was validated in Supporting Information S1: Figure S1. Acarbose, a well-established  $\alpha$ -glucosidase inhibitor, was selected as the positive control. The inhibition rate (%) was calculated by applying the subsequent equation:

$$\text{Inhibition rate (\%)} = 1 - \frac{A_s - A_{sc}}{A_c - A_{bc}} \times 100\%$$

$A_s$  and  $A_{sc}$  represent the absorption intensity of the sample group with or without  $\alpha$ -glucosidase, respectively.  $A_c$  and  $A_{bc}$  represent the absorption intensity of the control group (100%  $\alpha$ -glucosidase activity) and blank control group

without  $\alpha$ -glucosidase, respectively. All samples were evaluated in three independent replicates.

#### 2.3.2 | Pancreatic Lipid Inhibitory Assay

Pancreatic lipid inhibitory activity of the *A. grossedentata* extracts was assessed in accordance with our previous work with minor modification (Xu et al. 2023). Briefly, a 96-well microplate was sequentially loaded with a 150  $\mu$ L reaction mixture, consisting of equal volumes (50  $\mu$ L each) of 0.1 M phosphate buffer (pH 7.4), pancreatic lipase solution (0.5 mg/mL), and sample solutions at varying dilutions. The plate was incubated at 37°C for 10 min and then 50  $\mu$ L of substrate solution (*p*-NPB, 1.2 mM) was injected into the formulation. Then the formulation was incubated at 37°C for 20 min (linear phase) and the absorption intensity was detected at 405 nm by microplate spectrophotometer. Reaction linearity with time and enzyme concentration was validated in Supporting Information S1: Figure S2. Chinese herb Zhimaikang capsule was used as positive control. The equation used to calculate the inhibition rate (%) is given in section 2.3-1.

#### 2.3.3 | XOD Inhibitory Assay

The inhibitory activity of the extracts of *A. grossedentata* against XOD was determined according to our previous method with a slight modification (Liang et al. 2023). A 96-well microplate was, respectively, added with 90  $\mu$ L of phosphate buffer, 20  $\mu$ L of XOD solution (0.1 U/mL) and 10  $\mu$ L of diluted sample solutions at different concentrations, and then incubated at 37°C for 10 min with subsequently mixed by adding 80  $\mu$ L of substrate solution (xanthine, 0.4 mM). Following this, the formulation was maintained at 37°C for another 10 min (linear phase), after which the absorption intensity was assessed at 295 nm using a microplate spectrophotometer. Reaction linearity with time and enzyme concentration was validated in Supporting Information S1: Figure S3. Allopurinol was applied as the positive control. The inhibition rate (%) was determined following the equation described in section 2.3-1.

### 2.4 | Screening and Characterization of Prospective Hypoglycemic, Hypolipidemic and Hypouricemic Inhibitors Using UF-LC-MS/MS

#### 2.4.1 | Ultrahigh-Performance Liquid Chromatography Quadrupole Time-of-Flight Tandem Mass Spectrometry (UPLC-Q-TOF-MS/MS) Analysis

The chemical composition of *A. grossedentata* extract were comprehensively characterized by UPLC-Q-TOF-MS/MS assessment conducted on an Agilent 1290 ultra-high-performance liquid chromatography system connected to an Agilent 6545 quadrupole time-of-flight tandem mass spectrometry system.

#### 2.4.2 | Affinity Ultrafiltration

The screening process was performed based on previously established protocols with some modifications (Fan et al. 2024). First, *A. grossedentata* extract (5 mg/mL), powder dissolved in phosphate buffer was separately mixed with  $\alpha$ -glucosidase (6 U, pH 6.8), pancreatic lipase (2 mg/mL, pH 7.4), or XOD (6 U,

pH 7.4) at 37°C for 1 h, respectively. Subsequently, mixtures were moved into ultrafiltration membranes utilizing membranes with molecular weight cut-offs of 30 or 10 kD. Non-specifically bound constituents were eliminated through rinsing with phosphate buffer and centrifuging at 6000g for 10 min. In contrast, the inactivated  $\alpha$ -glucosidase, pancreatic lipase and XOD were employed as negative controls. Denaturation of these enzymes was achieved by heating them in boiling water for 15 min and the residual activity was confirmed in Supporting Information S1: Figures S1–S3. Non-specific binding regarding physical adsorption on the ultrafiltration membrane was evaluated in Supporting Information S1: Figure S4. Then, methanol (90%, v/v) was introduced and the mixture was allowed to react for 20 min to dissociate the complexes and elute the bound ligands. Recovery evaluations were demonstrated in Supporting Information S1: Figures S5–S8. All tubes stood for 10 min, and were centrifuged again. Three elution steps ensured maximal ligand recovery. At last, the collected elution was dried off by a mild stream of nitrogen and was redissolved in methanol and further analyzed.

## 2.5 | Molecular Docking Simulation

Molecular docking analysis and visualization were carried out to elucidate the mode of binding of prospective ligands with the target enzymes. Analysis was done following a previously reported procedure (Liang et al. 2023). Initially, the 3D structures of  $\alpha$ -glucosidase (PDB: 3A4A), pancreatic lipase (1LPB) and XOD (1FIQ) were downloaded from RCSB Protein database ([www.rcsb.org](http://www.rcsb.org)). The binding site was defined as a 60 × 60 × 60 grid encompassing the receptor cavity, with the grid box center coordinates set to X: 21.519, Y: −7.702, Z: 23.554 for  $\alpha$ -glucosidase; X: 5.998, Y: 18.427, Z: 37.754 for pancreatic lipase; and X: 26.544, Y: 10.162, Z: 113.365 for XOD. All remaining parameters were maintained at their default values. Subsequently, the docking conformations were sorted in accordance with their binding energies, and the pose exhibiting the minimum binding energy was selected for three-dimensional visualization and detailed binding mode analysis using PyMOL software.

## 2.6 | Statistical Analysis

All detection data are presented as the mean value  $\pm$  standard deviation (SD) based on three independent measurements. The half-maximal inhibitory concentration (IC<sub>50</sub>) was calculated by nonlinear regression fitting of the complete dose–response curves using a four-parameter logistic (4PL) model (SPSS Statistics 24.0). Statistical analysis and graphical representation were conducted using SPSS Statistics 24.0, Origin 2018, and ChemDraw V20.

## 3 | Results and Discussion

### 3.1 | Inhibitory Potential of *A. grossedentata* Extracts Towards $\alpha$ -glucosidase, Pancreatic Lipase and XOD

In vitro enzyme activity inhibition evaluations were carried out to examine the modulatory potential of *A. grossedentata* extract

towards  $\alpha$ -glucosidase, pancreatic lipase, and XOD. The results demonstrated that the IC<sub>50</sub> value of *A. grossedentata* extract for  $\alpha$ -glucosidase inhibition was  $15.36 \pm 0.24 \mu\text{g/mL}$  (Figure 1A), markedly reduced compared to the positive control acarbose ( $232.90 \pm 19.10 \mu\text{g/mL}$ ) (Figure 1B). In the pancreatic lipase inhibition assay, the IC<sub>50</sub> values of *A. grossedentata* extract and the Zhimaikang capsule was  $0.86 \pm 0.06 \text{ mg/mL}$  (Figures 1C) and  $4.25 \pm 0.04 \text{ mg/mL}$  (Figure 1D), respectively. The results indicated that the *A. grossedentata* extract exhibited remarked pancreatic lipase inhibitory activity, which was fivefolds higher than that of the positive control, Zhimaikang capsules (a type of traditional Chinese medicine used for lipid-lowering regulation). For XOD, *A. grossedentata* extract demonstrated potent inhibitory effects showing an IC<sub>50</sub> concentration of  $74.22 \pm 2.12 \mu\text{g/mL}$  (Figure 1E), which was comparable with the IC<sub>50</sub> value of the positive control allopurinol ( $3.63 \pm 0.07 \mu\text{g/mL}$ ) (Figure 1F). The *A. grossedentata* extract exhibited pronounced inhibitory activities against  $\alpha$ -glucosidase, pancreatic lipase and XOD, in contrast to fractionated dark tea water extracts, *Monascus purpureus*-fermented mulberry leaves extracts and commonly consumed vegetables or folk medicinal herb extracts, demonstrating its promise as a valuable resource of natural inhibitors for these enzymes (Ooi et al. 2021; Wang et al. 2023; Yu et al. 2025). These findings also suggest its promising prospects for the regulation and intervention of diabetes, obesity, and hyperuricemia.

