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ABSTRACT

Three new pregnane glycosides, drevoluosides O-Q (**1-3**) along with five known volubiloside C (**4**), dreageoside A11 (**5**), 17β-marsdenin (**6**), stavaroside H (**7**), and hoyacarnoside G (**8**) were isolated from the methanol extract of the *Dregea volubilis* leaves. Their structures were elucidated by chemical and spectroscopic methods. Compounds **6-8** showed significant anti α-glucosidase activity with the inhibitory percentages ranging from 32.6 to 47.1% at the concentration of 200 μM. Compound **3** showed significant inhibitory α-amylase activity with IC_{50} value of 51.3 ± 2.1 μM.

Dregea volubilis
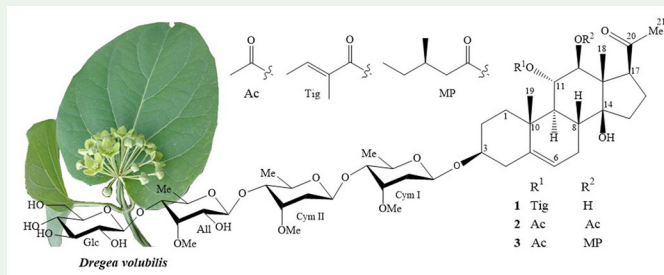
1. Introduction

Diabetes mellitus is a chronic disorder and considered as one of the most critical health issues in both developed and developing countries. Among hypoglycemic agents, drugs targets for α-glucosidase and α-amylase could control blood sugar and

therefore can be an important strategy in the management of blood glucose level in type 2 diabetic patients.

Dregea volubilis (L.f.) Benth. ex Hook. f. (Apocynaceae) is a perennial woody climber native to tropical and subtropical regions such as India, Africa, and southeast Asia. *D. volubilis* has been used in traditional medicine for the treatment of inflammation, rheumatic pain, fever, cough, and severe cold. The phytochemical investigation of this plant suggested the presence of pregnanes, pregnane glycosides (Yoshimura et al. 1985; Sahu et al. 2002), and flavonoids. The extract and compounds from this plant have been showed α-glucosidase, α-amylase, antioxidant (Das et al. 2017), and anti-inflammatory activities (Hossain et al. 2010). As a part of our ongoing investigation on anti-diabetic compounds from Vietnamese medicinal plants (Nhiem et al. 2010), we report herein the isolation, structural elucidation of pregnane-*type* glycosides from *D. volubilis* leaves and their anti-diabetic activity.

