Short communication

Wedtrilosides A and B, two new diterpenoid glycosides from the leaves of Wedelia trilobata (L.) Hitchc. with α-amylase and α-glucosidase inhibitory activities

Nguyen Thi Luyen a,b,c, Pham Thanh Binh a, Pham Thi Tham d, Ta Manh Hung e, Nguyen Hai Dang a,c, Nguyen Tien Dat b,c, Nguyen Phuong Thao a,*

* Institute of Marine Biochemistry (IMBC), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam
b Center for Research and Technology Transfer, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam
c Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam
d Faculty of Chemical Technology, Hanoi University of Industry, 298 Cau Dien, Bactulim, Hanoi, Viet Nam
e National Institute of Drug Quality Control (NIDQC), 48 Hai Ba Trung, Hoan Kiem, Hanoi, Viet Nam

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ABSTRACT

In the ongoing research to find new diabetes constituents from the genus Wedelia, the chemical constituent of Wedelia trilobata leaves, a Vietnamese medicinal plant species used to treat type 2 diabetes mellitus, was selected for detailed investigation. From a methanolic extract, two new ent-kaurane diterpenoids, wedtrilosides A and B (1 and 2), along with five known metabolites (3–7), were isolated from W. trilobata. The chemical structures of (1–7) were assigned via spectroscopic techniques (IR, 1D, 2D NMR and HR-QTOF-MS data) and chemical methods. The isolates were evaluated for α-amylase and α-glucosidase inhibitory activities compared to the clinical drug acarbose. Among them, compounds 4, 6, and 7 showed the most potent against α-glucosidase enzyme with $IC_{50}$ values of 27.54 ± 1.12, 173.78 ± 2.37, and 190.40 ± 2.01 μg/mL. While moderate inhibitory effect against α-amylase was observed with compounds 6 and 7 (with $IC_{50} = 181.97 ± 2.62$ and 52.08 ± 0.56 μg/mL, respectively). The results suggested that the antidiabetic properties from the leaves of W. trilobata are not simply a result of each isolated compound, but are due to other factors such as the accessibility of polyphenolic groups to α-amylase and α-glucosidase activities.

1. Introduction

Diabetes mellitus (DM) is a complex chronic illness requiring continuous medical care, with multifactorial risk-reduction strategies beyond glycemic control. The numbers concerning the prevalence of DM are alarming, about 415 million people worldwide are estimated to have diabetes [1]. The estimate for 2040 is that this number will rise to 642 million diabetics worldwide, with the greatest increase occurring in low and middle-income countries [2,3]. Natural products are an important source for lead compounds that play significant roles to serve as more effective antidiabetic agents, with reduced side effects, has attracted study interest [2]. Vietnam is found as one mega-biodiversity Southeast Asia countries, where can be expected to have many potential unexploited medicinal herbs to be studied as a source phytopharmacology or molecule targets/strategies and their main function medicine. Opportunity exploration of medicinal plants is still very wide open in line with the development of the herbal industry, herbal medicine, and phytopharmaca. Therefore, several reports showed to explore the promising antidiabetic agents as α-amylase and α-glucosidase inhibitions in the genus Wedelia [4]. The Wedelia genus is well-known medicinal plants with a long history of use in traditional medicine and has been studied extensively for its effectiveness in the anti-hyperglycemic effects [5,6].

The genus Wedelia, belonging to the family Asteraceae (formerly Compositae), comprises approximately 107 species in the world and among them, about 6 species are in Vietnam. They are all herbal plants and different geographic distributions (Asia, the Pacific islands, and South America) [7]. Wedelia trilobata (L.) Hitch. (synonym Sphagnetica trilobata (L.) Pruski) is a deciduous shrub, distributed mainly in several Asian countries as China, Korea, Japan, India, and Vietnam. In folk medicine, it is employed to treat arthritic painful joints, abdominal pains, backache, bronchitis, colds, dysmenorrheal, even as a fertility
enhancer, muscle cramps, rheumatism, stubborn wounds, sores and swellings [8,9]. Flowers and leaf part of this plant were used in the ladies for the purpose of amenorrhea, childbirth, abortion and to clear the placenta after birth [9,10]. Recently, pharmacological reports revealed that this plant has antioxidant [11], analgesic [12], antileishmaniasis [13], anti-inflammatory [11], antimicrobial [11], antitumor [11], hepatoprotective [6], larvicidal, wound healing [14], uterine contraction, diabetes [15], menstrual pain and reproductive problems in women [16]. Phytochemical work on this species has revealed the presence of a number of flavonoids, polyacetylenes, and terpenoids, as well as steroids [9]. Besides, the previous studies have indicated the presence of several other compounds as benzene derivatives were also reported in the species, but they appear to have a more limited distribution. To date, most of the studies have been focused on antioxidant and cytotoxic activities of extract or/and compounds from W. trilobata [9,11]. However, the α-amylase and α-glucosidase effects of compositions from this species have not been studied.

