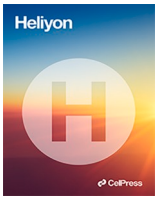
Heliyon 10 (2024) e34502

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Exploring anti-diabetic potential of compounds from roots of *Dendrobium polyanthum* Wall. ex Lindl. through inhibition of carbohydrate-digesting enzymes and glycation inhibitory activity

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ARTICLE INFO

Keywords:
Dendrobium polyanthum Wall. ex Lindl.
Alpha-glucosidase
Alpha-amylase
Advanced glycation end products
Molecular docking analysis
Kinetic study

ABSTRACT

Eight compounds, including one anthraquinone, two bibenzyls, one phenanthrene, three dihydrophenanthrenes, and one flavonoid, were isolated from the roots of *Dendrobium polyanthum* Wall. ex Lindl. Among these, six compounds were investigated for inhibitory activities against alpha-glucosidase, alpha-amylase, and advanced glycation end products (AGEs) production. Additionally, molecular docking was conducted to analyze the interactions of the test compounds with alpha-glucosidase. Moscatin, the only isolated phenanthrene, displayed the strongest anti–alpha-glucosidase activity with an IC_{50} of 32.45 ± 1.04 μM, approximately 10-fold smaller than that of acarbose. Furthermore, moscatilin most strongly inhibited alpha-amylase and AGEs production with IC_{50} values of 256.94 ± 9.87 and 67.89 ± 9.42 μM, respectively. Molecular docking analysis revealed the effective binding of all substances to alpha-glucosidase with smaller lowest binding energy values than acarbose. Moscatin was selected for kinetics studies, and it was identified as a non-competitive inhibitor with approximately 9-fold greater inhibitory capability than acarbose. This study represents the first report on the phytochemical constituents and antidiabetic potential of compounds derived from the roots of *D. polyanthum* Wall. ex Lindl.

1. Introduction

Diabetes, a chronic metabolic disorder, represents a substantial global health challenge. Its incidence is steadily increasing, with projections estimating that approximately 700 million individuals will be affected by 2045 [1]. Elevated blood sugar levels underlie

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https://doi.org/10.1016/j.heliyon.2024.e34502
Received 10 March 2024; Received in revised form 2 July 2024; Accepted 10 July 2024
Available online 11 July 2024

the disease, precipitating harm to diverse body tissues and systems, including cardiac, vascular, ocular, renal, and neural structures and functions. Alarmingly, diabetes ranks among the leading causes of mortality globally, accounting for around 1.5 million annual deaths [2].

At present, six primary classes of contemporary medications are used globally for blood glucose control, along with two classes of injectables. Oral diabetic drugs include biguanides (e.g., metformin), sulfonylureas (e.g., glibenclamide), thiazolidinediones (e.g., glitazones), meglitinides (e.g., glinides), alpha-glucosidase inhibitors (e.g., acarbose), and DPP-4 inhibitors (e.g., sitagliptin). Injectable options include incretin mimetics and insulin [3]. Multifaceted organ damage is a prevalent complication of hyperglycemia, adding complexity to comprehensive treatment. Most complications arise from the formation of advanced glycation end products (AGEs), which occur through the transformation of glucose in the bloodstream and/or glycated biomolecules. Currently, there are no approved drugs with anti-AGEs activity [4,5]. Despite the utilization of approved antidiabetic medicines, a definitive cure for diabetes remains elusive. Although existing medications display efficacy, managing diabetes without adverse side effects remains challenging [6]. Moreover, cost and accessibility challenges associated with many modern medicines have led to an increased focus on medicinal plants and their derivatives, which have been used for generations in traditional and folk medicine globally to manage diabetes [7–10]. This heightened interest is particularly pertinent because most diabetes treatments necessitate continuous and lifelong management.

Dendrobium, a substantial genus within the Orchidaceae family, encompasses approximately 1400 species [11], including 150 species in Thailand [12]. Renowned for their traditional medicinal properties, several *Dendrobium* species, particularly their stems, have been integral in traditional Chinese medicine. These treatments, known as "Shi-Hu," have been applied for their tonic, antipyretic, astringent, and anti-inflammatory attributes [13]. Previous investigations found that *Dendrobium* plants possess diverse secondary metabolites with biological activities such as cytotoxic, antioxidant, anticancer, antibacterial, antiviral, antimalarial, antifibrotic, neuroprotective, and hypoglycemic properties [14–17]. Recent studies have compiled data on *Dendrobium* plants exhibiting inhibitory activity against alpha-glucosidase, suggesting potential antidiabetic properties. Such properties have been identified in at least 10 species within this genus, e.g., *D. formosum*, *D. loddigesii*, and *D. scabrilingue*. Additionally, important substances isolated from these plants, such as confusarin, loddigesiinol B, and coelonin, have been studied extensively [18]. Additional antidiabetic effects, inferred from alpha-amylase inhibition, were detected in chemicals originating from *Dendrobium* species, such as dendronobiloside A from *D. nobile* [19] and 3,4-dihydroxy-4′,5-dimethoxybibenzyl from *D. officinale* [20]. The levels of AGEs, indicative of the complexity and progression of diabetes, have been observed to decrease upon treatment with the extracts of various *Dendrobium* plants, but the responsible compounds remain unidentified. Examples of *Dendrobium* species with anti-AGEs activity include *D. huoshanense* [21], *D. sulcatum* [22], *D. officinale* [23], and *D. parishii* [24].

D. polyanthum Wall. ex Lindl (Fig. 1A). is indigenous to the Himalayas, extending to China, particularly Southern Yunnan, and