### 3.2 | Screening of Potential Hypoglycemic, Hypolipidemic and Hypouricemic Ligands in *A. grossedentata* Extracts

Generally, the therapeutic effects of natural functional foods are primarily ascribed to their distinctive bioactive chemical constituents. As a traditional Chinese health-promoting food, *A. grossedentata* has been extensively studied for its potential in managing diabetes, obesity, and hyperuricemia (Zeng et al. 2023; Zhou et al. 2024; Luo et al. 2023). Nevertheless, its specific functional components and underlying mechanisms of action remain to be completely clarified. UF-LC-MS/MS is a molecular-based conjugator screening approach that potently circumvents the labor-intensive procedure of isolating and identifying non-bioactive constituents. It has been extensively employed for the efficient identification and characterization of biologically active constituents in intricate natural matrices (Li et al. 2022). In the present work, the glucose-lowering, lipid-reducing, and urate-lowering effects of *A. grossedentata* were examined through inhibition assays choosing  $\alpha$ -glucosidase, pancreatic lipase, and XOD as key enzymatic targets. The underlying mechanism of UF-LC-MS/MS employed in this work is summarized as detailed below. Initially, the target protein is allowed to react with candidate binding molecules extracted from the sample to generate enzyme–ligand conjugates via specific molecular associations. Then, the formed assemblies are effectively isolated from the remaining components using an highly selective filtration barrier. Lastly, 90% methanol is introduced to dissociate the ligands from the enzyme–ligand complexes, thereby facilitating their quantitative and qualitative assessment via UPLC-Q-TOF-MS/MS evaluation.

In particular, the intensity of bio-affinity binding is diverse for different kind of compounds which are demonstrated by peak

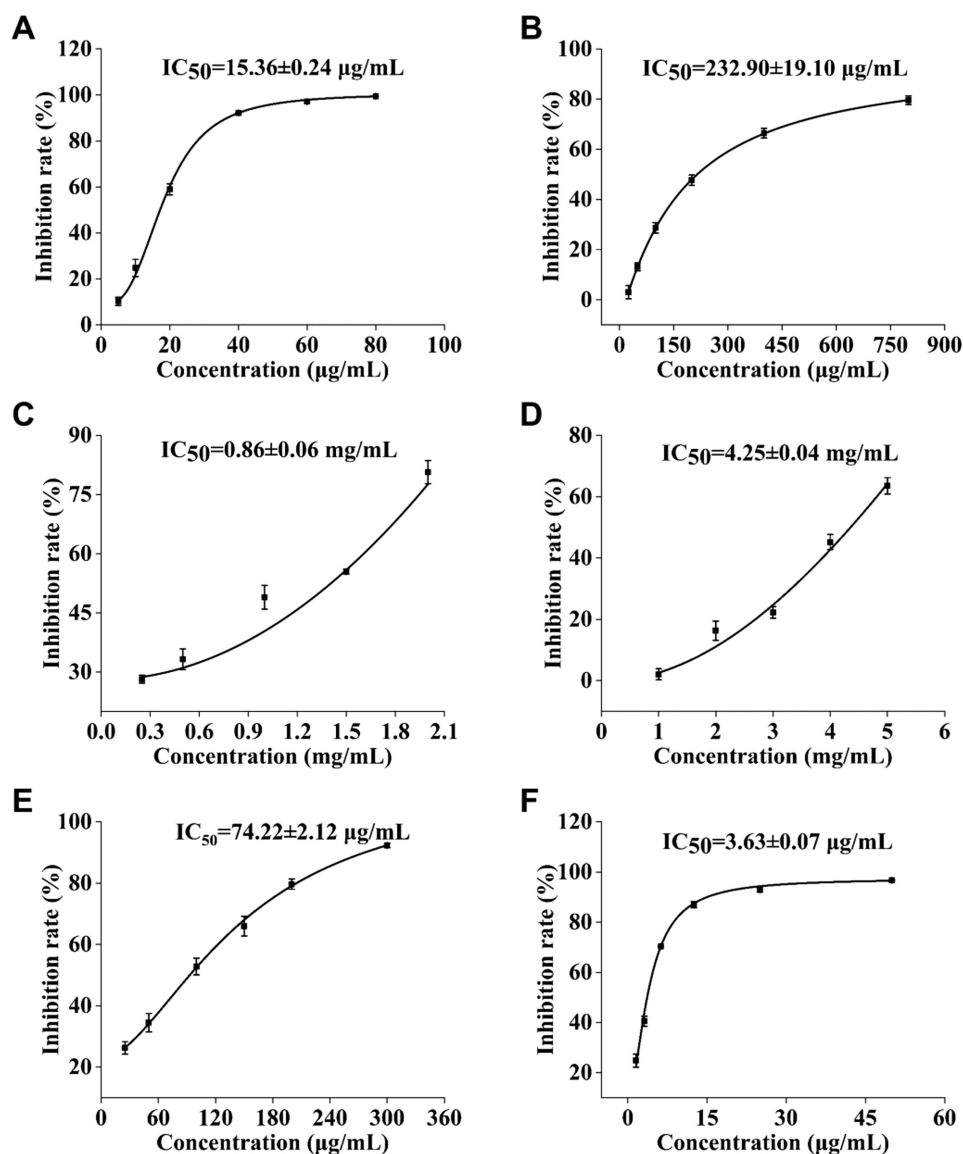


**FIGURE 1** | The  $\alpha$ -glucosidase inhibition potential of *Ampelopsis grossedentata* extract (A) and acarbose (B), lipase inhibitory activities of *A. grossedentata* extract (C) and Zhimaikang capsule (D), and XOD inhibitory activities of *A. grossedentata* extract (E) and allopurinol (F).

area of chromatogram as changed before and after incubation with respective enzyme (Figure 2). Binding degree (BD) is obtained as the relative intensity of the peak of compound following its incubation with intact versus inactive enzymes and is used as quantitative estimator of specific binding to given protein target (Zhang et al. 2025a). Thus, the potential ligands were found to have high binding affinities towards  $\alpha$ -glucosidase, pancreatic lipase and XOD based on their relatively higher BD values. In the present work, total of 6, 10, and 15 components in *A. grossedentata* were found to have different binding strength bioaffinity towards three enzymes, respectively (Table 1). For the inhibition of  $\alpha$ -glucosidase, peak 15 exhibited the highest value BD at 74.8%, while peaks 12 and 13 respectively showed BD 59.4% and 57.6%, respectively. For pancreatic lipase, peak 9 represented the greatest BD value (70.5%), then peak 10 (69.5%) and peak 6 (67.0%). As to XOD, peak 7 and peak 10 showed the higher BD value (61.8% and 58.9%, respectively), and then peaks 13 and 15 exhibited the same BD value of 58.5%.

The difference of BD values between ligands and the targeted enzymes reflects the intensity of competitively interactions to a certain extent (Zhang et al. 2025a). In order to further discuss the correlation between potential bioactive compounds detected from the extract of *A. grossedentata* with their corresponding targeted enzymes, a multi-component and multi-target interaction network diagram was drawn (Figure 3). In this figure, the color depth is representing the number of ingredients bound to each target protein, while the line width for each compound is representing the strength of the binding to its corresponding target. The results suggested that these possible active substances played important roles in the hypoglycemic, hypolipidemic and hypouricemic effects of extract of *A. grossedentata* via acting on several target enzymes. Furthermore, peak 15 demonstrated the highest BD values with  $\alpha$ -glucosidase and XOD, highlighting its critical role in the inhibitory activities of *A. grossedentata* extracts. Additionally, the compounds corresponding to peak 3 and peak 10 also exert a substantial influence in the component-target interaction network with respect to pancreatic lipase and XOD.