In ongoing phytochemical search for novel natural products from genus *Wedelia* regarding type 2 diabetes [4], described herein are the characterization of two new diterpenoids, named wedtrilosides A and B (1 and 2), along with the identification of five known compounds (3–7, Fig. 1) from the leaves of *W. trilobata*. The structures of new compounds were determined by the analyses of conventional NMR and HRMS data and chemical modification methods. These phytochemicals were evaluated for their α-amylase and α-glucosidase effects.

2. Experimental section

2.1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Tokyo, Japan) at 24 °C. IR spectra were obtained on a Bruker TENSOR 37 FT-IR spectrometer (Bruker, Billerica, MA, USA). The 1H NMR (500 MHz) and 13C NMR (125 MHz) spectra were recorded on an AVANCE III HD 500 spectrometer (MA, USA) with tetramethylsilane (TMS) used as an internal standard. The HR-QTOF-MS were acquired on an Agilent 6550 iFunnel Q-TOF LC/MS system (Emeryville, CA, USA). Medium pressure liquid chromatography (MPLC) was carried out on a Biotage-Isolera One system (SE-751 03 Uppsala, Sweden). Column chromatography (CC) was conducted using 65–250 or 230–400 mesh silica gel (Sorbent Technologies, Atlanta, GA, USA), porous polymer gel (Diaion HP-20, 20–60 mesh, Mitsubishi Chemical, Fig. 1. Structures of the compounds (1–7) isolated from *W. trilobata*.
2.2. Plant material

The leaves of W. trilobata were collected in September 2017 from Thai Binh province, Vietnam and taxonomically identified by Dr. Do Van Hai (Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (NCCB-2016.55.02) was deposited at the Herbarium of Institute of Marine Biochemistry and Institute of Ecology and Biological Resources, Van Hai (Institute of Ecology and Biological Resources). A voucher specimen (NCCB-2016.55.02) was deposited at the Herbarium of Institute of Marine Biochemistry and Institute of Ecology and Biological Resources, Van Hai (Institute of Ecology and Biological Resources).

2.3. Compounds

From the methanolic extract of W. trilobata, seven compounds (1–7) were isolated and structurally elucidated. Stock solutions of tested compounds in DMSO were prepared kept at –20 °C and diluted to the final concentration in fresh media before each experiment. To not affect the cell growth, the final DMSO concentration did not exceed 0.5% in all experiments.

2.4. Extraction and isolation

The dried leaves of W. trilobata (1.2 kg) were cut into pieces and extracted with 95% aqueous MeOH by percolation at room temperature to obtain 275 g of extract. The extract was partitioned into n-hexane-soluble (101.9 g) and ethyl acetate-soluble (15.2 g) fractions.

The EtOAc fraction was separated on silica gel MPLC (column: Biotage SNAP Cartridge, KP-Sil, 340 g) using the mobile phase of CH2Cl2-MeOH (0–5 min 50% MeOH, 6–65 min 50–100% MeOH, 66–90 min 100% MeOH, 15 mL/min, 90 min) to give three fractions (E-1 to E-3). This MPLC procedure was repeated 5 times using the same conditions before further isolation. By TLC monitoring, fractions E-1 (2.75 g) was further separated on a silica gel CC (Φ35 mm, L750 mm), using CHCl3-ace tone (95:5:70–30) as mobile phase, to give three subfractions (E-1.1 to E-1.3). Precipitates from fraction E-1.1 eluted by CH3Cl-EtOAc (85:15) were collected, dissolved in MeOH, and purified on Sephadex LH-20 (eluted with MeOH, Φ25 mm, L1250 mm) to yield compound 4 (10.5 mg). In a similar process to that described above, subfraction E-1.2 (2.2 g) was chromatographed over an open ODS column (Φ35 mm, L750 mm) eluted with MeOH-H2O (40%, 60%, v/v) to give one subfraction E-1.2.1 and compound 6 (13 mg). Similarly, subfraction E-1.3 was separated by passage over a Sephadex LH-20 column (Φ25 mm, L1250 mm) and then applied to repeated YMC RP-18 column (Φ45 mm, L1900 mm) using MeOH-H2O (1:1.5), to yield compound 5 (6.9 mg).