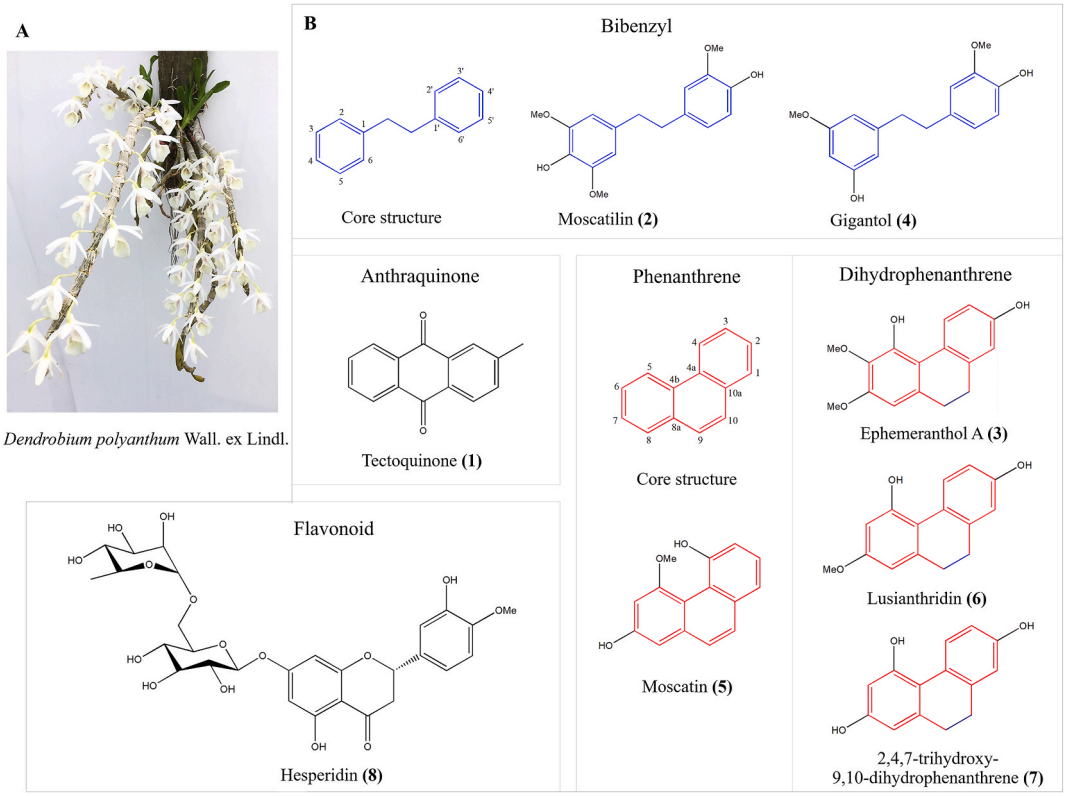

Fig. 1. (**A**) *Dendrobium polyanthum* Wall. ex Lindl. (**B**) The structures of isolated compounds (**1**–**8**).

Indo–China [25]. Prior research identified chemical constituents isolated from the stem of *D. polyanthum* Wall. ex Lindl [26]. Nevertheless, the biological activity of extracts or isolated substances from parts other than the stem of this plant have not been studied. As part of our continuous exploration of bioactive constituents in *Dendrobium* species, a methanolic extract derived from the entire plant of *D. cretaceum* Lindl., identified as a synonym of *D. polyanthum* Wall. ex Lindl. [27–30], exhibited moderate inhibitory effects. At concentrations of 100 and 250 μg/mL, the extract inhibited alpha-amylase activity by more than 70 % and alpha-glucosidase activity by more than 50 % [24].

A recent review suggested that essential antidiabetic compounds might be concentrated in specific parts of the plant, underscoring the potential of plant roots and rhizomes in managing hyperglycemia [31]. However, comprehensive data on the roots of any plant within the *Dendrobium* genus exhibiting antidiabetic properties have not been reported. This study specifically focused on extracting essential substances from the roots of *D. polyanthum* Wall. ex Lindl., aiming to fill the gap in knowledge regarding the antidiabetic compounds present in the roots of this plant. This study represents the first exploration of chemical constituents isolated from the roots of *D. polyanthum* Wall. ex Lindl. Comprehensive analyses using *in vitro* assays to assess the inhibitory activity of compounds against alpha-amylase and alpha-glucosidase, the key enzymes responsible for metabolizing polysaccharides into absorbable glucose units in the bloodstream, were performed. An analysis of anti-AGEs production as a marker for decreased chronic diabetic complications was additionally conducted. Molecular docking analysis as an *in silico* methodology was performed to evaluate the potential anti-hyperglycemic properties of the compounds. An enzyme kinetics study was performed to reveal the mode of alpha-glucosidase inhibition for an isolated compound. Our findings highlight the potential of this plant as a promising resource for the development of novel antidiabetic agents.

2. Materials and methods

2.1. Plant material

The roots of *Dendrobium polyanthum* Wall. ex Lindl. was purchased from Chatuchak market, Bangkok, in August 2017. Plant identification was conducted by one of the authors (B. Sritularak). The plant specimen was comprehensively compared with herbarium specimens from the Department of National Parks, Wildlife, and Plant Conservation (DPN), Ministry of National Resources and Environment, Thailand. A voucher specimen (BS-DC-082560) is on deposit at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2.2. Extraction and isolation

Air dried and powdered roots (0.4 kg) of *Dendrobium polyanthum* Wall. ex Lindl. were macerated with MeOH (3 × 6 L) for 24 h at room temperature, giving a MeOH extract (47 g) after removing the solvent using a rotary evaporator at 40 °C. The MeOH extract was fractionated with vacuum liquid chromatography on Silica gel (EtOAc-hexane, gradient) to obtain 7 fractions (A-G). Fraction B (666 mg) was isolated by column chromatography (CC) on Silica gel (CH_2Cl_2-hexane) and then by Sephadex LH-20 (acetone) to give tectoquinone (**1**) (2 mg). Fraction C (2.1 g) was isolated by CC (silica gel, CH_2Cl_2-hexane, gradient) and further purified on Sephadex LH-20 (acetone) to give moscatilin (**2**) (52 mg). Fraction D (1.0 g) was fractioned on CC (Silica gel; EtOAc-hexane gradient) and then recrystallized from acetone to furnish ephemeranthol A (**3**) (8 mg). Fraction E (0.8 g) was subjected to Sephadex LH-20 (MeOH) to give 4 fractions (EI-EIV). Fraction E3 (213 mg) was separated by CC (Silica gel; EtOAc-hexane gradient) and further purified on Sephadex LH-20 (MeOH) to obtain gigantol (**4**) (24 mg). Fraction E4 (57 mg) was purified by CC over Silica gel (CH_2Cl_2) to give moscatin (**5**) (10 mg), lusianthridin (**6**) (7 mg) and 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**7**) (8 mg), respectively. Fraction G (23 g) was partitioned with butanol and water to give a butanol extract (1 g). Hesperidin (**8**) (4 mg) was obtained from the butanol extract after recrystallization from MeOH. The scheme of chromatographic conditions is illustrated in Supplementary Fig. S1. All isolated compounds were subjected to comprehensive analysis using spectroscopic instruments. Mass spectra were acquired using the Bruker MicrOTOF mass spectrometer (ESI-MS) (Billerica, MA, USA). NMR spectra were recorded using either the Bruker Avance DPX-300FT NMR spectrometer or the Bruker Avance III HD 500 NMR spectrometer (Billerica, MA, USA). The purity level exceeding 95 % of all isolated compounds utilized in our experiments was confirmed using NMR spectroscopy. The structures of the isolated compounds were identified by analyzing their spectroscopic data and comparing them with previously reported values, confirming their classification as the mentioned compounds.