2. Results and discussion

The molecular formula of **1** was determined as $C_{53}H_{84}O_{21}$ by the HR-ESI-MS at *m/z* 1055.5372 [M – H]$^-$ (Figures S2). The ^{1}H-NMR spectrum of **1** (CD$_3$OD) showed the following signals: one olefinic proton at δ_H 5.50 (1H, br d, $J = 5.5$ Hz), and three tertiary methyl groups at δ_H 1.00 (3H, s), 1.15 (3H, s), and 2.25 (3H, s) assigned to a pregnane aglycone, two methyl groups at δ_H 1.84 (3H, d, $J = 7.0$ Hz) and 1.88 (3H, s) and one olefinic proton at δ_H 6.91 (1H, q, $J = 7.0$ Hz), suggested the presence of tigloyl moiety, four anomeric protons at δ_H 4.37 (1H, d, $J = 8.0$ Hz), 4.60 (1H, d, $J = 8.0$ Hz), 4.81 (1H, overlapped signal), and 4.87 (1H, dd, $J = 2.0, 9.5$ Hz), indicated the presence of four sugar units. The ^{13}C-NMR and HSQC spectra (Figures S5 and S6) revealed the signals of 53 carbons, including 2 carbonyls, 5 non-protonateds, 26 methines, 9 methylenes, and 11 methyl carbons. Analysis of ^{1}H and ^{13}C NMR data indicated the structure of **1** was similar to those of volubiloside C (Sahu et al. 2002) except for the addition of a tigloyl moiety at C-11. The HMBC correlations (Figure S1) from H-21 (δ_H 2.25) to C-17 (δ_C 58.7)/C-20 (δ_C 218.4) suggested the ketone group at C-20. The HMBC correlations between H-18 (δ_H 1.00) and C-12 (δ_C 77.0)/C-13 (δ_C 56.4)/C-14 (δ_C 85.5)/C-17 (δ_C 58.7), between H-8 (δ_H 1.81)/H-9 (δ_H 1.65) and C-11 (δ_C 74.7), and COSY correlations of H-9 (δ_H 1.65)/H-11 (δ_H 5.33)/H-12 (δ_H)3.25 indicated three oxygenated groups at C-11, C-12, and C-14. The (*E*)-tigloyl moiety was confirmed by the HMBC correlations between tig H-5 (δ_H 1.88) and tig C-1 (δ_C 169.2)/tig C-2 (δ_C 130.3)/tig C-3 (δ_C 139.0) and between tig H-4 (δ_H 1.84) and tig C-2 (δ_C 130.3)/tig C-3 (δ_C 139.0) and also by the NOESY correlation between tig H-4 (δ_H 1.84) and tig H-5 (δ_H 1.88). The position of tigloyl moiety at C-11 was confirmed by the HMBC correlation between H-11 (δ_H 5.33) and tig C-1 (δ_C 169.2). It was further confirmed by the HR-ESI-MS/MS which showed an ion peak at 1055.5372 [M–H]$^-$ giving product ions at *m/z* 955.4853 [M-(C$_5$H$_8$O$_2$)-H]$^-$ and 99.0442 [C$_5$H$_7$O$_2$]$^-$. The *axial* orientations of H-11 and H-12 were confirmed by large coupling constants of H-9 and H-11, $J = 10.5$ Hz; H-11 and H-12, $J = 10.0$ Hz and the observation of NOESY correlations between H-11 (δ_H 5.33) and H-19 (δ_H 1.15); H-12 (δ_H 3.25) and H-17 (δ_H 3.52). The constitution of **1** was proven by the analysis of NOESY spectrum as well as by biogenetic pathway of pregnane from *Dregea* genus. The aglycone of **1** was supposed to have the same configurations as those of

known compounds, volubiloside C (**4**) (Sahu et al. 2002) and dreageoside A11 (**5**) (Yoshimura et al. 1983). The alkaline hydrolysis of **1** afforded an aglycone, identified as drevogenin P, [3β,11α,12β,14β-tetrahydroxypregn-5-en-20-one] proved by its physical and spectral data (Sauer et al. 1965; Sahu et al. 2002). The sugar moieties were determined as by acid hydrolysis. The acid hydrolysis of **1** gave three monosaccharides, which were identified as D-cymarose, 6-deoxy-3-O-methyl-D-allose, and D-glucose by comparing its specific rotation with those reported (Abe, Okabe, et al. 1999; Warashina and Noro 2000). The multiplicity of H-1 of monosaccharide units, Cym: δ_H 4.86 (dd, $J = 2.0, 9.5$ Hz), All: δ_H 4.60 (d, $J = 8.0$ Hz), and Glc: δ_H 4.37 (d, $J = 8.0$ Hz) suggested the configurations of monosaccharides as β-D-cymaropyranosyl, 6-deoxy-3-O-methyl-β-D-allopyranosyl, and β-D-glucopyranosyl. The HMBC correlations from Glc H-1 (δ_H 4.37) to All C-4 (δ_C 83.8), All H-1 (δ_H 4.60) to Cym II C-4 (δ_C 84.0), Cym II H-1 (δ_H 4.81) to Cym I C-4 (δ_C 83.8), and from Cym I H-1 (δ_H 4.86) to C-3 (δ_C 78.4) indicated the sugar linkage as β-D-glucopyranosyl-(1→4)-6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and at C-3 of aglycone, a sugar linkage previously reported from *Dregea volubilis* (Sahu et al. 2002). Consequently, the structure of **1** was elucidated to be 11α-tigloyloxy-3β,12β,14β-trihydroxy-pregn-5(6)-en-20-one   3-O-β-D-glucopyranosyl-(1→4)-6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, a new compound named drevoluoside O.

