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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

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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 South-East Asia countries, where can be expected to have many potential unexploited medicinal herbs to be studied as a source phytopharmica 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.) Hitchn. (synonym *Sphagneticola 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

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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 ^{1}H NMR (500 MHz) and ^{13}C 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 on 65–250 or 230–400 mesh silica gel (Sorbent Technologies, Atlanta, GA, USA), porous polymer gel (Diaion HP-20, 20–60 mesh, Mitsubishi Chemical,

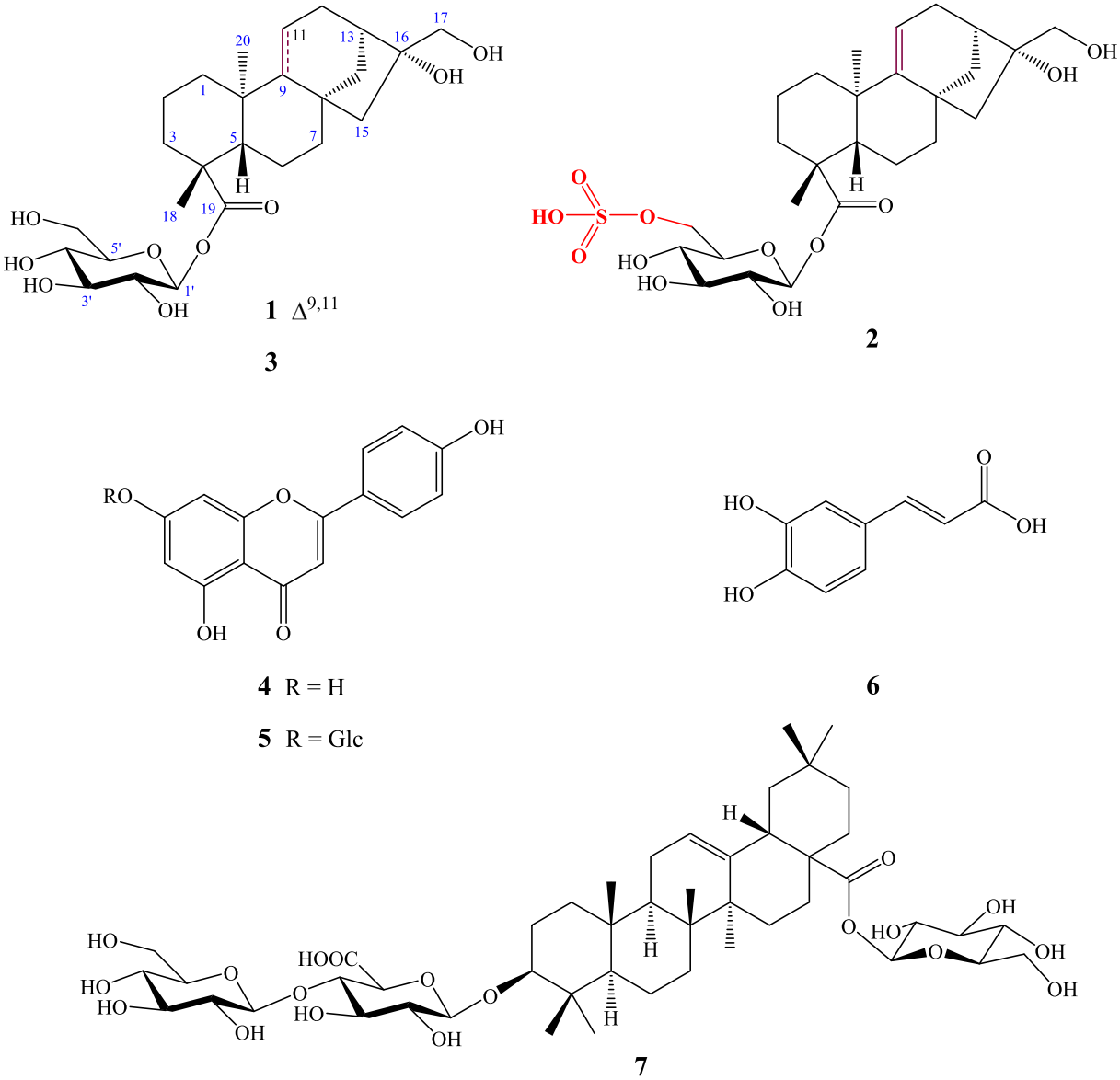

Fig. 1. Structures of the compounds (**1**–**7**) isolated from *W. trilobata*.