2.3. In vitro alpha-amylase inhibition assay

The *in vitro* alpha-amylase inhibition activity of separated compounds from *Dendrobium polyanthum* Wall. ex Lindl. roots was assessed using the 2-chloro-4-nitrophenol (CNP) colorimetric assay employing a method previously illustrated [24]. Test samples were dissolved in 5 % DMSO, and solution of alpha-amylase from *Aspergillus oryzae* (Sigma-Aldrich) was prepared by dissolving 0.5 U/mL of alpha-amylase in 0.04 M phosphate buffer (pH 6.8). The assays were conducted by combining 50 μL of the test sample with 50 μL of the alpha-amylase solution in a 96-well plate. The mixture was pre-incubated at 37 °C for 10 min. Subsequently, 50 μL of 1 mM CNPG3 substrate (Sigma-Aldrich) was addedto the mixture, which was then incubated at 37 °C for 20 min. The reaction was stopped by adding 100 μL of 0.1 mM Na_2CO_3 solution. Absorbance was measured at 405 nm using a microplate reader (BioTek, USA Model Epoch2 Gen5). The negative control comprised 5 % DMSO, while acarbose served as the positive control and was treated under identical conditions to the samples. Each experiment was conducted in triplicate, and the data were presented as mean ± SD. The IC_{50} values for

alpha-amylase inhibition were determined from the graph by plotting percent inhibition against tested concentrations.

2.4. In vitro alpha-glucosidase inhibition assay

The alpha-glucosidase activity was determined following a previously described method [24]. The assay relies on the inhibition of alpha-glucosidase enzyme from *Saccharomyces cerevisiae* recombinant (Sigma-Aldrich) in the sample, leading to the release of *p*-nitrophenol (PNP) from the substrate *p*-nitrophenol-α-D-glucopyranoside (PNPG) (Sigma-Aldrich) through hydrolysis. The assay employed 0.2 U/mL of the enzyme and 2 mM of *p*-nitrophenol-α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.8). For IC_{50} determination, test samples were dissolved in 5 % DMSO, and a twofold serial dilution was conducted for each sample. In brief, the test was initiated by adding 50 μL of the sample solution in phosphate buffer (pH 6.8) and 50 μL of α-glucosidase in phosphate buffer (pH 6.8) to a 96-well plate. The mixture was pre-incubated at 37 °C for 10 min. Subsequently, 50 μL of *p*-nitrophenol-α-D-glucopyranoside was added, and the reaction proceeded at 37 °C for 20 min. Finally, Na_2CO_3 (100 μL, 0.1 mM) solution was added, and the mixture was measured with a microplate reader at 405 nm (BioTek, USA Model Epoch2 Gen5). Acarbose served as the positive control. Each experiment was performed in triplicate, and data are presented as mean ± SD. IC_{50} values were determined by plotting the percent inhibition of α-glucosidase against various concentrations of the tested compounds.

2.5. In vitro advanced glycation end products (AGEs) inhibition assay

The inhibitory effects of isolated compounds from *Dendrobium polyanthum* Wall. ex Lindl. roots on the formation of advanced glycation end products (AGEs) were analyzed using a method previously reported [24]. The AGEs reaction solution comprised bovine serum albumin (BSA) at 10 mg/mL, D-glucose at 0.2 M, D-fructose at 0.2 M, and 0.02 % w/v of sodium azide in a phosphate buffer (0.05 M, pH 7.4). The reaction mixture (450 μL) of the AGEs reaction solution was incubated at 37 °C for seven days in the dark, with or without 50 μL of the test sample dissolved in the phosphate buffer. Aminoguanidine served as the positive control. Noted that all chemicals in this assay were purchased from Merck. The fluorescence intensity (FI) of the reaction products was measured using a fluorescence spectrometer (PerkinElmer model LS-55) with excitation and emission wavelengths set at 355 and 450 nm, respectively. The IC_{50} of the extracts was determined based on the percent inhibition of AGEs formation derived from the graph.

2.6. Docking analysis of bioactive compounds on the conformation of alpha-glucosidase

The three-dimensional structure of alpha-glucosidase (source: *Saccharomyces cerevisiae*) was retrieved from Protein Data Bank (PDB code:3A4A) and imported into the AutoDockTools (ADT, version 1.5.6). Water molecules were removed from the protein, and hydrogen molecules were added to ensure correct structure preparation for docking studies. The three-dimensional (3D) structures of all ligands were retrieved from PubChem database as sdf formats and then changed into pdb and pdbqt formats by BIOVIA Discovery Studio Visualizer 2021 and ADT, respectively. All hydrogen molecules were added to the ligands, followed by computation of Gasteiger charges. The receptor grid box for docking was centered on the active site of alpha-glucosidase, with dimensions set to 50 x 50 x 50 grid points and grid center at coordinates 22.625, −8.069, 24.158 using a grid spacing of 0.375 Å, as configured in AutoGrid 4.0 [32]. The compound was docked inside a grid using AutoDock 4.0, with all other docking parameters set to their default values [33]. The AutoDock docking runs were conducted on a system equipped with an Intel® CoreTM i5- 7200U CPU running at 2.50 GHz processor, paired with 4 GB RAM, running on the Windows10 with 64-bit operating system. After molecular docking, the conformation of ligand at the lowest binding energy was obtained. BIOVIA Discovery Studio Visualizer 2021 was used for visualization of protein-ligand interactions. The inhibition constant (Ki) of each ligand was calculated from the binding energy using the equation: $\Delta G = RT\ln Ki$, where ΔG represents the binding energy in calories per mole (cal/mol), R is gas constant (1.987 cal/K mol), and T denotes the temperature in Kelvin (310.15 K) [34].

2.7. Kinetics of moscatin and acarbose against alpha-glucosidase

Kinetic experiments were carried out following Thant's method with some modifications [35]. The kinetic study of enzyme inhibition was accessed using the double reciprocal Lineweaver–Burk plot (1/V versus 1/[S]). The experiment setup involved varying concentrations of substrate *p*-nitrophenol-α-D-glucopyranoside (0.25, 0.5, 1.0, 2.0 mM) in the absence or presence of moscatin **(5)** (30 and 60 μM) and acarbose (200 and 400 μM). The reaction progress was monitored at 5-min intervals over a duration of 35 min, and absorbance was measured at 405 nm using a microplate reader. Each experiment was achieved in triplicate. The inhibition constant (K_i) value was determined by constructing a secondary plot of the slopes from the double-reciprocal lines against inhibitor concentration.