The H2O fraction was separated using a Diaion HP-20 column (Φ100 mm, L500 mm) and was eluted with a gradient solvent mixture of MeOH-H2O-H2O (gradient 25:75, 50:50, 65:35, 75:25, to pure MeOH, stepwise) to yield three fractions (W-1 to W-3), based on TLC analysis. The fractionation W-1 was separated via silica gel CC (Φ250 mm, L1250 mm), and then an open YMC RP-18 column (Φ15 mm, L500 mm) and was eluted repeatedly with CHCl3-MeOH-H2O, 3:2:0.3 (v/v) and was sprayed with a 10% MeOH solution of 1% phosphomolybdic acid with CHCl3 (n→100%) to afford compounds 1 (7.5 mg), 2 (5.1 mg), and 3 (16.3 mg). Finally, when the same steps were repeated as above, compound 7 (25.6 mg) was also obtained by purifying sub fraction W-3 on YMC RP-18 column (Φ90 mm, L700 mm) and followed by passage over a Sephadex LH-20 column (Φ45 mm, L1900 mm) using mixtures of MeOH-H2O (1:2:5) and MeOH-0.15 M HCl (1:1).

2.5. Physical and spectroscopic data of new compounds

**Wedtriloside A (1):** White, amorphous powder; [α]D20 = 61.6 (c 0.15, MeOH); IR νmax (KBr) 3219, 2926, 2865, 1727, 1630, 1461, 1381, 1319, 1250, 1224, 1080, and 992 cm−1; HR-QTOF-MS (negative-ion mode) m/z 575.2190 [M – H]− (Calcd. for C26H39O12S, 575.2192); 1H NMR (CD3OD, 500 MHz) and 13C NMR (CD3OD, 125 MHz) spectroscopic data, see Table 1.

**Wedtriloside B (2):** White, amorphous powder; [α]D20 = 370 (c 0.10 MeOH); IR νmax (KBr) 3379, 2926, 2862, 1721, 1632, 1458, 1378, 1248, 1227, 1065, 1030, 986, 884, and 767 cm−1; HR-QTOF-MS (negative-ion mode) m/z 575.2190 [M – H]− (Calcd. for C26H39O12S, 575.2192); 1H NMR (CD3OD, 500 MHz) and 13C NMR (CD3OD, 125 MHz) spectroscopic data, see Table 1.
sulphuric acid solution containing 2% vanillin), by the positive value of optical rotation (\(\{\alpha\}^D_{D} + 10.5\) (c 0.15, H₂O) in comparison with \(\alpha\)-glucose standard, as well as GC analysis with a modification of the protocol previously described [18,19]. The addition of Ba(OH)₂ to the aqueous solution produced a white precipitate. The precipitate was not soluble in 2 M HCl, indicating the presence of \(\text{SO}_4^{2-}\) anions.

2.7. Assay for \(\alpha\)-amylase inhibition

The porcine pancreas \(\alpha\)-amyrase (A3176, Sigma-Aldrich) enzyme inhibitory activity was carried out according to the standard method with minor modification by Kusano et al. with slight modifications [20]. The substrate was prepared by boiling 100 mg potato starch in 5 mL phosphate buffer (pH 7.0) for 5 min, then cooling to room temperature. The sample (2 mL dissolved in DMSO) and the substrate (50 mL) were mixed in 30 mL of 0.1 M phosphate buffer (pH 7.0). After 5 min pre-incubation, 5 mg/mL \(\alpha\)-amylase solution (20 mL) was added and the solution was incubated at 37 °C for 15 min. The reaction was stopped by adding 50 mL 1 M HCl, and then 50 mL iodine solution was added. The absorbances were measured at 650 nm by a microplate reader. Acarbose was used as a positive control.

The inhibitory activity was calculated by the following equation: \(\alpha\)-amyrase inhibitory activity (%) = (1 − \(A/A_\text{dd}\)) × 100, where \(A\) is the absorbance of the sample and \(A_\text{dd}\) is the absorbance of the blank, respectively. \(IC_{50}\) value was calculated by GraphPad Prism.