Table 1
^{1}H and ^{13}C NMR spectroscopic data (δ ppm) of **1** and **2** in CD_3OD.

No.	Wedtriloside A (**1**)		Wedtriloside B (**2**)	
	δ_C[a]	δ_H[b] mult. (*J* in Hz)	δ_C[a]	δ_H[b] mult. (*J* in Hz)
1	42.2, CH_2	1.20 m/1.95 m	42.2, CH_2	1.21 m/1.97 m
2	21.2, CH_2	1.93 m/1.50 m	21.2, CH_2	1.92 m/1.47 m
3	39.4, CH_2	2.23 m/1.07 m	39.3, CH_2	2.23 m/1.06 m
4	46.2, C	–	46.1, C	–
5	48.1, CH	1.66 dd (8.5, 11.0)	48.2, CH	1.65 dd (8.5, 11.0)
6	19.6, CH_2	2.56 m/1.98 m	19.5, CH_2	2.56 m/1.85 m
7	31.3, CH_2	2.03 m/1.51 m	31.3, CH_2	2.01 m/1.50 m
8	43.9, C	–	43.9, C	–
9	159.0, C	–	159.0, C	–
10	39.9, C	–	39.9, C	–
11	114.9, CH	5.20 t (3.0)	114.9, CH	5.19 t (3.0)
12	31.2, CH_2	2.22 m/1.48 m	31.2, CH_2	2.21 m/1.49 m
13	45.0, CH	2.16 m	45.0, CH	2.15 br d (3.5)
14	44.2, CH_2	2.06 m/1.45 m	44.2, CH_2	2.05 m/1.46 m
15	55.8, CH_2	1.96 m/1.54 m	55.8, CH_2	1.97 m/1.54 d (13.0)
16	85.6, C	–	85.6, C	–
17	68.9, CH_2	3.51 d (11.0) 3.55 d (11.0)	68.9, CH_2	3.50 d (11.0) 3.55 d (11.0)
18	28.4, CH_3	1.28 s	28.4, CH_3	1.23 s
19	177.7, C	–	177.4, C	–
20	24.5, CH_3	1.02 s	24.5, CH_3	1.02 s
1′	95.4, CH	5.47 d (8.0)	95.2, CH	5.47 d (8.0)
2′	74.0, CH	3.36 dd (8.0, 9.0)	73.9, CH	3.37 dd (8.0, 9.0)
3′	78.5, CH	3.43 dd (9.0, 9.0)	78.0, CH	3.43 br d (9.0)
4′	71.1, CH	3.38 t (9.0)	70.7, CH	3.49 t (9.0)
5′	78.7, CH	3.40 m	76.7, CH	3.53 m
6′	62.4, CH_2	3.83 dd (2.0, 11.5) 3.71 dd (4.5, 11.5)	67.6, CH_2	4.25 dd (2.0, 11.5) 4.21 dd (4.5, 11.5)

Assignments were made using the HMQC, HMBC, COSY, and NOESY spectra.
[a] 125 MHz.
[b] 500 MHz.

Tokyo, Japan), Sephadex LH-20 (Supelco, Bellefonte, PA, USA), octadecyl silica (ODS, 50 μm, Cosmosil 140 C_{18}-OPN, Nacalai Tesque), and YMC RP-18 resins (30–50 μm, Fuji Silysia Chemical). Analytical thin layer chromatography (TLC) systems were performed on precoated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck) and the isolated compounds were visualized by spraying with 10% H_2SO_4 in water and then heating for 1.5–2 min. All procedures were carried out with solvents purchased from commercial sources that were used without further purification.

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). A voucher specimen (NCCB-2016.55.02) was deposited at the Herbarium of Institute of Marine Biochemistry and Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

2.3. Compounds

From the methanolic extract of *W. trolobata*, 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 CH_2Cl_2-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 CH_2Cl_2-acetone (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 CH_2Cl_2-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-H_2O (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 (Φ15 mm, L900 mm) using MeOH-H_2O (1:1.5), to yield compound **5** (6.9 mg).