2.8. Statistical analysis

A minimum of at least triplicate samples was examined in each experiment except for molecular docking investigation. The results were presented as the mean ± standard deviation (SD). Statistical significance was assessed using the Student's *t*-test with GraphPad software, where *p*-values less than 0.05 were considered indicative of a significant difference.

3. Results

3.1. Structure identification

The isolation of substances from the methanolic root extracts of *D. polyanthum* Wall. ex Lindl. resulted in the identification of eight known compounds including one anthraquinone, two bibenzyls, one phenanthrene, three dihydrophenanthrenes, and one flavonoid. The quantity of substances isolated under specific chromatographic conditions is illustrated in Supplementary Fig. S1. The structures of these compounds were confirmed through spectroscopic analyses, including one-dimensional (1D)- and 2D-NMR, as well as HR-ESI-MS experiments. The results were then compared with previously reported data, leading to compound identification as follows: tectoquinone (**1**) [36], moscatilin (**2**) [37], ephemeranthol A (**3**) [38], gigantol (**4**) [39], moscatin (**5**) [40], lusianthridin (**6**) [41], 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**7**) [42], and hesperidin (**8**) [43], as presented in Fig. 1B. Detailed information regarding the appearance and spectroscopic data of the isolated substances are documented in Supplementary Table S1.

In accordance with a recent review article summarizing the latest research progress on natural stilbenes in *Dendrobium* species, certain compounds identified in our study corresponded to previously documented substances [44]. Specifically, compounds **4**–**7** have been investigated and documented for their inhibition of alpha-glucosidase, whereas no such reports exist for compounds **1**–**3** and **8**. The activities of all isolates against alpha-amylase, a pivotal enzyme crucial in the treatment of disorders associated with excessive carbohydrate intake, remain incompletely elucidated. Moreover, there are no preexisting data on the ability of the compounds to inhibit the production of AGEs, recognized as markers of complications in diabetes. Nevertheless, despite the successful separation and structural analysis of the isolated substances, limitations were encountered during the subsequent biological activity analyses. Specifically, inadequate quantities of **1** and **8** impeded their inclusion in the subsequent experiments.

3.2. Analysis of the inhibition of carbohydrate-hydrolyzing enzymes

Two carbohydrate-hydrolyzing enzymes, namely alpha-amylase and alpha-glucosidase, were employed to evaluate the antidiabetic potential of chemicals isolated from the methanolic root extract of *D. polyanthum* Wall. ex Lindl. Six isolates (compounds **2**–**7**) were available in sufficient quantities for subsequent experiments. Each compound was assessed at a concentration of 100 μg/mL. Excluding **5**, all compounds inhibited the activities of both enzymes by more than 50 %. The determined IC_{50} values are summarized in Table 1.

In the evaluation of alpha-amylase inhibition, all tested substances exhibited weaker inhibitory effects than the positive control acarbose ($IC_{50} = 53.68 \pm 1.51$ μM). Compounds **2** and **4**, which are bibenzyl compounds, demonstrated appreciable inhibitory activity against alpha-amylase with IC_{50} values of 256.94 ± 9.87 and 332.13 ± 4.23 μM, respectively. Regarding the dihydrophenanthrenes, **3**, **6**, and **7** similarly exhibited weaker activity than the positive control with IC_{50} values of 279.68 ± 9.76, 372.19 ± 2.74, and 617.08 ± 11.04 μM, respectively. Remarkably, **5**, the only phenanthrene isolate, did not inhibit alpha-amylase activity.

During the assessment of alpha-glucosidase inhibition, notable outcomes were observed, as presented in Table 1. Compounds **2** and **4** exhibited considerable inhibitory activity with IC_{50} values of 173.13 ± 5.84 and 230.62 ± 11.30 μM, respectively. Among the dihydrophenanthrenes, **3** and **6** exhibited greater efficacy in inhibiting this enzyme than acarbose ($IC_{50} = 361.44 \pm 5.34$ μM), with IC_{50} values of 62.51 ± 2.85 and 63.89 ± 0.27 μM, respectively. Meanwhile, **5**, the only isolated phenanthrene, exhibited remarkable activity with an IC_{50} of 32.45 ± 1.04 μM, approximately 10-fold lower than that of acarbose.

3.3. Analysis of antiglycation activity

The antiglycation activity of the isolated compounds was initially assessed by examining the inhibition of glycated bovine serum albumin (BSA) formation at 37 °C in the dark. Among the six compounds isolated in sufficient quantities, only **2** and **7** inhibited glycated BSA formation. In the presence of these compounds, the fluorescence intensity significantly decreased, indicating reduced formation of glycated BSA. Compound **2** exhibited stronger inhibitory activity than the positive control aminoguanidine (IC_{50} =

Table 1
Alpha-amylase and alpha-glucosidase inhibitory activities of compounds **1**–**8**.

Compounds	$IC_{50} \pm$ S.D. (μM)	
	alpha-amylase	alpha-glucosidase
1	ND	ND
2	256.94 ± 9.87^{b}	173.13 ± 5.84^{c}
3	279.68 ± 9.76^{c}	62.51 ± 2.85^{b}
4	332.13 ± 4.23^{d}	230.62 ± 11.30^{d}
5	NA	32.45 ± 1.04^{a}
6	372.19 ± 2.74^{e}	63.89 ± 0.27^{b}
7	617.08 ± 11.04^{f}	421.58 ± 7.00^{f}
8	ND	ND
Acarbose	53.68 ± 1.51^{a}	361.44 ± 5.34^{e}

NA refers no inhibitory activity; ND refers not determined.
Data with a different letter are significantly different with a p value less than 0.05 ($p < 0.05$).
All the findings are displayed as mean ± SD ($n = 3$).

327.37 ± 5.95 μM), with an IC_{50} of 167.89 ± 9.42 μM. Meanwhile, **7**, with an IC_{50} of 410.81 ± 14.96 μM, displayed weaker activity than **2** and aminoguanidine, as illustrated in Table 2.

3.4. Docking analysis to assess alpha-glucosidase inhibitory activity

Given that alpha-glucosidase is the ideal target for alleviating postprandial hyperglycemia and our experiments indicated that the isolated chemicals exhibit stronger effects on this enzyme than on alpha-amylase, our primary focus in molecular docking experiments was alpha-glucosidase. Access to the Protein Data Bank allowed the retrieval of the precise 3D structure of alpha-glucosidase from *Saccharomyces cerevisiae* (PDB ID: 3A4A), which served as the main enzyme source for the previously conducted biochemical assays. The active site residues were identified as ASP69, HIS112, ARG213, ASP215, GLU277, HIS351, and ASP352 [45].