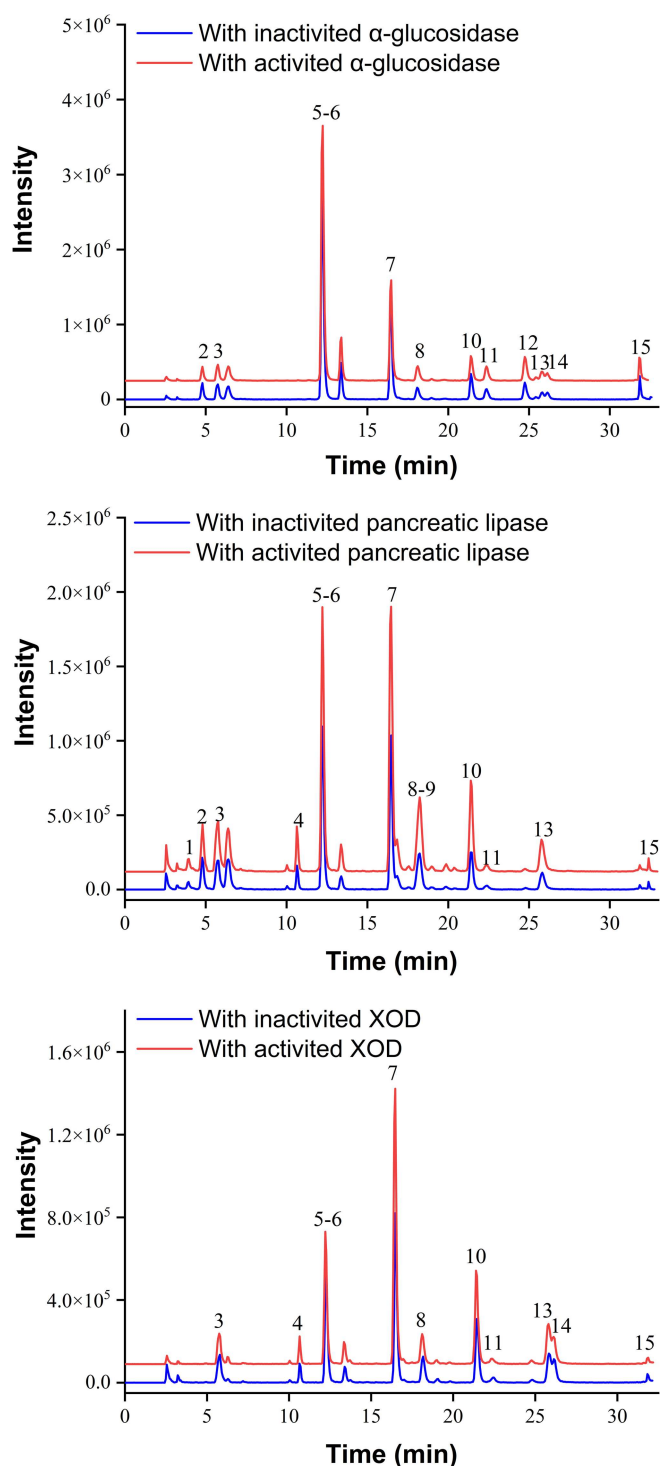


**FIGURE 2** | The HPLC fingerprint patterns of the bioactive components in *Ampelopsis grossedentata* detected at 280 nm following ultrafiltration. The red and blue line correspond to samples incubated with activated and inactivated  $\alpha$ -glucosidase, pancreatic lipase, and XOD, respectively.

### 3.3 | Identification of Potential Inhibitors in *A. grossedentata* Extracts

The chemical components of *A. grossedentata* extracts were comprehensively characterized using UPLC-Q-TOF-MS/MS and 13 peaks were preliminarily characterized in total (except

peaks of 4 and 9). In principle, each peak can be identified based on its RT, quasi-molecular ions ( $[M-H]^-$ ) and distinctive fragmentation pathways through comparison with those reported in previous literature or obtained from standard substances. Detailed mass spectrometry data for each compound are presented in Table 1, and the respective corresponding molecular structures are depicted in Figure 4. Most of these components were identified as flavonoid compounds, including dihydromyricetin, quercetin-3-O-(2-caffeoyl)-glucoside, myricitrin, taxifolin, quercitrin, myricetin-3-O-gallate, apigenin-7-glucoside and quercetin. These findings align with those previously recorded in the prior investigation (Zhang et al. 2025b).

Peak 1 generated a precursor ion at  $m/z$  331.06  $[M-H]^-$  along with a diagnostic fragment ion of  $m/z$  169.01 which was associated with the absence of a glucose group  $[M-Glu-H]^-$ . The specific fragment ion of  $m/z$  169.01 then further lost  $-COOH$  to generate  $m/z$  125.00  $[M-COOH]^-$ . Furthermore, the fragment ion at  $m/z$  169.01 also underwent Diels-Alder reaction (RDA) cleavage in negative ion mode to generate the distinctive fragment ion at  $m/z$  151.00. Peak 1 was initially characterized as  $\beta$ -glucogalin through comparison to the mass spectrum fragmentation fragments in previous study (Han et al. 2022). For peak 2, the parent ion was determined to be  $m/z$  169.01  $[M-H]^-$ , with characteristic fragment ions at  $m/z$  125.02. The ion at  $m/z$  125.02 was formed by the absence of a  $CO_2$  group from the parent ion. The comparison with standard substance confirmed that peak 2 corresponded to gallic acid (Liu et al. 2020; Ren et al. 2021). Peak 3 displayed a parent ion at  $m/z$  153.01  $[M-H]^-$  with two characteristic fragment ions at  $m/z$  125.02  $[M-CO-H]^-$  and  $m/z$  107.01  $[M-CO-H_2O-H]^-$ , which originated from the depletion of the CO group and further release of the  $H_2O$  group. Peak 3 was determined as 3,4-dihydroxybenzoic acid through comparison with the MS fragments of standard substance (mass error < 10 ppm) (Liu et al. 2020). Peak 5 showed a parent ion at  $m/z$  319.04  $[M-H]^-$  with three diagnostic fragment ions at  $m/z$  301.03,  $m/z$  193.01, and  $m/z$  125.02. The ion at  $m/z$  301.03  $[M-H_2O-H]^-$  was generated through the removal of a  $H_2O$  group from the precursor ion, and the further RDA cleavage led to the generation of ion at  $m/z$  151.00. The diagnostic fragment peaks detected at  $m/z$  193.01 and  $m/z$  125.02 were derived from the C-ring cleavage of the parent ion. Through comparison of the MS fragmentation patterns with standard substance (purity 98%), Peak 5 was identified as dihydromyricetin (Wang, Tan, et al. 2025). Dihydromyricetin is a major bioactive constituent of *A. grossedentata* and has been reported to possess lipid-lowering, antioxidant, and hepatoprotective activities, which are closely associated with metabolic regulation (Cheng et al. 2025). Peak 6 exhibited a parent ion at  $m/z$  639.05  $[M-H]^-$ . It produced a signature fragment ion at  $m/z$  319.04  $[M-C_{16}H_{16}O_7-H]^-$  subsequent to the removal of a caffeoyl glucose glycoside group. Then, a characteristic fragment ion at  $m/z$  193.01 was formed through C-ring fragmentation. Based on the MS fragments information from the literature, it is tentatively characterized as quercetin-3-O-(2-caffeoyl)-glucoside (Kiselova-Kaneva et al. 2022). For peak 7, the parent ion was identified as  $m/z$  463.08  $[M-H]^-$ , exhibiting a distinctive fragment ion at  $m/z$  316.02  $[M-C_6H_{11}O_4-H]^-$ . These fragments were verified against standard substances and confirmed that peak 7 corresponded to myricitrin. Myricitrin has been documented to exhibit

**TABLE 1** | The BD results and the characterization of the biologically active compounds from *Ampelopsis grossedentata* that bind to  $\alpha$ -glucosidase, pancreatic lipase, and XOD targets.