Compound **2** was obtained as a white amorphous powder and its molecular formula was determined as $C_{52}H_{82}O_{22}$ by HR-ESI-MS (Figure S10) at m/z 1057.5165 [M – H]$^-$ (calcd. for $[C_{52}H_{81}O_{22}]^-$, 1057.5225). The ^{1}H- and ^{13}C-NMR spectra of **2** (Figures S12 and S13) showed the presence of a pregnane aglycone, four sugar moieties, and two acetoxy groups. The ^{13}C-NMR and HSQC spectra revealed the signals of 56 carbons, including 3 carbonyls, 4 non-protonateds, 25 methines, 9 methylenes, and 11 methyl carbons. Analysis of ^{1}H- and ^{13}C- NMR data indicated the structure of **2** was similar to those of **1** except for the position of two acetoxy groups at C-11 and C-12. In addition, the aglycone of **2** was found to be similar to those of hoyacarnoside D (Abe, Fujishima, et al. 1999). The position of two acetoxy groups at C-11 and C-12 were confirmed by HMBC correlations from H-11 (δ_H 5.38) to Ac I (δ_C 171.8) and from H-12 (δ_H 4.85) to Ac II (δ_C 172.5). The monosaccharides were identified as D-cymarose, 6-deoxy-3-O-methyl-D-allose, and D-glucose by the acid hydrolysis of **2**. The glucose in terminal sugar linkages was confirmed by the HR-ESI-MS/MS fragment ions at m/z 161.0449 $[C_6H_9O_5]^-$ and 895.4685 [M-glc]$^-$ from molecular ion peak at 1057.5165 [M-H]$^-$. The sugar linkage at C-3 of aglycone was suggested   as   O-β-D-glucopyranosyl-(1→4)-6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside   by   HMBC   correlations between Glc H-1 (δ_H 4.37) and All C-4 (δ_C 83.8), All H-1 (δ_H 4.60) and Cym II C-4 (δ_C 84.1), Cym II H-1 (δ_H 4.80) and Cym I C-4 (δ_C 83.8), and Cym I H-1 (δ_H 4.86) and C-3 (δ_C 78.6). Thus, the structure of **2** was determined as 11α,12β-diacetoxy-3β,14β-dihydroxypregn-5(6)-en-20-one   3-O-β-D-glucopyranosyl-(1→4)-6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and named drevoluoside P.

The HR-ESI-MS of **3** (Figure S18) gave a pseudo-molecular ion peak at m/z 1113.5790 [M – H]$^-$ (calcd. for $[C_{56}H_{89}O_{22}]^-$, 1113.5851) corresponding to the molecular formula of $C_{56}H_{90}O_{22}$. The ^{1}H- and ^{13}C-NMR spectra of **3** (Figures S20 and S21) showed the presence of a pregnane aglycone, four sugar moieties, one acetoxy group, and one 3-

methylpentanoyl group [the typical signals: two methyl groups at δ_H 0.96 (dt, $J = 1.5$, 7.5 Hz) and 1.02 (d, $J = 6.0$ Hz), and four methylene protons at δ_H 1.31 (m), 1.44 (m), 2.15 (dd, $J = 8.0$, 15.5), and 2.44 (dd, $J = 6.0$, 15.5), one methine proton at 1.92 (m)]. Analysis of ^{1}H- and ^{13}C-NMR data indicated the structure of **3** was similar to those of **2** with the replacement of an acetoxy group by 3-methylpentanoyloxy group at C-12. This was confirmed by HMBC correlations from H-12 (δ_H 4.85) to Mep C-1 (δ_C 174.6), Mep H-5 (δ_H 0.96) to Mep C-3 (δ_C 32.8)/Mep C-4 (δ_C 30.4), Mep H-6 (δ_H 1.02) to C-2 (δ_C 42.3)/C-3 (δ_C 32.8)/C-4 (δ_C 30.4). In addition, the acid hydrolysis of **3** gave 3-methylpentanoic acid. This moiety was further proved by the HR-ESI-MS/MS fragment ions at m/z 115.0751 [$C_6H_{11}O_2$]$^-$ from molecular ion peak at 1113.5790 [M-H]$^-$. The stereochemistry of 3-methylpentanoic acid was proved as S by comparing its specific rotation ([α]$_D^{25}$ = +5.6 (c 1.0, MeOH)) with those reported: (3S)- methylpentanoic acid: [α]$_D$ = +5.8 (c 1.4, MeOH) (Roeder et al. 1999) and (3 R)-methylpentanoic acid: [α]$_D^{25}$ = −5.5 (c 1.0, MeOH) (Yang et al. 2017).