The results of molecular docking using AutoDockTools, including the lowest binding energy (LBE) of the test compounds (with the corresponding PubChem IDs), as well as their inhibition constants and conformations, are presented in Table 3 and Fig. 2 (A-G). Table 4 presents the interactions of the test compounds with the amino acid residues of alpha-glucosidase. The LBE values were −6.10, −7.52, −6.57, −5.45, −5.58, −5.93, and −4.25 kcal/mol for compounds **2**–**7** and acarbose, respectively. The inhibition constants for compounds **2**–**7** and acarbose were 50.26, 5.02, 23.44, 144.31, 116.86, 66.23, and 1011.47 μM, respectively. The 2D interactions between the amino acid residues of alpha-glucosidase and the tested compounds are illustrated in Fig. 2 (A-G). ASP352 emerged as a crucial component in the interaction with all compounds investigated in this study. Hydrogen bonding at ASP352 served as the binding mode for **2**, **5**, and acarbose, whereas hydrophobic bonds were implicated in the engagement of **3**, **6**, and **7**. Compound **4** formed both hydrogen and hydrophobic bonds with this amino acid residue. In addition, a carbon–hydrogen interaction was observed exclusively with acarbose at ASP352.

3.5. Kinetics of alpha-glucosidase inhibition

To elucidate the mechanism underlying the inhibition of alpha-glucosidase by **5** as the most potent alpha-glucosidase inhibitor in the aforementioned experiment, a kinetics investigation was conducted. The experiment involved varying the substrate concentration of *p*-nitrophenol-α-D-glucopyranoside (0.25, 0.5, 1.0, 2.0 mM) in the presence of **5** at concentrations of 30 and 60 μM, along with acarbose at concentrations of 200 and 400 μM. Fig. 3 (A, B) illustrates the Lineweaver–Burk diagram outlining the alpha-glucosidase inhibitory effects of **5** and acarbose, as summarized in Table 5. Both **5** and acarbose exhibited a consistent pattern, namely decreases in V_{max} without changes in K_m, indicative of their non-competitive inhibition. Remarkably, **5** displayed a K_m ranging from 1.62 to 1.66 mM, indicating higher binding affinity for the enzyme than acarbose, which had a K_m of 4.93–4.98 mM. Furthermore, the K_i of **5** was 31.75 μM, which was significantly lower than that of acarbose (280.68 μM), indicating that **5** has approximately 9-fold greater potency in inhibiting the activity of alpha-glucosidase.

4. Discussion

Two pivotal carbohydrate-hydrolyzing enzymes, namely alpha-amylase and alpha-glucosidase, play crucial roles in postprandial hyperglycemia. Alpha-amylase initiates the digestion of carbohydrates by hydrolyzing 1,4-glycosidic linkages in polysaccharides such as starch and glycogen, converting them into disaccharides. Subsequently, alpha-glucosidase catalyzes the transformation of disaccharides into monosaccharides, facilitating the absorption of molecules and contributing to an increase in blood sugar levels. Inhibitors targeting these enzymes are essential for controlling hyperglycemia, as they hinder carbohydrate digestion, thereby reducing postprandial plasma glucose levels. Our findings align with prior data indicating that methanolic extracts of the whole plant of *D. polyanthum* Wall. ex Lindl. exhibited insignificant alpha-amylase inhibitory potency but appreciable alpha-glucosidase inhibitory activity [24]. Despite the limited impact on alpha-amylase, a combined effect of suppressing carbohydrate digestion persists through

Table 2
Advanced glycation end product inhibitory activity of compounds **1**–**8**.

Compounds	IC_{50} ± S.D. (μM)
1	ND
2	167.89 ± 9.42[a]
3	NA
4	NA
5	NA
6	NA
7	410.81 ± 14.96[c]
8	ND
Aminoguanidine	327.37 ± 5.95[b]

NA refers no inhibitory activity; ND refers not determined.
Data with a different letter are significantly different with a p value less than 0.05 ($p < 0.05$).
All the findings are displayed as mean ± SD ($n = 3$).

Table 3
The lowest binding energy of test compounds to alpha-glucosidase.

Compounds	PubChem ID	Binding energy (kcal/mol)	Estimated inhibition constant (μM)
2	176096	−6.10	50.26
3	44445444	−7.52	5.02
4	3085362	−6.57	23.44
5	194774	−5.45	144.31
6	442702	−5.58	116.86
7	21678577	−5.93	66.23
Acarbose	444254	−4.25	1011.47

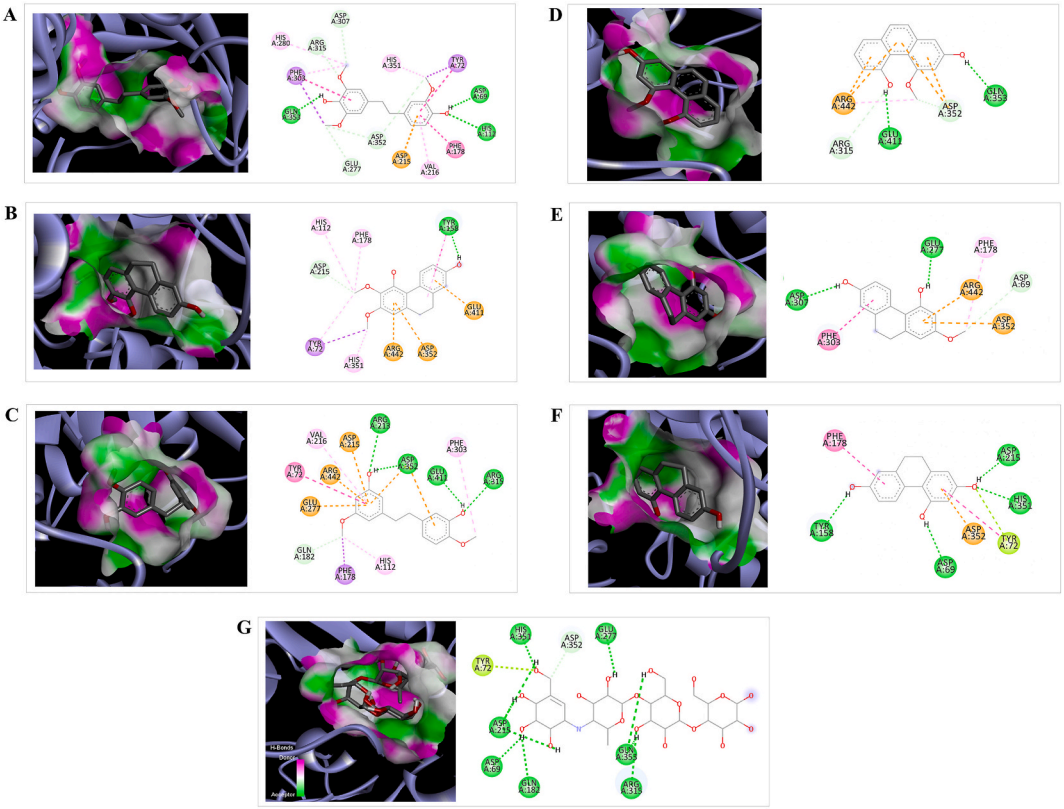

Fig. 2. The 3D and 2D illustrations of amino acid residues of alpha-glucosidase interacted with (**A**) moscalitin (**2**), (**B**) ephemeranthol A (**3**), (**C**) gigantol (**4**), (**D**) moscatin (**5**), (**E**) lusianthridin (**6**), (**F**) 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**7**) and (**G**) acarbose at the lowest binding energy conformation.

these two mechanisms because of the determination of the inhibitory activities of compounds isolated from this plant.