2.8. Assay for \(\alpha\)-glucosidase inhibition

The yeast \(\alpha\)-glucosidase (G0660, Sigma-Aldrich) inhibition assay was performed using the substrate \(p\)-nitrophenyl-\(\alpha\)-D-glucopyranoside according to the previously described method [21]. Briefly, samples and acarbose were prepared by dissolving at 2 mg/mL (with extracts) or 0.8 mM (with pure compounds) in DMSO and 0.5 U/mL \(\alpha\)-glucosidase (40 mL) were mixed in 120 mL of 0.1 M phosphate buffer (pH 6.8). After 5 min pre-incubation, 5 mg/mL \(\alpha\)-amylase solution (20 mL) was added and the solution was incubated at 37 °C for 30 min. Pipette the following reagents into 96 wells plate. Each concentration of the sample was carried out in triplicate. The absorbance of released 4-nitrophenol was measured at 405 nm by using a microplate reader (Xmark, Biorad, USA). Acarbose was used as the positive control.

The inhibitory activity was calculated by the following equation: \(\alpha\)-glucosidase inhibitory activity (%) = (1 − \(A/A_\text{dd}\)) × 100, where \(A\) is the absorbance of the sample and \(A_\text{dd}\) is the absorbance of the blank, respectively. \(IC_{50}\) value was calculated by GraphPad Prism.

2.9. Data expression and statistical analysis

Data were expressed as mean value ± standard deviation (SD) of blood glucose. Data were evaluated using two way ANOVA followed by Dunnett’s multiple comparison test and groups were considered significantly different if \(P < 0.05\). All data are presented as means ± SD.

3. Results and discussion

3.1. Identification of compounds 1–9

A MeOH extract from the leaves of \(W.\ trilobata\) was suspended in \(H_2O\) and fractionated successively with EtOAc-soluble fraction and then each fraction was evaluated for \(\alpha\)-amylase and \(\alpha\)-glucosidase activities. Since the EtOAc fraction significantly reduced \(\alpha\)-amylase and \(\alpha\)-glucosidase activities (with 52.7 ± 1.31% and 68.0 ± 0.84%, respectively, at a concentration of 200 μg/mL). The EtOAc-soluble fraction and water layer were chosen for subsequent studied and resulted in the isolation of two new diterpenoids (1 and 2), together with five known compounds (3–7, Fig. 1). Moreover, compound 7 was reported for the first time from the genus Wedelia.
characteristic resonances of two tertiary methyl groups, an olefinic methine proton, two oxymethylene while its 13C NMR and HSQC data (Table 1) feature 26 carbon resonances including two methyl groups, an olefinic group, nine methylene (two oxygenated), eight methine (five oxygenated), and six none-protonated carbons (one carbonyl). On the basis of its NMR data and the structures of the diterpenoids previously isolated from Wedelia species [27,28], compound 2 was tentatively assigned as an ent-kauranoid [9].

The NMR data of 2 displayed features similar to those of 1 (Table 1). The chemical shifts and correlations in the 2D spectra were mostly comparable; except for major differences in the resonances associated with a methylene group of the sugar moiety in the 1H NMR spectrum [δH 4.25 (1H, dd, J = 2.0, 11.5 Hz, H-6′a) and 4.21 (1H, dd, J = 4.5, 11.5 Hz, H-6′b)] and a 13C NMR signal [δC 67.6 (C-6′)] caused by the presence of a sulfate group at this carbon. This was also supported by the downfield shift of C-6′ resonance (δC 67.6 in 2 instead of 62.4 in 1), together with the HMBC correlations between the signal at δC 3.53 (H-5) with the carbon resonance at δC 67.6 (C-6′) confirmed the presence of an sulfate group at C-6′. The position of attachment of the sugar was established based on the HMBC data in which the HMBC correlation between δH 5.47 (1H, d, J = 8.0 Hz, H-1′) and δC 177.4 (C-19) in compound 2 were used to establish the attachment of the glucose unit at C-19. Alkaline hydrolysis of 2 allowed identification of the aglycone as 16α,17-dihydroxy-ent-9(11)kaurane 19-acid by comparing its physical and spectroscopic data with published value [17]. The presence of α-glucose moiety in 1 was further confirmed by TLC, GC analysis, and comparisons with authentic α-glucose [18]. Moreover, when the addition of Ba(OH)2 to the aqueous solution resulted in a formation of a white precipitate, which was not soluble in 2 M HCl, indicating the presence of a sulfate group [29]. These results agreed with the mass spectral data. Additionally, the similar NOESY spectra observed for 1 and 2 revealed the identity of their relative configurations. Consequently, the structure of 2 was defined as 16α,17-dihydroxy-ent-9(11)-kaurene-19-19-ic acid-β-D-glucopyranosyl-6′-sulfate ester and the structure is as shown.