The position of the acetyloxy at C-11 was confirmed by HMBC correlation from H-11 (δ_H 5.38) to Ac (δ_C 171.7). The saccharide linkage was also found to be identical with those of **1** and **2** by the analysis of HMBC and COSY spectra (Figures S23 and S24) as well as acid hydrolysis. Thus, the structure of **3** was elucidated as 11α-acetoxy-12β-(3S)-methylpentanoyloxy-3β,14β-dihydroxypregn-5(6)-en-20-one 3-O-β-D-glucopyranosyl-(1→4)-6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside and named drevoluoside Q.

The known compounds were characterized as volubiloside C (**4**) (Sahu et al. 2002), dreageoside A11 (**5**) (Yoshimura et al. 1983), 17β-marsdenin (**6**) (Panda et al. 2003), stavaroside H (**7**) (El Sayed et al. 1995), and hoyacarnoside G (**8**) (Abe, Fujishima, et al. 1999) by spectroscopic data (Figure 1).

All isolates were evaluated for their inhibitory activities against α-glucosidase and α-amylase. (Table S2). First, the inhibitory α-glucosidase and α-amylase activities of compounds were screened at the concentration of 200 μM. Compounds **6-8** showed significant α-glucosidase with the inhibitory percentages ranging from 32.6 to 47.1%, similar to those of an anti-diabetes drug, acarbose with α-glucosidase inhibition of 32.7%. Regarding α-amylase activity, compound **3** showed α-amylase inhibition of 78.9 ± 1.1%, compared to those of acarbose with inhibition of 89.1 ± 3.7% at the concentration of 200 μM. Compound **3** was further evaluated on α-amylase assay at the concentrations of 200, 100, 50, 25, and 10 μM to get IC$_{50}$ value. Compound **3** showed significant inhibitory α-amylase with an IC$_{50}$ value of 51.3 ± 2.1 μM (acarbose, IC$_{50}$ of 36.3 ± 0.5 μM). To the best of our knowledge, α-glucosidase and α-amylase inhibitory activities of pregnane glycosides from *D. volubilis* has been reported for the first time

3. Experimental

3.1. General: see supporting information

3.2. Plant material

The leaves of *Dregea volubilis* were collected at Huu Lung, Lang Son, Viet Nam in September 2017 and identified by Dr. Nguyen The Cuong, Institute of Ecology and
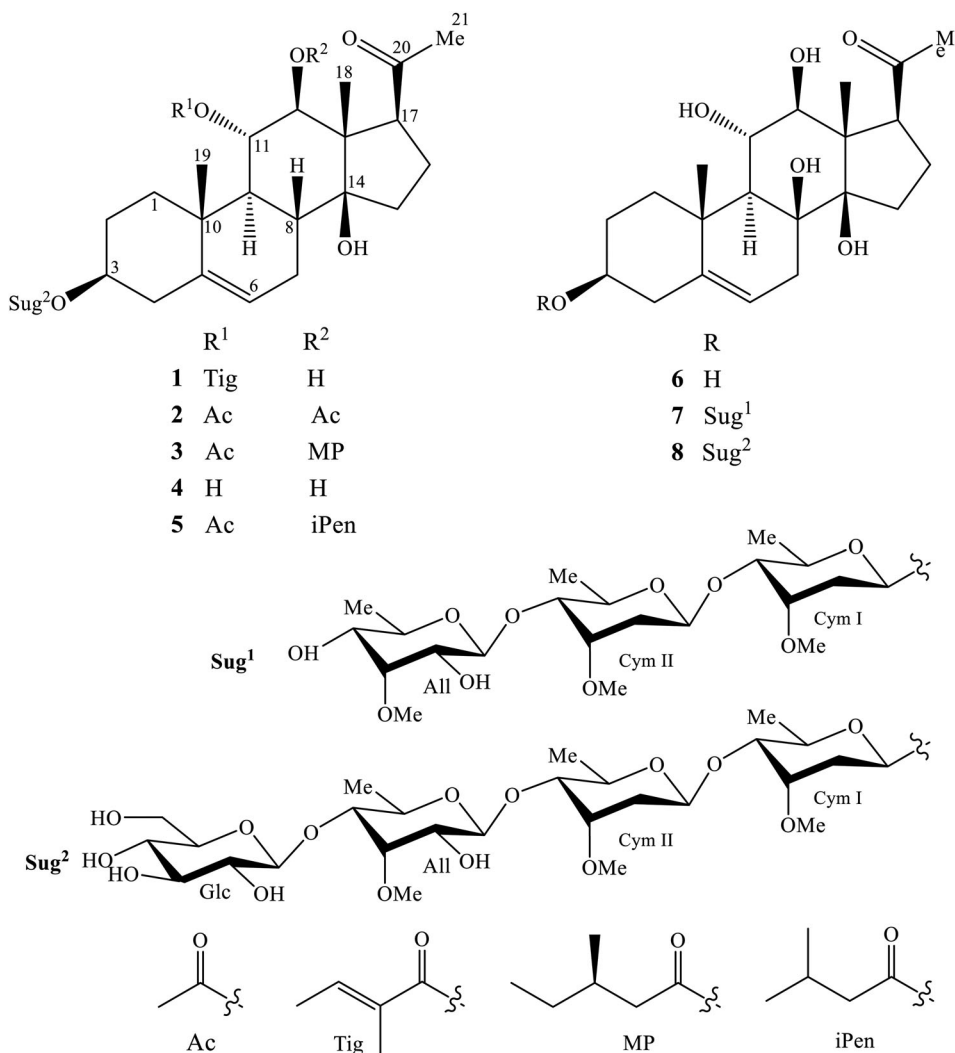
Figure 1. Chemical structures of compounds 1–8.