Extensive research has explored natural, semi-synthetic, and synthetic compounds for their inhibitory effects on alpha-glucosidase, aiming to develop effective and non-toxic antidiabetic agents. A comprehensive study revealed 290 chemically distinct molecules from 53 plants across to 27 different families exhibiting *in vitro* inhibition of alpha-glucosidase. Among these, terpenes and flavonoids were the most prevalent groups demonstrating this activity [46]. In our study, it was observed that the isolated substances with anti-alpha-glucosidase activity were primarily bibenzyls and phenanthrenes (compounds **2**–**6**). These compounds are prevalent in *Dendrobium* species and have shown potential inhibitory effects against this enzyme [18,47]. Nevertheless, inhibition values obtained from our experiments differ from those reported in previous studies. This variance may be due to differences in experimental conditions, variations in the enzymes used across different laboratories, and other factors. Therefore, interpreting results and comparing bioactivities from different studies becomes challenging, underscoring the need for standardized protocols in future research.

Numerous natural compounds, such as polyphenols, terpenoids, alkaloids, polysaccharides, vitamins, and peptides, particularly those with high hydroxyl substitution, were found to inhibit the formation of AGEs in previous studies [48]. Various anti-AGEs mechanisms include covering glycation sites on proteins, scavenging oxidative free radicals, trapping active dicarbonyl compounds, chelating metal ions, inhibiting aldose reductase, regulating receptors for AGEs, and lowering blood glucose levels [49]. This finding underscores the necessity for further in-depth experimental research to elucidate the potential anti-AGEs mechanisms of these

Table 4
Data for the molecular docking of test compounds in alpha-glucosidase.

Compounds	Interacting Amino Acid Residues	Ligand Interactions
2	HIS112	Conventional Hydrogen Bond
	ASP69	Conventional Hydrogen Bond
	GLN353	Conventional Hydrogen Bond
	ARG315	Carbon Hydrogen Bond
	ASP352	Carbon Hydrogen Bond
	GLU277	Carbon Hydrogen Bond
	ASP307	Carbon Hydrogen Bond
	ASP215	Pi-Anion
	TYR72	Pi-Sigma, Pi-Pi T-shaped
	PHE303	Pi-Sigma, Pi-Pi Stacked, Pi-Alkyl
	PHE178	Pi-Pi Stacked
	HIS280	Pi-Alkyl
	HIS351	Pi-Alkyl
	VAL216	Pi-Alkyl
3	TYR158	Conventional Hydrogen Bond, Pi-Pi T-shaped, Pi-Alkyl
	ASP215	Carbon Hydrogen Bond
	ARG442	Pi-Cation
	ASP352	Pi-Anion
	GLU411	Pi-Anion
	TYR72	Pi-Sigma, Pi-Alkyl
	HIS112	Pi-Alkyl
	PHE178	Pi-Alkyl
	HIS351	Pi-Alkyl
4	ARG213	Conventional Hydrogen Bond
	ARG315	Conventional Hydrogen Bond
	GLU411	Conventional Hydrogen Bond
	ASP352	Conventional Hydrogen Bond, Pi-Anion
	GLN182	Carbon Hydrogen Bond
	ARG442	Pi-Cation
	ASP215	Pi-Anion
	GLU277	Pi-Anion
	PHE178	Pi-Sigma
	TYR72	Pi-Pi T-shaped
	HIS112	Pi-Alkyl
	PHE303	Pi-Alkyl
	VAL216	Pi-Alkyl
5	GLN353	Conventional Hydrogen Bond
	GLU411	Conventional Hydrogen Bond
	ARG315	Carbon Hydrogen Bond
	ASP352	Carbon Hydrogen Bond, Pi-Anion
	ARG442	Pi-Cation, Alkyl
6	GLU277	Conventional Hydrogen Bond
	ASP307	Conventional Hydrogen Bond
	ASP69	Carbon Hydrogen Bond
	ARG442	Pi-Cation
	ASP352	Pi-Anion
	PHE303	Pi-Pi Stacked
	PHE178	Pi-Alkyl
7	HIS351	Conventional Hydrogen Bond
	ASP69	Conventional Hydrogen Bond
	ASP215	Conventional Hydrogen Bond
	TYR158	Conventional Hydrogen Bond
	ASP352	Pi-Anion
	TYR72	Pi-Lone Pair, Pi-Pi T-shaped
	PHE178	Pi-Pi T-shaped
Acarbose	ARG315	Conventional Hydrogen Bond
	HIS351	Conventional Hydrogen Bond
	GLU277	Conventional Hydrogen Bond
	ASP215	Conventional Hydrogen Bond
	ASP69	Conventional Hydrogen Bond
	GLN182	Conventional Hydrogen Bond
	GLN353	Conventional Hydrogen Bond
	ASP352	Carbon Hydrogen Bond
	TYR72	Pi-Lone Pair