By comparison of their experimental and reported NMR data, the known compounds were identified as paniculoside-IV (3) [30], 5,7,4′-trihydroxyflavone (4) [31], apigenin 7-O-β-D-glucopyranoside (5) [32], 3,4-dihydroxy-cinnamic acid (6) [33], and 3-O-[β-D-glucopyranosyl (1→4)-β-D-glucoronopyranosyl] oleanolic acid 28-O-β-D-glucopyranosyl ester (7) [34].

3.2. α-Amylase and α-glucosidase inhibitory activities of compounds 1–7

The quest for safe, economical, and pharmacologically active molecules with multifunctional attributes towards diabetes management is necessary. This can be achieved by screening natural sources such as medicinal plants. Moreover, there is considerable interest in the application of traditional plants due to their natural origin, easily cultivable with minimum or no side effects. Previously, the extracts of W. trilobata have been reported to possess antihyperglycemic effects [5,6].

According to our results obtained, the extracts from this plant demonstrated moderate inhibitory activities against intestinal α-glucosidase and pancreatic α-amylase. Therefore, the antidiabetic effects of the plant may be associated with the components isolated from W. trilobata. In this report, we utilized the in vitro α-amylase [EC 3.2.1.1] and α-glucosidase [EC 3.2.1.20] inhibitory activities to evaluate the isolated compounds 1–7. Acarbose was used as the positive control in this study, which is an oligosaccharide of microbial origin (Actinoplanes) that potently inhibits in vitro and in vivo such brush-border enzymes as glucoamylase, dextrinase, maltase, and sucrose as well as the pancreatic α-amylase [35].

The new compounds 1 and 2 showed significant inhibition against α-amylase enzyme with IC50 values of 112.20 ± 2.87 and 87.10 ± 1.89 μg/mL, respectively. While the known compounds 4, 6, and 7 exhibited the most potent effects against α-glucosidase with IC50 values of 27.54 ± 1.12, 173.78 ± 2.37, and 190.40 ± 2.01 μg/mL, respectively (Table 2 and Fig. 3), when compared with that of a standard.

Table 2 Inhibitory effects of selected compounds against α-amylase and α-glucosidase activities (IC50 ± SD, μg/mL).

<table>
<thead>
<tr>
<th>Compoundsa</th>
<th>α-Amylase IC50 ± SD (μg/mL)</th>
<th>α-Glucosidase IC50 ± SD (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112.20 ± 2.87</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>87.10 ± 1.89</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>27.54 ± 1.12</td>
</tr>
<tr>
<td>6</td>
<td>181.97 ± 2.62</td>
<td>173.78 ± 2.37</td>
</tr>
<tr>
<td>7</td>
<td>52.08 ± 0.56</td>
<td>190.40 ± 2.01</td>
</tr>
<tr>
<td>Acarboseb</td>
<td>6.78 ± 0.32</td>
<td>450.56 ± 2.31</td>
</tr>
</tbody>
</table>

(–): No inhibition (less than 25% inhibition).

a Compounds tested in a set of experiments three times. P < 0.05 different versus control group.
b Acarbose was used as a positive control.

Fig. 3. IC50 values of compounds 4, 6, 7, and acarbose against α-glucosidase. Activity data points (absorbance) were plotted as mean ± SD (n = 3).
dard reference drug, acarbose. Additionally, moderate inhibitory effect against α-amylase was observed with compounds 6 and 7 (with IC\textsubscript{50} values of 181.97 ± 2.62 and 52.08 ± 0.56 μg/mL, respectively). In contrast, compound 3 showed weak inhibition on both enzymes with IC\textsubscript{50} values larger than 200 μg/mL. On the basis of the above activity values of 181.97 ± 2.62 and 52.08 ± 0.56 μg/mL, respectively). In standard reference drug, acarbose. Additionally, moderate inhibitory effect of remaining compounds. These results indicated that the diterpenoid and phenolic derivatives exhibited high inhibitory activity, but were not sufficient to clarify the structure-activity relationships between derivatives. Further research is required to clarify their potential selective α-amylase and α-glucosidase activities.

4. Conclusion

In conclusion, compounds 1, 2, and 7 were reported for the first time from the genus Wedelia. The present work reported for the first time the α-amylase and α-glucosidase inhibitory effects of W. trilobata, in support of their ethnomedicinal use for diabetes. The report partly defines the reason on why these medicinal plants possess antidiabetic properties and could propose a scientific warrant for its application as natural health and supplementary products for diabetes treatment and preventive interventions.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioxir.2019.01.010.

References