The H_2O fraction was separated using a Diaion HP-20 column (Φ100 mm, L500 mm) and was eluted with a gradient solvent mixture of MeOH-H_2O (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 (Φ45 mm, L750 mm) and eluted repeatedly with CH_2Cl_2-MeOH (0 → 100%) to yield three subfractions (W-1.1 to W-1.3). Subfraction W-1.1 was subjected to a silica gel CC (Φ20 mm, L800 mm) with a solvent mixture of CH_2Cl_2-MeOH, 5:1), passage over a Sephadex LH-20 column (Φ15 mm, L950 mm), and then an open YMC RP-18 column (Φ15 mm, L800 mm, 65 → 100%, H_2O-MeOH) 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 subfraction W-3 on YMC RP-18 column (Φ20 mm, L700 mm) and followed by passage over a Sephadex LH-20 column (Φ15 mm, L900 mm) using mixtures of MeOH-H_2O (1:5).

2.5. Physical and spectroscopic data of new compounds

Wedtriloside A (1): White, amorphous powder; $[\alpha]_D^{24}$ −61.6 (*c* 0.15, MeOH); IR ν_{max} (KBr) 3423, 2941, 2865, 1727, 1630, 1461, 1381, 1319, 1250, 1224, 1080, and 992 cm^{-1}; HR-QTOF-MS (negative-ion mode) *m/z* 541.2659 $[M+HCOO]^-$ (Calcd. for $C_{27}H_{41}O_{11}^-$, 541.2654) and 531.2367 $[M+Cl]^-$ (Calcd. for $C_{26}H_{40}O_9Cl^-$, 531.2366); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1.

Wedtriloside B (2): White, amorphous powder; $[\alpha]_D^{24}$ −370 (*c* 0.10 MeOH); IR ν_{max} (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 $C_{26}H_{39}O_{12}S^-$, 575.2192); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1.

2.6. Alkaline hydrolysis of compounds 1–2

A solution of compounds **1** and **2** (3 mg) in 10% KOH/EtOH (5 mL) was heated, stirred and refluxed at 70 °C for 3 h. After acidification with HOAc, the solvent was removed and the residue was partitioned between EtOAc and H_2O (10 mL × 3). The EtOAc fraction concentrated in vacuo and then was chromatographed on silica gel and purified to yield a white powder, which was identified as 16β,17-dihydroxy-kauran-19-oic acid by analysis of their NMR spectroscopic data as well as comparison with the reported values [17]. The sugar product in the aqueous layer was identified as D-glucose by silica gel TLC (Rf 0.3 developed with $CHCl_3$-MeOH-H_2O, 3:2:0.3 (v/v) and was sprayed with a 10%

sulphuric acid solution containing 2% vanillin), by the positive value of optical rotation ($[\alpha]_D^{24}$ +10.5 (*c* 0.15, H_2O) in comparison with D-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 SO_D^{2-}anions.

2.7. Assay for α-amylase inhibition

The porcine pancreas α-amylase (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 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 α-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: α-amylase inhibitory activity (%) = $(1 - A/A_0) \times 100$, where A is the absorbance of the sample and A_0 is the absorbance of the blank, respectively. IC_{50} value was calculated by GraphPad Prism.

2.8. Assay for α-glucosidase inhibition

The yeast α-glucosidase (G0660, Sigma-Aldrich) inhibition assay was performed using the substrate *p*-nitrophenyl-α-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 α-glucosidase (40 mL) were mixed in 120 mL of 0.1 M phosphate buffer (pH 6.8). After 5 min pre-incubation, 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution (40 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 samples 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: α-glucosidase inhibitory activity (%) = $(1 - A/A_0) \times 100$, where A is the absorbance of the sample and A_0 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 α-amylase and α-glucosidase activities. Since the EtOAc fraction significantly reduced α-amylase and α-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*.