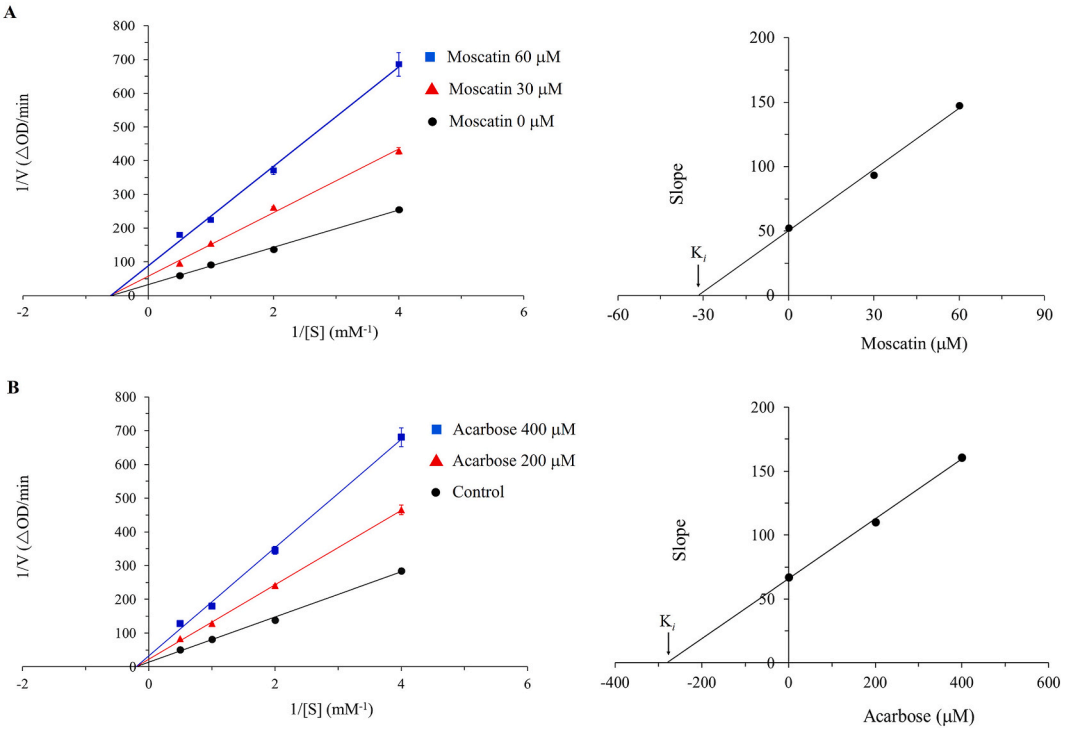

Fig. 3. Lineweaver–Burk plots of (**A**) moscatin (**5**) and (**B**) acarbose. The secondary plot of each compound is on the right.

Table 5
Enzyme inhibition kinetics parameters of alpha-glucosidase inhibition by Moscatin **(5)**.

Inhibitors	Dose (μM)	V_{max} (△OD/min)	K_m (mM)	K_i (μM)
	0	0.030	1.62	
5	30	0.017	1.62	31.75
	60	0.011	1.66	
	0	0.073	4.93	
Acarbose	200	0.045	4.98	280.68
	400	0.030	4.96	

V_{max} refers maximum rate of velocity; K_m refers Michaelis constant; K_i refers inhibitor constant.

substances.

Considering the complex nature of diseases associated with specific AGEs, a practical approach involves evaluating the inhibition of sugar binding to a representative protein, such as BSA. This method enables the observation of reduced fluorescence for glycated BSA in the presence of an inhibitor, providing valuable insights into the potential antiglycation properties of the tested compounds. Fluorescent AGEs are generated through interactions with reducing sugars and proteins. This is of considerable importance in evaluating antiglycation effects, albeit with ongoing challenges related to sensitivity, quantification, and precision in systematic validation [50]. Because of the considerable heterogeneity of AGEs, the establishment of a standardized approach for their simultaneous quantification has yet to be achieved [51,52]. Various substances, such as glyceraldehyde, glucose, methylglyoxal, and carboxymethyllysine, are known to induce the formation of AGEs, resulting in fluorescence and the production of multiple classes of AGEs [53]. These fluorescent AGEs exhibit different sensitivities for verification. Previous research found that glyceraldehyde-derived AGEs demonstrated superior sensitivity compared to other AGEs, while glucose-derived AGEs exhibited moderate sensitivity [54]. This information implies that if compounds 3–6 exhibit anti-AGEs properties, their effects are expected to be moderate. This would lead to reduced AGEs production and potentially negligible fluorescence intensities, possibly falling below the detection limit of the analytical instrument, as illustrated in the experiment. Additionally, alternative methods for evaluating anti-AGEs properties, such as immunological measurements [55] and blocking the binding of AGEs to various receptors [56], might offer increased accuracy and relevance to preventing diabetic complications.

Molecular docking is a computational tool employed to predict the binding affinity and strength between a molecule of interest or ligand and a protein in a database. It utilizes calculations to assess binding characteristics. Additionally, the analysis involves examining LBE, which reflects binding affinity, particularly if the value is small. From a previous study, tertiary structure analysis of alpha-glucosidase indicated a configuration of 589 amino acids arranged into three domains. GLU277 and ASP352 were identified as

key catalytic residues for hydrolysis, and the entrance to the active site pocket is restricted by TYR158, HIS280, and the 310–315 loop [45]. In our study, we performed molecular docking analysis to investigate the predicted binding sites of the isolated compounds with alpha-glucosidase. The significance of ASP352 as a crucial binding site was revealed, evidenced by its interactions with every tested substance. ARG442 appeared to be another key residue for interactions between the enzyme and compounds **3**–**6**. These observable results align with findings from prior studies [32,57].

In scientific investigations, especially in molecular docking, inconsistencies may arise from several factors. These include algorithmic randomness, sampling bias, and scoring function variance. Furthermore, parameters configured in research programs, such as solvent effects, protein conformational flexibility, and numerical precision, can also impact data presentation [58]. In our experiments, the obtained LBE values were not directly correlated with the outcomes of prior biochemical experiments. Specifically, compound **5**, which displayed the lowest IC_{50} for inhibiting alpha-glucosidase, exhibited a higher LBE than the other isolated compounds, which acted as weaker inhibitors. Additionally, the positive control acarbose exhibited a high LBE, significantly differing from those of all tested substances in our experiment. One factor contributing to the high binding energy of acarbose is its chemical structure, which includes highly flexible regions allowing for significant torsional rotation. Previous studies have reported a variable binding energy for acarbose against alpha-glucosidase, ranging from low to high [59–61], suggesting a similar underlying reason. As a result, conducting additional runs becomes imperative to explore all potential conformations for effective binding with the enzyme. Despite variations in enzyme inhibition values, acarbose did not emerge as the weakest inhibitor in our enzymatic experiments. In addition, since our study utilizes a non-human source of alpha-glucosidase, it is pertinent to address the differences in binding energy observed compared to the acarbose-human alpha-glucosidase complex. Acarbose is specifically designed as an antagonist for human alpha-glucosidase, and thus, the binding energy in our non-human model may differ due to species-specific variations in enzyme structure and interaction dynamics. This phenomenon raises questions and calls for further in-depth exploration, possibly through the application of alternative tools such as molecular dynamics simulations. These simulations provide valuable insights into elucidating the functional mechanisms of proteins, peptides, and other biomolecules, thereby overcoming the inherent sampling limitations encountered in docking analysis [62]. In future studies, the molecular docking analysis could be further strengthened by incorporating molecular dynamics simulations or other complementary computational approaches to provide more robust predictions of binding interactions. Nevertheless, these data hold utility for predicting the conformational characteristics of the test compounds within the binding sites. These insights can guide future investigations into the structural modification of bibenzyl derivatives and phenanthrenes.