| No. | RT (min) | [M-H] <sup>-</sup> (m/z) | MS/MS (m/z)                                                    | BD            |       |       | Identification                         |
|-----|----------|--------------------------|----------------------------------------------------------------|---------------|-------|-------|----------------------------------------|
|     |          |                          |                                                                | $\alpha$ -Glu | PL    | XOD   |                                        |
| 1   | 3.951    | 331.06                   | 331.06, <b>169.01</b> , 151.00, 125.00                         | —             | 0.648 | —     | $\beta$ -Glucogalin                    |
| 2   | 4.801    | 169.01                   | 169.01, 126.02, <b>125.02</b>                                  | 0.449         | 0.590 | 0.399 | Gallic acid <sup>a</sup>               |
| 3   | 5.748    | 153.01                   | <b>153.01</b> , 151.01, 125.02, 109.02, 107.01                 | 0.494         | 0.601 | 0.525 | 3,4-Dihydroxybenzoic acid <sup>a</sup> |
| 4   | 10.635   | 431.19                   | 431.19, 385.18, 205.12, <b>153.09</b> , 125.02                 | 0.547         | 0.658 | 0.566 | Unknown                                |
| 5   | 12.231   | 319.04                   | 319.04, 301.03, 257.04, <b>193.01</b> , 175.00, 151.00, 125.02 | 0.549         | 0.658 | 0.537 | □ Dihydromyricetin <sup>a</sup>        |
| 6   | 12.233   | 639.05                   | <b>319.04</b> , 301.03, 193.01                                 | 0.576         | 0.670 | 0.558 | Quercetin-3-O-(2-caffeoyl)-glucoside   |
| 7   | 16.470   | 463.08                   | 463.08, 348.54, <b>316.02</b> , 317.02                         | 0.557         | 0.662 | 0.618 | Myricitrin <sup>a</sup>                |
| 8   | 18.110   | 303.05                   | 303.05, <b>285.04</b> , 275.05, 125.02                         | 0.552         | 0.631 | 0.538 | □ Taxifolin <sup>a</sup>               |
| 9   | 18.293   | 637.08                   | 637.08, 511.04, <b>385.01</b>                                  | 0.501         | 0.705 | 0.537 | Unknown                                |
| 10  | 21.390   | 447.09                   | 447.09, <b>301.03</b> , 300.02, 271.02, 178.99, 151.00         | 0.499         | 0.695 | 0.589 | □ Quercitrin                           |
| 11  | 22.305   | 303.05                   | 303.05, 285.04, <b>151.00</b> , 123.29                         | 0.568         | 0.621 | 0.504 | Taxifolin isomer                       |
| 12  | 24.735   | 471.05                   | 471.05, <b>319.04</b> , 193.01                                 | 0.594         | —     | —     | Myricetin-3-O-gallate                  |
| 13  | 25.831   | 301.03                   | 301.03, 283.02, 273.03, 191.03, <b>151.00</b>                  | 0.576         | 0.660 | 0.585 | Quercetin isomer                       |
| 14  | 26.135   | 431.09                   | <b>431.09</b> , 271.09                                         | 0.503         | 0.661 | 0.545 | Apigenin-7-glucoside                   |
| 15  | 31.815   | 301.03                   | 301.03, 273.03, 178.99, <b>151.00</b> , 121.02, 107.01         | 0.748         | 0.659 | 0.585 | □ Quercetin <sup>a</sup>               |

Abbreviations:  $\alpha$ -Glu,  $\alpha$ -glucosidase; [M-H]<sup>-</sup>, quasi-molecular ions; PL, pancreatic lipase; RT, retention time; XOD, xanthine oxidase.  
<sup>a</sup>Standard, confirmed based on the characteristic fragment ions of standard substances (purity  $\geq$  98%) analyzed under identical LC/MS conditions, whereas peaks without an asterisk were tentatively identified based on MS/MS fragmentation patterns. The bolded entries indicate the ion fragments exhibiting the highest signal intensities. “—” indicates no detectable binding under the experimental conditions. “Unknown” indicates indefinable substances.

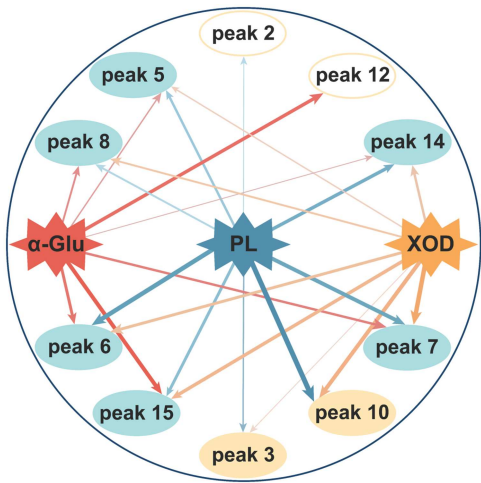


**FIGURE 3** | A network illustration depicting the interactions between multiple potential bioactive constituents isolated from *Ampelopsis grossedentata* and their associated target enzymes.

antioxidant, anti-inflammatory, and antihyperglycemic activities, suggesting its potential contribution to the observed enzyme inhibitory effects (Zhang et al. 2016). Peak 8 shared common precursor ion of  $m/z$  303.05 [M-H]<sup>-</sup> with peak 11, indicating that they share the same molecular formula yet exhibit distinct structural configurations. The loss of a group of OH or CO resulted in characteristic fragmentation ions at  $m/z$  285.04 or  $m/z$  275.05. Through comparison of the MS fragmentation patterns with

standard substance, Peak 8 was identified as taxifolin and Peak 11 was identified as taxifolin isomer (Li et al. 2018). For peak 10, the parent ion was determined to be  $m/z$  447.09 [M-H]<sup>-</sup>, featuring a distinctive fragment ion at  $m/z$  301.03 by the loss of a rhamnoside group. Then, through RDA reaction cleavage, it generated characteristic fragmentation ions at  $m/z$  178.99 and  $m/z$  151.00. Compared with standard, peak 10 was assigned to quercitrin.  $M/z$  471.05 [M-H]<sup>-</sup> was the precursor ion of peak 12 with diagnostic fragment ions at  $m/z$  319.04 and  $m/z$  193.01. The formation of  $m/z$  319.04 could be due to removal of galloyl moiety from the parent ion followed by RDA reaction leading to  $m/z$  193.01 fragment. Combined with fragmentation profile and comparison to published data, this ingredient was provisionally characterized as myricetin-3-O-gallate (Peixoto Araujo et al. 2020). Peak 14 presented a parent ion at  $m/z$  431.09 [M-H]<sup>-</sup> and the absence of glucoside group formed a diagnostic fragment ion at  $m/z$  271.09 [M-C<sub>6</sub>H<sub>8</sub>O<sub>5</sub>-H]<sup>-</sup>. By comparing the fragmentation patterns with that reported in previous study, Peak 14 was identified as apigenin-7-glucoside (Tahir et al. 2012). For peak 15, the quasi-molecular ion was determined to be  $m/z$  301.03 [M-H]<sup>-</sup> with signature fragment ions of  $m/z$  273.03 [M-CO-H]<sup>-</sup>,  $m/z$  151.00, and  $m/z$  178.99. Peak 13 shared common parent ion with peak 15, indicating that they might be structural isomers. The characteristic fragment ion at  $m/z$  273.03 [M-CO-H]<sup>-</sup> corresponds to the elimination of a CO moiety from parent ion, whereas  $m/z$  151.00 and  $m/z$  178.99 were generated via RDA reactions. Quercetin possesses a wide range of biological activities, including antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, and urate-lowering effects, and has been demonstrated to play an important role in