Wedtriloside A (**1**) was obtained as a white, amorphous powder, with a negative optical rotation $[\alpha]_D^{24}$ −61.6 (*c* 0.15, MeOH). Its molecular formula was found to be $C_{26}H_{40}O_9$ via the NMR data and the HR-QTOF-MS ion at *m/z* 541.2659 $[M+HCOO]^-$ and 531.2367 $[M+Cl]^-$ in the negative-ion mode. The major IR absorption bands indicated a double bond (1630 cm^{-1}), a carboxyl group (1727 cm^{-1}), and hydroxy groups (3423 cm^{-1}). Analysis of the ^1H, ^{13}C NMR, and HSQC spectroscopic data belonging to the *ent*-kaurane diterpenoid moiety of the aglycon of **1** showed the resonances of protons and carbons of two tertiary methyl groups [δ_H 1.28 (3H, s, H_3-18)/δ_C 28.4 (C-18) and δ_H 1.02 (3H, s, H_3-20)/δ_C 24.5 (C-20)], an olefinic methine proton on a triple-substituted double bond [δ_H 5.20 (1H, t, J = 3.0 Hz, H-11)/δ_C 114.9 (C-11)], and an oxymethylene [δ_H 3.51 (1H, d, J = 11.0 Hz, H-17a), 3.55 (1H, d, J = 11.0 Hz, H-17b)/δ_C 68.9 (C-17)], together with a glucose moiety as evidenced by the presence of the anomeric proton [δ_H 5.47 (1H, d, J = 8.0 Hz, H-1′)/δ_C 95.4 (C-1′)] and other proton signals [δ_H 3.36 (1H, dd, J = 8.0, 9.0 Hz, H-2′)/δ_C 74.4 (C-2′), δ_H 3.43 (1H, dd, J = 9.0, 9.0 Hz, H-3′)/δ_C 78.5 (C-3′), δ_H 3.38 (1H, t, J = 9.0 Hz, H-4′)/δ_C 71.1 (C-4′), δ_H 3.40 (1H, m, H-5′)/δ_C 78.7 (C-5′), and δ_C 3.83 (1H, dd, J = 2.0, 11.5 Hz, H-6′a), 3.71 (1H, dd, J = 5.0, 11.5 Hz, H-6′b)/δ_C 62.4 (C-6′)] (Table 1).

These findings indicated that the structure of **1** was similar to the known 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-al [22], except for an ester carbonyl group in **1** instead of an aldehyde group in 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-al and the presence of an additional glucose moiety. The glycosylation site of the D-glucosyl unit was determined based on an HMBC experiment, which showed a cross-peak between the H-1′ doublet (δ_H 5.47, J = 8.0 Hz) and the C-19 carbon (δ_C 177.7) of the *ent*-kaurane aglycon. Moreover, the value of the spin-coupling pattern of the sugar proton signals ($J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 9.0 Hz, and $J_{3',4'}$ = 9.0 Hz) and carbon chemical shifts indicated that the glucoside was the β-anomer. Furthermore, alkaline hydrolysis of **1** afforded glucose and an aglycone, which was identified as 16α,17-dihydroxy-*ent*-9(11)kaurane 19-acid by comparing its physical data with those of published data [17]. The presence of D-glucose moiety in **1** was further confirmed by TLC, GC analysis, and comparisons with authentic D-glucose [18]. Additionally, HMBC correlations of the methyl protons at δ_H 1.28 (H_3-18) with C-3/C-4/C-5/C-19 and of H_3-20 (δ_H 1.02) with C-1/C-5/C-9/C-10 were observed. The correlations of an olefinic proton δ_H 5.20 (H-11) with C-8/C-9/C-13 were also observed. Detailed analysis of the other HMBC and COSY correlations (Fig. 2) unambiguously identified the planar structure of **1**. Thus, the 2D structure of **1** was established.

The relative configuration of **1** was determined by analyzing its NOESY data and based on the generally comparable NMR data with previous reports. The α-orientation of H_2C-14 was indicated by the NOESY correlation of H-14a (δ_H 1.45) and H_3-20 (δ_H 1.02), while the association between δ_H 1.28 (H_3-18) and δ_H 1.66 (H-5), however, no correlation between of δ_H 1.28 (H_3-18)/δ_H 1.66 (H-5) with H_3-20 (δ_H 1.02), indicated the β-orientations of H_3-18 and H-5. Additionally, the chemical shifts of C-16 (δ_C 85.6) and C-17 (δ_C 68.9) corresponded well to the similar signals observed in the ^{13}C NMR spectra with 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-al [δ_C 84.6 (C-16) and 68.4 (C-17)] [22] and the major difference from the ^{13}C NMR spectrum with 16β,17-dihydroxy configurations of 16β,17-hydroxy-(-)-kauran-19-oic acid-β-D-glucopyranosyl ester [δ_C 79.8 (C-16) and 70.3 (C-17)] [23]. These data clearly indicated the presence of 16α,17-dihydroxy configurations in **1**. From all of this information, the structure of **1** was established as 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-oic acid-β-D-glucopyranosyl ester.