Biological Resources, VAST. A voucher specimen (NCCT-P75) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried powder of leaves of *D. volubilis* (5.0 kg) was sonicated 3 times with hot methanol and then removed solvent under reduced pressure to yield 640 g of a dark solid extract. The extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane, ethyl acetate giving *n*-hexane (DV1, 90 g), dichloromethane (DV2, 200 g), ethyl acetate extracts (DV3, 23 g) and water layer (DV4). DV2 extract was chromatographed on a silica gel column eluting with *n*-hexane/acetone (100:0, 50:1, 20:1, 10:1, 5:1, 2.5:1, v/v) to give six sub-fractions DV2A-DV2F. DV2D was chromatographed on an RP-18 column eluting with methanol/water (2/1, v/v) to give six smaller

fractions, DV2D1-DV2D6. DV2D1 fraction was chromatographed on an RP-18 column eluting with acetone/water (1,2/1, v/v) to give two smaller fractions, DV2D1A-DV2D1B. DV2D1A was further chromatographed on HPLC using J'sphere ODS M-80 column (150 mm length × 20 mm ID), 35% ACN in H_2O with a flow rate of 3 mL/min to yield compounds **7** (28.4 mg). DV2D3 was chromatographed on an RP-18 column eluting with acetone/water (1.5/1, v/v) to give three smaller fractions, DV2D3A-DV2D3C. The DV2D3B was purified on HPLC J'sphere ODS M-80 column (150 mm length × 20 mm ID) eluting with 40% ACN in water to yield **1** (28.4 mg) and **2** (13.5 mg). DV2D5 was chromatographed on an RP-18 column eluting with acetone/water (1.7/1, v/v) to give two smaller fractions, DV2D5A-DV2D5B. Compound **5** (26.9 mg) was obtained from DV2D5A by chromatography on HPLC J'sphere ODS M-80 column (150 mm length × 20 mm ID) eluting with 55% ACN in water and a flow rate of 3 mL/min. DV2D5B was further chromatographed on HPLC, using J'sphere ODS M-80 column (150 mm length × 20 mm ID) eluting with 53%ACN in H_2O to yield **3** (21.6 mg). DV2F was chromatographed on an RP-18 column eluting with acetone/water (1/1.8, v/v) to give three smaller fractions, DV2F1-DV2F3. DV2F3 fraction was chromatographed on an RP-18 column eluting with methanol/water (1/1, v/v) to give two smaller fractions, DV2F3A-DV2F3B. Compound **4** (24.0 mg) was obtained from DV2F3B by chromatography on HPLC using J'sphere ODS M-80 column (150 mm length × 20 mm ID), eluting with 24%, ACN in H_2O and a flow rate of 3 mL/min. The water layer was chromatographed on a Diaion HP-20 column eluting with water to remove sugar components, then increase the concentration of methanol in water (25, 50, 75 and 100%, v/v) to obtain four fractions, DV4A-DV4D. The DV4D fraction was chromatographed on a silica gel column eluting with dichloromethane/methanol (20/1, 10/1, 5/1, 2.5/1, v/v) to give four sub-fractions DV4D1-DV4D4. DV4D2 was chromatographed on an RP-18 column eluting with methanol/water (1/2, v/v) to give two smaller fractions, DV4D2A-DV4D2B. The DV4D2B was purified on HPLC J'sphere ODS M-80 column (150 mm length × 20 mm ID), eluting with 20% acetonitrile in water to yield **6** (16.0 mg). DV4D4 was chromatographed on an RP-18 column eluting with methanol/water (1/1, v/v) to give two smaller fractions, DV4D4A-DV4D4B. Finally, compound **8** (61.0 mg) was obtained from DV2F3B chromatographed on HPLC using J'sphere ODS M-80 column (150 mm length × 20 mm ID), eluting with 24% ACN in H_2O and a flow rate of 3 mL/min.

