Chemical Constituents from *Mentha haplocalyx* Briq. (*Mentha canadensis* L.) and Their α-Glucosidase Inhibitory Activities

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**Abstract**

*Mentha haplocalyx* (*Mentha canadensis*) is widely used as a medicinal plant in traditional Chinese medicine, and the extracts of its aerial parts are found to significantly inhibit the activity of α-glucosidase with an IC₅₀ value of 21.0 μg/mL. Bioactivity-guided isolation of the extracts afforded two new compounds (1 and 2), together with 23 known ones (3–25). Their structures were established by extensive spectroscopic analyses (1D and 2D NMR, MS, IR and UV). Compounds 1–17 and 21–25 were evaluated for their α-glucosidase inhibitory activities. Compound 11 was the most active ones with an IC₅₀ values of 83.4 μM. These results verify the α-glucosidase inhibitory activity of *M. haplocalyx* (*M. canadensis*) and specify its active compounds for the first time.

**Graphical Abstract**

**Keywords** *Mentha haplocalyx* Briq. (*Mentha canadensis* L.) · Lamiaceae · α-Glucosidase inhibitor

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**1 Introduction**

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin [1]. Such a deficiency results in an increased blood glucose level and in turn damages body systems including blood vessels and nerves [2]. It is one of the most serious diseases worldwide and developing with an increase in obesity and ageing [3]. An efficiently therapeutic approach is to retard absorption of glucose through the inhibition of carbohydrate-hydrolysing enzymes such as α-amylase and α-glucosidase in the digestive organs [4]. Clinically, acarbose and voglibose have been
used as effective α-glucosidase inhibitors to delay glucose absorption [5].

_Mentha haplocalyx_ Briq. (_Mentha canadensis_ L.), a perennial herbaceous plant of the family Lamiaceae, is widely distributed in southwest of China and popularly used in food, cosmetics and medicines. As a traditional Chinese medicine, it is clinically used to treat diseases in the nerve center, breath, procreation and digestive systems [6]. Pharmacological studies of _M. haplocalyx_ (_M. canadensis_) revealed various biological activities, such as antimicrobial, anti-inflammatory, antioxidant, antitumor, gastrointestinal protective, and hepatoprotective activities [7]. A large number of volatile compounds were reported from _M. haplocalyx_ (_M. canadensis_), as well as a few polyphenolic acids, flavonoids, monoterpenoids, and glycosides, which might contribute to the medicinal benefits of this plant [8].

Several findings have depicted the potential antidiabetic capability of genus _Mentha_. _M. piperita_ could alleviate hyperglycemia induced by streptozotocin–nicotinamide-induced type 2 diabetes in rats, and cause a reduction of glycemia in human [9, 10]. However, no report has referred to the active compounds of _Mentha_ responsible for its anti-glycemia in human [9, 10].