Wedtriloside B (**2**) displayed a deprotonated ion peak at *m/z* 575.2190 $[M-H]^-$, as determined by HR-QTOF-MS data, which supports the molecular formula $C_{26}H_{39}O_{12}S$ (Calcd. for 575.2192). The IR spectrum of **2** displayed the S—O stretching band (1227 and 1248 cm^{-1}) and C—O—S banding (767, 819, and 884 cm^{-1}) characteristic for the sulfate group [24–26]. Its ^1H NMR data (Table 1) show

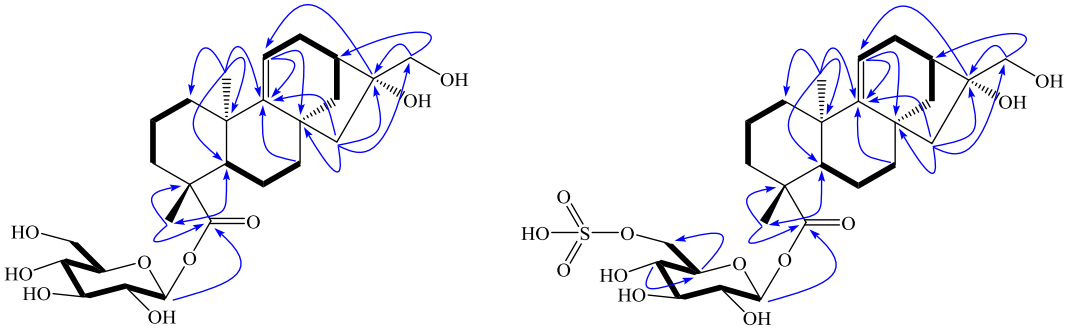

Fig. 2. Key HMBC (⟶) and COSY (▬) correlations of **1** and **2**.

characteristic resonances of two tertiary methyl groups, an olefinic methine proton, two oxymethylene while its ^{13}C 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 ^{1}H NMR spectrum [δ_H 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 ^{13}C NMR signal [δ_C 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 (δ_C 67.6 in **2** instead of 62.4 in **1**], together with the HMBC correlations between the signal at δ_H 3.53 (H-5′) with the carbon resonance at δ_C 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 ^{3}J HMBC correlation between δ_H 5.47 (1H, d, J = 8.0 Hz, H-1′) and δ_C 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 D-glucose moiety in **1** was further confirmed by TLC, GC analysis, and comparisons with authentic D-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 **2** and **1** revealed the identity of their relative configurations. Consequently, the structure of **2** was defined as 16α,17-dihydroxy-*ent*-9(11)-kaurene-19-oic 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 IC_{50} 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 IC_{50} 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 stan-

Table 2
Inhibitory effects of selected compounds against α-amylase and α-glucosidase activities (IC_{50} ± SD, μg/mL).

Compounds[a]	α-Amylase	α-Glucosidase
1	112.20 ± 2.87	–
2	87.10 ± 1.89	–
4	–	27.54 ± 1.12
6	181.97 ± 2.62	173.78 ± 2.37
7	52.08 ± 0.56	190.40 ± 2.01
Acabose[b]	6.78 ± 0.32	450.56 ± 2.31

(–): No inhibition (less than 25% inhibition).
[a] Compounds tested in a set of experiments three times. $P < 0.05$ different versus control group.
[b] Acabose was used as a positive control.

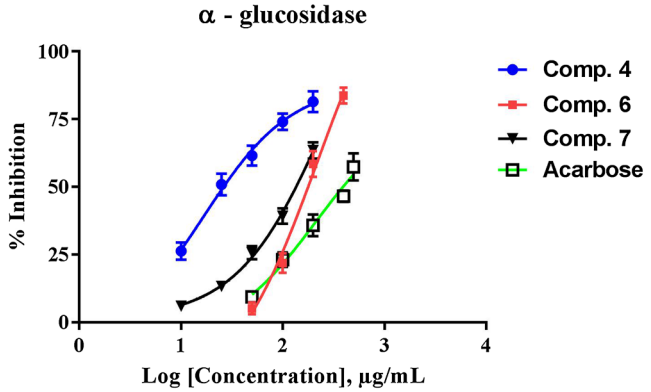

Fig. 3. IC_{50} values of compounds **4**, **6**, **7**, and acarbose against α-glucosidase. Activity data points (absorbance) were plotted as mean ± SD (n = 3).

dard reference drug, acarbose. Additionally, moderate inhibitory effect against α-amylase was observed with compounds **6** and **7** (with IC_{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_{50} values larger than 200 μg/mL. On the basis of the above activity results, a preliminary structure-activity relationship was deduced. The substitution on the hydroxy atom can increase the inhibitory activity, as indicated by comparing the inhibitory activity of **4**, **6**, and **7** with that 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.bioorg.2019.01.010.

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