3.3.1. Drevoluoside O (1)
White amorphous powder; $[\alpha]_D^{25}$ − 45.5 (c 0.1, MeOH); $C_{53}H_{84}O_{21}$, HR-ESI MS *m/z*: 1055.5372 [M − H]$^-$ (calcd. for $[C_{53}H_{83}O_{21}]^-$, 1055.5432); ^{1}H (CD$_3$OD, 500 MHz) and ^{13}C NMR (CD$_3$OD, 125 MHz) data, see Table S1.

3.3.2. Drevoluoside P (2)
White amorphous powder; $[\alpha]_D^{25}$ − 38.1 (c 0.1, MeOH); $C_{52}H_{82}O_{22}$, HR-ESI MS *m/z*: 1057.5165 [M − H]$^-$ (calcd for $[C_{52}H_{81}O_{22}]^-$, 1057.5225); ^{1}H (CD$_3$OD, 500 MHz) and ^{13}C NMR (CD$_3$OD, 125 MHz) data, see Table S1.

3.3.3. Drevoluoside Q (3)

White amorphous powder; $[\alpha]_D^{25}$ – 70.2 (c 0.1, MeOH); $C_{56}H_{90}O_{22}$, HR-ESI MS m/z: 1113.5790 [M – H]⁻ (calcd. for $[C_{56}H_{89}O_{22}]^-$, 1113.5851); ¹H (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz) data, see Table S1.

3.4. Acid hydrolysis

Each compound (**1–3**, 10.0 mg) was separately dissolved in 1.0 N HCl (dioxane–H₂O, 1:1, v/v, 1.0 mL) and heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N₂ overnight. After extraction with CHCl₃, the aqueous layer was concentrated to dryness using N₂ to give an aqueous residue (A). The aqueous residue (A) was separated by silica gel CC eluting with CH₂Cl₂–MeOH (10:1, v/v) and then further fractionated by RP-18 CC using a stepwise gradient of MeOH–H₂O (6:4, 7:3, and 8:2, v/v) to give the saccharides. The specific rotation of these sugars was determined. The specific rotation ($[\alpha]_D^{25}$) of sugars was determined after dissolving in H₂O for 24 h and compared to the literature (lit): D-cymarose: found +50.0 (c 0.4, H₂O), lit +51.8 (Warashina and Noro 2000); 6-deoxy-3-O-methyl-D-allose: found + 11.0 (c 0.4, H₂O); lit +10.0 (Abe, Okabe, et al. 1999); and D-glucose: found + 49.1 (c 0.4, H₂O); lit + 48.0 (Abe, Okabe, et al. 1999). Based on the above evidence and experiments, sugar components were found in compounds **1–3**: D-cymarose, 6-deoxy-3-O-methyl-D-allose, and D-glucose.

3.5. α-Gucosidase assay

see Supporting information (Trang et al. 2020)

3.6. α-Amylase assay

see Supporting information (Hanh et al. 2014)

Disclosure statement

No potential conflict of interest was reported by the authors

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