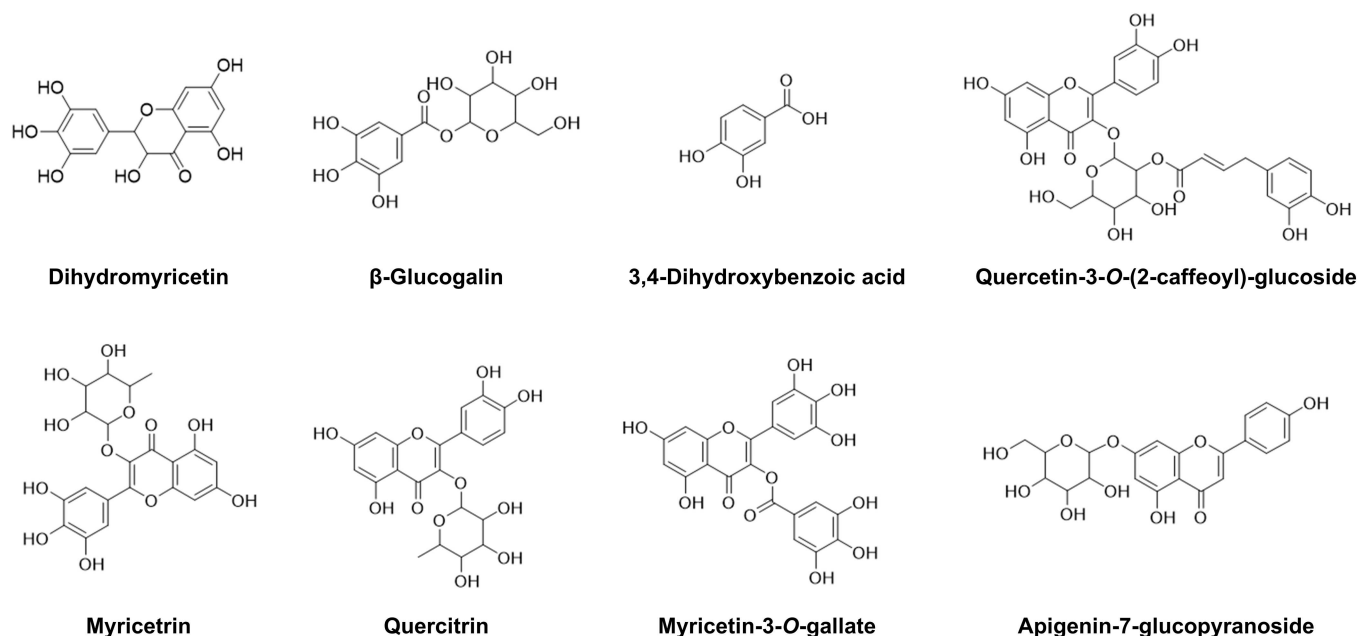


**FIGURE 4** | Structural configurations of the bioactive chemical constituents isolated and characterized from *Ampelopsis grossedentata*.

the prevention and management of diabetes (Alizadeh and Ebrahimzadeh 2022). Peak 15 was assigned as quercetin according to the signature fragment ion patterns of quercetin standard substance (purity 98%) analyzed under identical LC-MS/MS conditions.

### 3.4 | Molecular Docking

In this study, computational docking simulation was employed to attain a deeper and more comprehensive understanding of the molecular binding mechanisms among the candidate ligands and their respective target proteins. Binding energy (BE) refers to the predicted change in free energy upon formation of the ligand–protein complex during molecular docking calculations and is typically expressed in kcal/mol. In general, lower (more negative) BE values indicate more stable binding and stronger affinity between the ligand and the target protein. Four potential ligands fished out in Table 1 were selected to conducted virtual screening analysis. The docking data of the ligands with three enzyme targets were summarized in Table 2, including binding energy (BE), estimated  $IC_{50}$  values, and hydrogen bond formation details. Moreover, the 3D docking simulations of quercetin and myricetrin bound to  $\alpha$ -glucosidase, pancreatic lipase, and XOD were illustrated in Figure 5. As shown in Table 2 and Figure 5, Peak 15 (quercetin) exhibited varying binding affinities toward its corresponding enzymes, which are reflected by differences in BE values. Specifically, lower binding energy indicates stronger ligand–enzyme interactions. For  $\alpha$ -glucosidase, quercetin demonstrated a BE parameter of  $-5.99$  kcal/mol and an  $IC_{50}$  result of  $40.34$   $\mu$ M, exhibited comparable values to those of the positive control acarbose. In addition, Figure 5A reveals that quercetin could readily access the active site cavity and establish hydrogen bonds with residues such as GLN279, GLU277, ASP215, ASP69, GLU411 and ARG422. GLN279 and GLU277 have also been documented as  $\alpha$ -glucosidase binding residues (Liu et al. 2025; Sadeghi et al. 2024). For pancreatic lipase, quercetin exhibited a

BE value of  $-5.96$  kcal/mol and an  $IC_{50}$  concentration of  $42.99$   $\mu$ M, while forming hydrogen bonds with residues including LYS23, ASP249, ASP25, ASP331 and LYS268. For XOD, quercetin showed a BE value of  $-7.19$  kcal/mol, an  $IC_{50}$  value of  $5.38$   $\mu$ M, and interacted via hydrogen bonding with residues including GLU1261, ARG880, ALA1079 and THR1010. Among these, ALA1079 was likewise recognized as a probable XOD interaction residue in a previous reported study (Song et al. 2023). In summary, the molecular docking results aligned well with the ultrafiltration data. Furthermore, the interaction details of myricetrin with the three target enzymes were also presented in Table 2 and Figure 5, where the BE value with  $\alpha$ -glucosidase was  $-5.18$  kcal/mol and the  $IC_{50}$  value was  $159.24$   $\mu$ M. This can be attributed to the generation of hydrogen bonding interactions involving residues of GLN279, GLU277, ASP307, GLN353, ARG315 and ARG442. Regarding pancreatic lipase inhibition, myricetrin presented a BE value of  $-5.06$  kcal/mol and an  $IC_{50}$  concentration of  $196.29$   $\mu$ M, which was contributed to the hydrogen bonds generation with residues of ASP247, ASP31, ASP331, ARG38 and LEU41. In the case of XOD, myricetrin demonstrated a BE value of  $-5.98$  kcal/mol and an  $IC_{50}$  value of  $41.47$   $\mu$ M, attributed to its ability to readily access the active site pocket and establish hydrogen bonding interactions with the residues of ASN768, ALA1079, THR1010 and SER876. These findings suggested that quercetin and myricetrin could interact with their respective enzymes through hydrogen bonding with varying binding affinities. These hydrogen bonds may induce conformational changes in the enzymes, thereby inhibiting their activities. Moreover, the binding relationship among the putative biologically active molecules and the three target enzymes was also significantly influenced by van der Waals forces, alkyl interactions, H-bond, and pi-pi stacking interactions (Ha et al. 2025). In addition, the bioactive compounds dihydromyricetin and 3,4-dihydroxybenzoic acid also exhibited significant inhibitory effects against all three target enzymes. Specifically, dihydromyricetin displayed the lowest BE value of  $-6.03$  kcal/mol and an  $IC_{50}$  concentration of  $38.15$   $\mu$ M against  $\alpha$ -glucosidase, which can be attributed to hydrogen bonding with

**TABLE 2** | The computational docking simulations of the characteristic active phytochemicals in *Ampelopsis grossedentata* and reference inhibitors towards  $\alpha$ -glucosidase, pancreatic lipase, and XOD enzymes.

| No. | Compound                  | Target protein | BE <sup>a</sup> (kcal/mol) | IC <sub>50</sub> ( $\mu$ M) | H-bond atoms <sup>b</sup>                      |
|-----|---------------------------|----------------|----------------------------|-----------------------------|------------------------------------------------|
| 1   | Quercetin                 | $\alpha$ -Glu  | −5.99                      | 40.34                       | GLN279, GLU277, ASP215, ASP69, GLU411, ARG422  |
|     |                           | PL             | −5.96                      | 42.99                       | LYS23, ASP249, ASP25, ASP331, LYS268           |
|     |                           | XOD            | −7.19                      | 5.38                        | GLU1261, ARG880, ALA1079, THR1010              |
| 2   | Myricetrin                | $\alpha$ -Glu  | −5.18                      | 159.24                      | GLN279, GLU277, ASP307, GLN353, ARG315, ARG442 |
|     |                           | PL             | −5.06                      | 196.29                      | ASP247, ASP31, ASP331, ARG38, LEU41            |
|     |                           | XOD            | −5.98                      | 41.47                       | ASN768, ALA1079, THR1010, SER876               |
| 3   | Dihydromyricetin          | $\alpha$ -Glu  | −6.03                      | 38.15                       | ASP69, ASP215, ASP352, ARG442, ARG315, GLN279  |
|     |                           | PL             | −6.67                      | 12.87                       | GLN29, ASP31, ASP331, CYS39, ASP247, LYS239    |
|     |                           | XOD            | −6.84                      | 9.73                        | ALA1079, GLU1261, THR1010, ARG880              |
| 4   | 3,4-Dihydroxybenzoic acid | PL             | −5.19                      | 157.15                      | ASP257, ASP331, LYS239, ASP249, ARG65          |
|     |                           | XOD            | −5.5                       | 95.85                       | ARG880, THR1010, GLU802                        |
|     |                           | $\alpha$ -Glu  | −9.32                      | 0.15                        | ASP307, ARG315, LYS156, SER241, ASP242         |
| 5   | Acarbose <sup>c</sup>     |                |                            |                             | LYS268, SER333                                 |
| 6   | Orlistat <sup>d</sup>     | PL             | −6.93                      | 8.38                        |                                                |
| 7   | Allopurinol <sup>e</sup>  | XOD            | −6.09                      | 34.62                       | THR1010, GLU802, SER1008                       |

<sup>a</sup>BE, binding energy; <sup>b</sup>H-bond, hydrogen bond; <sup>c</sup>, <sup>d</sup>, <sup>e</sup>positive drug.