2 Results and Discussion

2.1 Structural Identification

Compound 1 was obtained as colorless caramelized solid, with the molecular formula of C_{16}H_{24}O_{7} from its HREIMS at m/z 351.1404 [M+Na]^{+}. The IR absorptions suggested the existence of a hydroxyl group (3427 cm\(^{-1}\)) and an α,β-un saturated carbonyl group (1635 cm\(^{-1}\)). The \(^{13}\)C NMR (DEPT) data (Table 1) displayed sixteen carbon signals, including one methyl, five methylenes, seven methines, and three quaternary carbons. Among them, a glucose unit (δ\(_{\text{C}}\) 104.1, 75.2, 78.2, 71.7, 78.2, 62.8) and an α,β unsaturated carbonyl group (δ\(_{\text{C}}\) 136.6, 149.4, 201.0) were observed. \(^{1}\)H NMR data (Table 1) exhibited a singlet methyl proton (δ\(_{H}\) 1.78, H-10), a trisubstituted olefinic proton (δ\(_{H}\) 7.23, H-2), and one terminal double bond proton (δ\(_{H}\) 4.82, H-9a; 4.80, H-9b). The glucose unit protons [δ\(_{H}\) 4.30 (d, J = 7.8 Hz, H-1′); 3.19–3.85 (6H, H-2′–H-6′)] were also present. All the above information suggested that compound 1 was a menthane-monoterpenic glycoside. Apart from the glucose unit, the NMR data of 1 closely resembled those of the reported compound (R)-7-hydroxy carvone [11]. Whereas the major differences between their NMR signals at C-7 position [δ\(_{\text{C}}\) 4.49, 4.28 and δ\(_{\text{H}}\) 67.0 for 1; δ\(_{\text{C}}\) 4.27 and δ\(_{\text{H}}\) 61.8 for (R)-7-hydroxy carvone] suggested that 7-OH in 1 might be glycosylated, which was further confirmed by the HMBC correlation of H-7 (δ\(_{H}\) 4.99 and 4.28) with C-1′ (δ\(_{\text{C}}\) 104.1) (Fig. 2). The specific rotation of 1 ([\(\alpha\)]\(_{20}^{D}\) = −22.0) was opposite to (4R)-7-hydroxyisopiperitenone7-β-D-glucopyranoside ([\(\alpha\)]\(_{20}^{D}\) + 53.9°), and similar to the (4S)-7-hydroxyisopiperitenone 7-Ο-β-D-glucopyranoside ([\(\alpha\)]\(_{20}^{D}\) = −14.4) [12], tentatively determining its (4S)-configuration. Therefore, compound 1 was elucidated as (4S)-7-hydroxy-carvone 7-Ο-β-D-glucopyranoside.

Compound 2, white powder, had a molecular formula of C_{47}H_{82}O_{33} from its HREIMS (m/z 694.6258 [M]+). The \(^{13}\)C NMR (DEPT) data (Table 2) displayed 47 carbon signals, including ten methyls, 23 methylenes, six methines and eight quaternary carbons. Among them, a methoxyl (δ\(_{\text{C}}\) 53.4), two oxy-methines (δ\(_{\text{C}}\) 80.4, C-3; 75.8, C-11), a trisubstituted double bond (δ\(_{\text{C}}\) 121.8, 149.7) were observed. A palmitoyl group (δ\(_{\text{C}}\) 173.7, C-1′; 34.7, C-2′; 25.2, C-3′; 29.2–29.7, C-4′–C-13′; 31.9, C-14′; 22.7, C-15′; 14.1, C-16′) was also present. The \(^{1}\)H NMR data (Table 2) exhibited eight singlet methyls (δ\(_{H}\) 0.85–1.21), a triplet methyl (δ\(_{H}\) 0.88, t, J = 7.8 Hz), a methoxyl (δ\(_{H}\) 3.25), and a trisubstituted olefinic proton (δ\(_{H}\) 5.32, d, J = 3.0 Hz, H-12). These NMR data suggested that 2 was an oleanane-type triterpenic acid with a palmitoyl group, and closely resembled those of the reported compound (3β,11α)-11-hydroxy-olean-12-en-3-yl palmitate [13]. The NMR signals at C-11 of the known compound (δ\(_{\text{C}}\) 4.50; δ\(_{\text{H}}\) 81.6) were upfield shifted in 2 (δ\(_{\text{C}}\) 3.89; δ\(_{\text{H}}\) 75.8) and a methoxyl group (δ\(_{\text{C}}\) 3.25; δ\(_{\text{H}}\) 53.4) further appeared, indicating the hydroxyl group at C-11 was methylated in 2, which was further confirmed by the HMBC (Fig. 2) correlation of OMe (δ\(_{\text{H}}\) 3.25) with C-11 (δ\(_{\text{C}}\) 75.8). Finally, compound 2 was established as (3β,11α)-3-hydroxy-11α-methoxy-olean-12-en-3-yl palmitate.