Our findings in the kinetics study support the potential application of compound **5** as a non-competitive inhibitor, offering advantages over competitive inhibitors. Non-competitive inhibitors can bind to the enzyme's allosteric site, thereby inhibiting its activity independently of the substrate concentration [63]. Additionally, non-competitive inhibitors exert inhibitory effects at lower concentrations compared to competitive inhibitors [64]. Therefore, the use of potential non-competitive inhibitors, such as compound **5**, in the treatment of individuals with diabetes might prove safer because of the lower exposure to chemical compounds.

As previously mentioned, plants within the *Dendrobium* genus have a rich history of traditional applications, particularly in the realm of Chinese herbal medicine, addressing a myriad of health concerns. Commonly utilized plant parts include the stem and the entire plant. However, the application of the entire plant or stem can result in substantial damage to the plant, as these structures serve as crucial support systems for branches, leaves, and flowers, which are integral for the overall functionality of the plant. If feasible, the utilization of other bioactive parts, such as roots, might offer distinct advantages compared to the stem. This is primarily attributable to their rapid growth, enabling them to locate water sources and minerals, especially under water-deficient conditions [65]. Importantly, the use of roots does not interfere with photosynthesis, a critical process for plant nutrition and energy production. Hence, exploitation of the roots of *D. polyanthum* Wall. ex Lindl. represents a promising avenue for the identification and development of novel pharmaceuticals.

Our comparative analysis of important substances isolated from the roots and stem of *D. polyanthum* Wall. ex Lindl., in reference to previous research [26], revealed the presence of three common compounds in both the stem and roots: **2**, **4**, and **5**. However, an additional five substances were exclusively identified: **1**, **3**, **6**, **7**, and **8**. These compounds were reported for the first time as chemicals isolated from the roots of this plant. In-depth examination of these substances in this study, particularly compounds **2**–**7**, revealed their potential in combating diabetes through distinct mechanisms with varying efficacy. Through exhaustive information retrieval, it was reported that compound **1** induced toxicity in specific species of termites and mosquitoes [66,67] and inhibited the main protease of SARS-CoV-2, a crucial component of the COVID-19 pathogen [68]. Compound **8** was comprehensively investigated for biological activities and health-promoting benefits [69,70]. This compound has been used in the treatment of cancer, cardiovascular diseases, neurological and psychiatric disorders, dermatological issues, infection, and type 2 diabetes [71–76]. Altogether, six of the eight substances (compounds **2**–**7**) isolated from the roots of this plant in our study are presumed to hold potential benefits for the treatment of diabetes.

In the field of herbal medicine, the concept of synergistic effects denotes that the individual components of a single or multi-extract formulation can affect multiple targets [77]. Consequently, they synergistically collaborate to amplify the effect beyond the sum of the individual responses. An additive effect might occur when combining individual compounds, resulting in a cumulative impact as this phenomenon typically arises when the compounds act on the same target. Recent research suggests that synergistic effects drive the action of numerous phytochemicals in biological systems. These compounds interact intricately to target multiple cellular and molecular pathways, yielding significant biological effects [78,79]. In our study, compounds isolated from *D. polyanthum* roots exhibited varying abilities to inhibit the two carbohydrate-digesting enzymes and neutralize AGEs. Specific substances displayed significant efficacy against distinct targets, such as compound **5** effectively inhibiting alpha-glucosidase but not alpha-amylase and AGEs production. Conversely, compound **2** demonstrated superior inhibition against AGEs production, followed by compound **7** as a moderate AGEs inhibitor, whereas other substances in our experiment did not exhibit this function. Adhering to the principle of synergism, the

concurrent administration of compounds **5** and **2** in diabetes treatment could potentially elicit a synergistic effect. Alternatively, when substances targeting the same mechanisms, such as AGEs production inhibition by compounds **2** and **7**, are combined for diabetes treatment, they will possibly exhibit an additive effect. Similarly, compounds **2**–**4**, **6**, and **7** could produce additive effects on anti-amylase activity when utilized in combination for diabetes treatment. The use of a comprehensive extract from the roots of this plant could result in enhanced antidiabetic efficacy compared to the effects of the individual substances. Therefore, an approach utilizing a holistic extract from the roots of this plant is advantageous given the multifaceted nature of complicated disorders including diabetes, which involve dysfunction across various organ or cellular levels [77,80]. This could be attributed to the additive and/or synergistic effects resulting from the combination of bioactive compounds with diverse biological activities in the root extract.

5. Conclusions

To the best of our knowledge, bioactive compounds with antidiabetic properties were isolated from the roots of *D. polyanthum* Wall. ex Lindl. for the first time. Both *in vitro* and *in silico* analyses underscored the potential of specific compounds with promising anti-diabetic mechanisms. The majority of isolated chemicals exhibited significant anti-amylase activity, with some showing specificity for other diabetes-related targets. Compound **5** was identified as a potent inhibitor of alpha-glucosidase via a non-competitive mechanism, while compound **2** demonstrated robust anti-AGE activity. Molecular docking analyses indicated interactions of all tested compounds with alpha-glucosidase, albeit with minor differences in affinity. Overall, our findings suggest that the beneficial effects of *D. polyanthum* are likely attributed to synergistic effects within the whole root extract rather than individual compounds, emphasizing the importance of a holistic approach in diabetes management. Moreover, compound **5** showed potent efficacy compared to the clinical drug acarbose, while compound **2** emerged as a promising candidate for further investigation due to its superior anti-AGE activity. These findings hold promise for achieving therapeutic effects with reduced dosages, minimizing chemical exposure and promoting overall health. However, comprehensive safety assessments and effective administration strategies are necessary before considering these compounds for clinical use.

Data availability statement

All data generated and analyzed are included in this research article. Data generated during this study is included in this published article.

Funding statement

This research was partly supported by the Chulalongkorn University grant to the Center of Excellence in Natural Products for Ageing and Chronic Diseases to Boonchoo Sritularak and Center of Excellence in DNA Barcoding of Thai Medicinal Plants to Taksina Chuanasa.

CRediT authorship contribution statement

Thaniwan Cheun-Arom: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Tharita Kitisripanya:** Software, Methodology, Investigation, Formal analysis. **Poomraphie Nuntawong:** Methodology, Investigation. **Boonchoo Sritularak:** Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Taksina Chuanasa:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the Faculty of Science, Ramkhamhaeng University and the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, for kindly providing the equipment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34502.

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