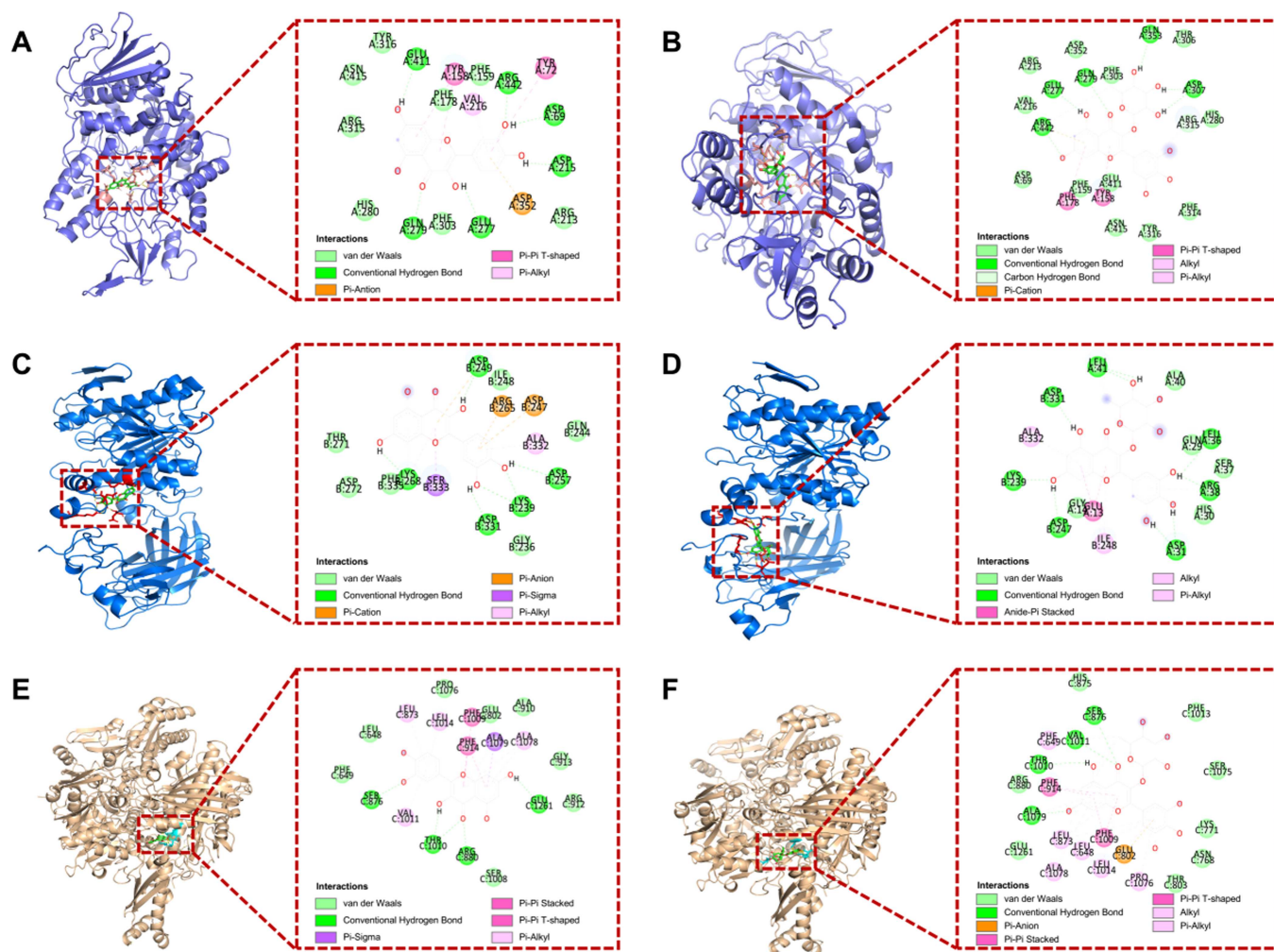


**FIGURE 5** | Interactions between  $\alpha$ -glucosidase and quercetin (A) and myricitrin (B), pancreatic lipase and quercetin (C) and myricitrin (D), XOD and quercetin (E) and myricitrin (F) by molecular docking analysis. H-bonds, hydrogen bonds.

the residues of ASP69, ASP215, ASP352, ARG442, ARG315, and GLN279. In the context of pancreatic lipase inhibition, dihydromyricitrin showed a BE of  $-6.67$  kcal/mol and an  $IC_{50}$  concentration of  $12.87 \mu M$ , resulting from the formation of hydrogen bonds with the residues of GLN29, ASP31, ASP331, CYS39, ASP247, and LYS239. For XOD, dihydromyricitrin demonstrated a BE value of  $-6.84$  kcal/mol and an  $IC_{50}$  of  $9.73 \mu M$ , owing to its capacity to efficiently penetrate the active site pocket and establish hydrogen bonding interactions with ALA1079, GLU1261, THR1010, and ARG880.

### 3.5 | Potential Ligand Inhibition Validation In Vitro

Computational docking analysis demonstrated quercetin and myricitrin had the ability to interact with the catalytic sites of  $\alpha$ -glucosidase, pancreatic lipase and XOD, respectively. To further validate the inhibitory potential of the screened ligands, in vitro enzyme inhibition assays were performed using the corresponding pure compounds. As summarized in Table 3, quercetin displayed pronounced inhibitory activities against  $\alpha$ -glucosidase, pancreatic lipase, and XOD, with  $IC_{50}$  values of  $4.26 \pm 0.26$ ,  $85.45 \pm 3.45$ , and  $2.59 \pm 0.13 \mu g/mL$ , respectively, which corresponded to their

lowest values of predicted  $IC_{50}$  and demonstrated its strong multi-target inhibitory capacity. These results were also in a good agreement with the BD values of quercetin against  $\alpha$ -glucosidase, pancreatic lipase and XOD. In contrast, myricitrin exhibited moderate inhibitory effects toward the three enzymes, with  $IC_{50}$  values of  $202.35 \pm 8.86 \mu g/mL$  for  $\alpha$ -glucosidase,  $273.64 \pm 11.06 \mu g/mL$  for pancreatic lipase, and  $270.59 \pm 10.26 \mu g/mL$  for XOD.

Taken together, the results from UF-LC-MS/MS bioaffinity screening, molecular docking simulations, and in vitro enzyme inhibition assays consistently indicated that quercetin represents the major multi-target bioactive constituent in *A. grossedentata* responsible for its hypoglycemic, hypolipidemic, and hypouricemic effects. Myricitrin may act as a secondary or synergistic inhibitor contributing to the overall bioactivity of the extract. Within the multi-components system of *A. grossedentata* extract, the coexistence of these constituents enables potential “multi-site occupancy” or “complementary binding” at distinct functional domains of the same target enzyme, potentially resulting in a more potent and stable inhibitory effect compared to individual compounds (Proença et al. 2017). The high consistency among the three independent analytical approaches further supported the reliability of the multi-target screening strategy employed in this study.

**TABLE 3** | IC<sub>50</sub> values of the bioactive ingredients screened from *Ampelopsis grossedentata* towards  $\alpha$ -glucosidase, pancreatic lipase, and XOD enzymes.

| Compound                  | IC <sub>50</sub> ( $\mu$ g/mL) |                      |                    |
|---------------------------|--------------------------------|----------------------|--------------------|
|                           | $\alpha$ -Glu                  | PL                   | XOD                |
| Quercetin                 | 4.26 $\pm$ 0.26                | 85.45 $\pm$ 3.45     | 2.59 $\pm$ 0.13    |
| Myricetrin                | 202.35 $\pm$ 8.86              | 273.64 $\pm$ 11.06   | 270.59 $\pm$ 10.26 |
| Dihydromyricetin          | 222.36 $\pm$ 6.32              | 1500.00 $\pm$ 50.00  | 83.61 $\pm$ 3.94   |
| Taxifolin                 | 193.73 $\pm$ 8.53              | 3160.00 $\pm$ 210.00 | 131.18 $\pm$ 4.40  |
| 3,4-Dihydroxybenzoic acid | —                              | 4530.00 $\pm$ 300.00 | 243.12 $\pm$ 8.37  |

Note: The results are demonstrated as the mean  $\pm$  SD, of three replicates. “—” indicates that the IC<sub>50</sub> value was greater than 5 mg/mL, suggesting no detectable inhibitory activity or only very weak activity under the experimental conditions.

4 | Conclusion

So far, research has shown that *A. grossedentata* is effective against diabetes, dyslipidemia and hyperuricemia. However, the detailed potent bioactive ingredients and their specific molecular mechanism processes remain not well and completely revealed. Here we reported the bioactive activity of *A. grossedentata* and explored its chemical composition, physicochemical properties and interaction mechanisms. *A. grossedentata* exhibited strikingly suppressive activities against  $\alpha$ -glucosidase, pancreatic lipase and XOD in vitro, indicating its potential as a promising agent for hypoglycemic, lipidaemic-reducing and anti-hyperuricemic therapies. The proposed multi-targeting UF-LC-MS/MS methodology facilitated the high-throughput and high-efficiency natural ligand screening, and accurately identified several candidate active moieties against the three significant key enzymes. The molecular docking results confirmed that active moieties might form the interactions with the active sites of these enzymes via H-bonding, hydrophobicity interaction and related mechanisms. It is worthwhile mentioning that quercetin and myricetin, the bioactive components with the widest recognition, possessed an exceptional binding affinity and enzyme inhibitory ability in this study. Besides, myricitrin was newly proved to be a multifunctional active ingredient in *A. grossedentata* with exceptional hypoglycemic, lipid-lowering and uric acid-lowering activity. The interaction network of quercetin and myricitrin with three enzymes was in silico described, thus revealing preliminary insight on its molecular mechanisms of actions. Overall, this work systematically revealed the key bioactive constituents and related mechanisms of *A. grossedentata* with hypoglycemic, lipid-lowering, and hypouricemic activities from a perspective of multi-target enzyme inhibition. These findings offer a robust empirical foundation supporting the advancement and practical use of *A. grossedentata* as a health-promoting functional food in the approaching future.

Author Contributions

**Siyang Mao:** investigation, data curation, formal analysis, software, validation, and writing – original draft. **Chu Chu:** data curation, formal analysis, software, and validation. **Yanzhi Xiong:** formal analysis, software, and visualization. **Mengjia Xu:** data curation, formal analysis, validation, writing – review and editing, funding acquisition. **Jian Yang:** conceptualization, methodology, and validation. **Mingquan Guo:** conceptualization, methodology, project administration, supervision, writing – review and editing, and funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alizadeh, S. R., and M. A. Ebrahimzadeh. 2022. “Quercetin Derivatives: Drug Design, Development, and Biological Activities, a Review.” *European Journal of Medicinal Chemistry* 229: 114068.

Borghi, C., E. Agabiti-Rosei, R. J. Johnson, et al. 2020. “Hyperuricaemia and Gout in Cardiovascular, Metabolic and Kidney Disease.” *European Journal of Internal Medicine* 80: 1–11.

Chen, Y., S. Hu, Y. Guo, et al. 2025. “Apple Polyphenols Alleviate Hyperuricemia by Inhibiting Xanthine Oxidase and Promoting Uric Acid Excretion.” *Journal of Functional Foods* 134: 107052.

Cheng, K., G. Hou, S. Mei, et al. 2025. “Structural Characterization and Bioactivity Evaluation of Selenium-Modified Dihydromyricetin From Vine Tea.” *Foods* 14, no. 10: 1735.

Fang, Y., W. Mei, C. Wang, et al. 2024. “Dyslipidemia and Hyperuricemia: A Cross-Sectional Study of Residents in Wuhu, China.” *BMC Endocrine Disorders* 24, no. 1: 2.

Feng, C., N. Zhang, D. Zhou, et al. 2018. “Determination of the Content of 4 Flavonoids in *Ampelopsis grossedentata* Leaves by HPLC.” *Science and Technology of Food Industry* 39, no. 24: 240–245.

Feng, S., S. Wu, F. Xie, C. S. Yang, and P. Shao. 2022. “Natural Compounds Lower Uric Acid Levels and Hyperuricemia: Molecular Mechanisms and Prospective.” *Trends in Food Science & Technology* 123: 87–102.

Ha, M. T., T. T. Le, D. Y. Lee, et al. 2025. “Chemical Constituents of *Himantormia lugubris* Collected From Antarctica and Their PTP1B and  $\alpha$ -glucosidase Inhibitory Activities.” *Phytochemistry Letters* 66: 91–99.

Han, Z., M. Wen, H. Zhang, L. Zhang, X. Wan, and C. T. Ho. 2022. “LC-MS Based Metabolomics and Sensory Evaluation Reveal the Critical Compounds of Different Grades of Huangshan Maofeng Green Tea.” *Food Chemistry* 374: 131796.

- Hu, Y., M. Li, L. Ding, et al. 2023. "Ampelopsis grossedentata improves Type 2 Diabetes Mellitus Through Modulating the Gut Microbiota and Bile Acid Metabolism." *Journal of Functional Foods* 107: 105622.
- Hua, X., M. Xu, M. Yang, et al. 2024. "Hypoglycemic and Hypolipidemic Bioactive Compounds From Edible Traditional Chinese Medicines and Their Action of Mechanisms Explored by Multitarget Affinity Ultrafiltration With Liquid Chromatography-Mass Spectrometry." *Food Frontiers* 6, no. 1: 65–85.
- Kiselova-Kaneva, Y., B. Galunska, M. Nikolova, I. Dincheva, and I. Badjakov. 2022. "High Resolution LC-MS/MS Characterization of Polyphenolic Composition and Evaluation of Antioxidant Activity of *Sambucus ebulus* Fruit Tea Traditionally Used in Bulgaria as a Functional Food." *Food Chemistry* 367: 130759.
- Li, J., K. Jiang, L. J. Wang, et al. 2018. "HPLC-MS/MS Determination of Flavonoids in *Gleditsiae Spina* for Its Quality Assessment." *Journal of Separation Science* 41, no. 9: 1752–1763.
- Li, Y. J., G. Z. Wan, F. C. Xu, Z. H. Guo, and J. Chen. 2022. "Screening and Identification of  $\alpha$ -glucosidase Inhibitors From *Cyclocarya paliurus* Leaves by Ultrafiltration Coupled With Liquid Chromatography-Mass Spectrometry and Molecular Docking." *Journal of Chromatography A* 1675: 463160.
- Liang, C., Y. Xu, M. Fan, et al. 2023. "Potential Antioxidative and Anti-Hyperuricemic Components in *Rodgersia podophylla* A. Gray Revealed by Bio-Affinity Ultrafiltration With SOD and XOD." *Frontiers in Pharmacology* 14: 1298049.
- Liu, Y., R. Zeng, C. Yang, J. Peng, and H. Yan. 2020. "Analysis of Chemical Composition in Sancao Baogan Decoction Based on UPLC-ESI-HRMS." *Chinese Journal of Experimental Traditional Medical Formulae* 26, no. 22: 141–147.
- Liu, Z., X. Kuang, L. Xu, X. Fan, and W. Lou. 2025. "Insights Into Novel  $\alpha$ -glucosidase ( $\alpha$ -GLU) Inhibitory Peptides From Sturgeon Fish Muscle Protein Based on In-Silico and In-Vitro Investigation." *Food Wellness* 1, no. 1: 100010.
- Lu, Y., X.-X. Fan, S.-L. Zhao, Y. Ishii, B.-Y. Yu, and R.-S. Li. 2025. "Supplements Extracted From *Lophatherum gracile* Brongn. Ameliorates Hyperuricemia by Regulating Nucleotide Metabolic Enzymes and Urate Transporters." *Food & Medicine Homology* 3, no. 2: 9420099. <https://doi.org/10.26599/FMH.2026.9420099>.
- Luo, Q., J. Qiu, M. Chen, et al. 2025. "Vine Tea (*Ampelopsis grossedentata*) Ameliorates Chronic Alcohol-Induced Hepatic Steatosis, Oxidative Stress, and Inflammation via YTHDF2/PGC-1 $\alpha$ /SIRT3 Axis." *Food Research International* 209: 116321.
- Luo, Q. J., W. C. Zhou, X. Y. Liu, et al. 2023. "Chemical Constituents and  $\alpha$ -glucosidase Inhibitory, Antioxidant and Hepatoprotective Activities of *Ampelopsis grossedentata*." *Molecules* 28, no. 24: 7956.
- Ooi, K. L., R. Zakaria, M. L. Tan, and S. F. Sulaiman. 2021. "The Influence of Chemical Composition of Potent Inhibitors in the Hydrolyzed Extracts of Anti-Hyperuricemic Plants to Their Xanthine Oxidase Activities." *Journal of Ethnopharmacology* 278: 114294.
- Peixoto Araujo, N. M., H. S. Arruda, F. N. dos Santos, D. R. de Moraes, G. A. Pereira, and G. M. Pastore. 2020. "LC-MS/MS Screening and Identification of Bioactive Compounds in Leaves, Pulp and Seed From *Eugenia calycina* Cambess." *Food Research International* 137: 109556.
- Proença, C., M. Freitas, D. Ribeiro, et al. 2017. " $\alpha$ -Glucosidase Inhibition by Flavonoids: An In Vitro and In Silico Structure–Activity Relationship Study." *Journal of Enzyme Inhibition and Medicinal Chemistry* 32, no. 1: 1216–1228.
- Ren, H., X. M. Cui, J. Hu, X. M. Liu, and Z. Y. Chen. 2021. "Analysis on Chemical Constituents in Rhizomes of *Bergenia scopolosa* by UHPLC-Q Exactive Focus MS/MS." *Chinese Journal of Experimental Traditional Medical Formulae* 27, no. 1: 118–128.
- Saag, K. G., N. Dalbeth, C. Hsu, et al. 2025. "Evaluation of the Efficacy and Safety of a Novel Xanthine Oxidase Inhibitor, Tigulixostat, in Gout Patients With Hyperuricemia: Design of the EURELIA 1 and EURELIA 2 Studies." *Contemporary Clinical Trials* 151: 107843.
- Sadeghi, M., M. Miroliaei, and M. Ghanadian. 2024. "Drug Repurposing for Diabetes Mellitus: In Silico and In Vitro Investigation of DrugBank Database for  $\alpha$ -glucosidase Inhibitors." *International Journal of Biological Macromolecules* 270: 132164.
- Song, H., J. Lu, and Q. Chu. 2024. "Polyphenols From *Prunus salicina* L. Alleviate Weight Gain, Obesity-Related Hyperlipidemia, Hepatic Steatosis, Hyperglycemia, and Modulate Gut Microbiota in Mice Fed a High-Fat Diet." *Nutrition Research* 132: 152–163.
- Song, J., M. Chen, F. Meng, et al. 2023. "Studies on the Interaction Mechanism Between Xanthine Oxidase and Osmundacetone: Molecular Docking, Multi-Spectroscopy and Dynamical Simulation." *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy* 299: 122861.
- Tahir, N. I., K. Shaari, F. Abas, G. K. A. Parveez, Z. Ishak, and U. S. Ramli. 2012. "Characterization of Apigenin and Luteolin Derivatives From Oil Palm (*Elaeis guineensis* Jacq.) Leaf Using LC-ESI-MS/MS." *Journal of Agricultural and Food Chemistry* 60, no. 45: 11201–11210.
- Wang, B., C. Wang, Y. Duan, et al. 2023. "The Effects of *Monascus purpureus* Fermentation on Metabolic Profile,  $\alpha$ -glucosidase Inhibitory Action, and In Vitro Digestion of Mulberry Leaves Flavonoids." *LWT* 188: 115449.
- Wang, W., G. Tan, Q. Lei, J. Chen, and Z. Qian. 2025. "Rapid and Environmentally Friendly Quantification of Myricetin and Dihydromyricetin in Vine Tea Using Direct Infusion–Tandem Mass Spectrometry." *Journal of Food Composition and Analysis* 140: 107193.
- Wang, Y.-X., X.-L. Chen, K. Zhou, et al. 2025. "Fucoidan Dose-Dependently Alleviated Hyperuricemia and Modulated Gut Microbiota in Mice." *Food & Medicine Homology* 3: 9420095. <https://doi.org/10.26599/FMH.2026.9420095>.
- Xu, M., H. Feng, and M. Guo. 2023. "Potential Hypoglycemic and Hypolipidemic Bioactive Constituents in *Cyclocarya paliurus* Leaves Explored by Affinity Ultrafiltration With  $\alpha$ -glucosidase and Pancreatic Lipase." *eFood* 5, no. 3: e123.
- Xue, H., M. Xu, D. Gong, and G. Zhang. 2023. "Mechanism of Flavonoids Inhibiting Xanthine Oxidase and Alleviating Hyperuricemia From Structure-Activity Relationship and Animal Experiments: A Review." *Food Frontiers* 4, no. 4: 1643–1665.
- Yang, T., L. Luo, X. Luo, and X. Liu. 2025. "Metabolic Crosstalk and Therapeutic Interplay Between Diabetes and Hyperuricemia." *Diabetes Research and Clinical Practice* 224: 112204.
- Yang, Y., J. Zhang, and Q. Zhou. 2020. "Targets and Mechanisms of Dietary Anthocyanins to Combat Hyperglycemia and Hyperuricemia: A Comprehensive Review." *Critical Reviews in Food Science and Nutrition* 62, no. 4: 1119–1143.
- Yang, Z., B. Lyu, B. Ma, Y. Gao, and D. Qin. 2024. "Screening of the Effective Sites of *Cichorium glandulosum* Against Hyperuricemia Combined With Hyperlipidemia and Its Network Pharmacology Analysis." *Computational Biology and Chemistry* 110: 108088.
- Yu, J., Y. Wu, C. Zeng, et al. 2025. "Phytochemicals From Fractioned Dark Tea Water Extract Enhance the Digestive Enzyme Inhibition, Antioxidant Capacities and Glucose-Lipid Balance." *Food Research International* 204: 115957.
- Zeng, T., Y. Song, S. Qi, R. Zhang, L. Xu, and P. Xiao. 2023. "A Comprehensive Review of Vine Tea: Origin, Research on Materia Medica, Phytochemistry and Pharmacology." *Journal of Ethnopharmacology* 317: 116788.
- Zhang, H., G. Chen, Y. Zhang, F. W. Muema, J. Ding, and M. Guo. 2024. "Screening for Bioactive Compounds From Mulberry Leaves Combining Bio-Affinity Ultrafiltration With  $\alpha$ -glucosidase and Pancreatic Lipase." *Journal of Analysis and Testing* 8, no. 3: 315–326.

Zhang, H., M. Xu, M. Yang, J. Ding, and M. Guo. 2025a. "Bioactive Compounds With Anti-Obesity Potential From *Cassia obtusifolia* L. Explored by Lipase-Immobilized Magnetic Metal-Organic Frameworks and UPLC-Q-TOF-MS/MS." *Food Research International* 217: 116779.

Zhang, J., S. Yamada, E. Ogihara, et al. 2016. "Biological Activities of Triterpenoids and Phenolic Compounds From *Myrica cerifera* Bark." *Chemistry & Biodiversity* 13, no. 11: 1601–1609.

Zhang, Y., T. Wang, S. Wu, et al. 2025b. "Vine Tea (*Ampelopsis grossedentata*)—A Different Kind of Tea Hidden Deep in the Mountains of China: A Comprehensive Review of the Nutritional Profile, Functional Effect, and Diverse Applications as a Novel Raw Material in Food Practices." *Trends in Food Science & Technology* 159: 104939.

Zhou, H. J., L. Zhang, T. Z. Li, and B. Li. 2021. "Screening and Identification of Natural Inhibitors of Tyrosinase From *Ampelopsis grossedentata* Using Ultra-Filtration Affinity-Liquid Chromatography-Mass Spectrometry." *Chinese Traditional and Herbal Drugs* 52, no. 22: 6796–6805.

Zhou, Q. X., Q. Zhou, P. Zhang, et al. 2024. "Integrating Multi-Level Interactive Network and In Vivo/Vitro Studies to Explore the Protective Mechanism of *Ampelopsis grossedentata* in Hyperuricemia." *Fitoterapia* 172: 105718.

### Supporting Information

Additional supporting information can be found online in the Supporting Information section.  
Supporting Information (2).