Table 1: $^1$H NMR (600 MHz, CD$_3$OD) and $^{13}$C NMR (150 MHz, CD$_3$OD) data for compound 1

| No | $\delta_H$       | $\delta_C$   | $^1$H–$^1$H COSY | HMBC                  |
|----|------------------|--------------|-------------------|-----------------------|
| 1  |                  | 136.6 s      | H-3a, 3b, 7a, 7b  | C-1, 3, 4, 6, 7       |
| 2  | 7.23 m           | 149.4 d      |                   |                       |
| 3a | 2.67 m           | 32.2 t       | H-2, 3b           | C-1, 2, 4, 5          |
| 3b | 2.41 ddq (18.0, 10.2, 1.8) | 43.6 d | H-5a, 5b         | C-3, 5, 6, 8, 9, 10  |
| 4  | 2.74 ddd (16.2, 10.2, 4.8) | 44.2 t       | H-4               | C-3, 4, 6, 8          |
| 5a | 2.50 ddd (16.2, 5.4, 1.2) | 2.48 m       | H-4               | C-3, 4, 6, 8          |
| 5b |                  | 201.0 s      |                   |                       |
| 6  |                  | 4.49 dq (13.2, 1.2) | 67.0 t       | C-1, 2, 6, 1’         |
| 7a |                  | 4.28 dd (13.2, 1.2) | 7a           | C-1, 2, 6, 1’         |
| 7b |                  | 148.3 s      |                   |                       |
| 8  | 4.82 m           | 111.3 s      | H-10              | C-4, 8, 10            |
| 9a | 4.80 m           | 20.7 q       | H-9a              | C-4, 8, 9             |
| 10 | 1.78 s           | 104.1 d      | H-2’              | C-7, 2’, 3’, 5’       |
| 1’ | 4.30 d (7.8)     | 75.2 d       | H-1’, 3’          | C-3’                  |
| 2’ | 3.19 dd (9.6, 8.4) | 78.2 d     | H-2’              | C-2’, 4’              |
| 3’ | 3.34 t (9.0)     | 71.7 d       | H-5’              | C-5’, 6’              |
| 4’ | 3.24 m           | 78.2 d       | H-4’, 6’a, 6’b    | C-1’, 4’, 5’          |
| 5’a| 3.85 dd (12.0, 1.8) | 62.8 t     | H-5’, 6’b        | C-4’, 5’              |
| 6’a| 3.66 dd (12.0, 5.4) |            | H-5’, 6’a        |                       |

![Fig. 2 Key $^1$H–$^1$H COSY and HMBC correlations of compounds 1 and 2](image)

By comparing their physical and spectroscopic data with those reported in the literatures, the known compounds (Fig. 1) were elucidated as (4R,6R)-carveol β-δ-glucoside (3) [14], (4R,6S)-carveol β-δ-glucoside (4) [14], (+)-neo-dihydrocarvyl β-δ-glucoside (5) [15], (−)-dihydrocarvy β-δ-glucoside (6) [15], uroterpenol β-δ-glucoside (7) [15], spicatoside A (8) [16], spicatoside B (9) [16], (35,6S)-cis-linalool-3,7-oxide (10) [17], (3R,9S)-megastigman-5-en-3,9-diol 3-O-β-δ-glucopyranoside (11) [18], larinarioside A (12) [19], 1,1,5-trimethyl-6-(3-hydroxy)cyclohexene-5-yl-1-β-δ-pyranoglucone (13) [20], larinarioside B (14) [21], (9S)-larinarioside B (15) [21], (+)-jasmololone glycoside (16) [14], (−)-5’-(β-δ-glucopyranosyloxy) jasmonic acid (17) [22], maniladiol (18) [23], 3β,28-dihydroxy-olean-12-yl palmitate (19) [24], olean-12-ene-28-arboxy-3-palmitate (20) [25], ursolic acid (21) [26], 1-(β-δ-ribofuranosyl)-1H-1,2,4-triazone (22) [27], naphthodoxazol A (23) [28], menthalactone (24) [29], 6-amino-9-[1-(3,4-dihydroxy phenyl)ethyl]-9H-purine (25) [30], respectively.

### 2.2 α-Glucosidase Inhibitory Activity

In the preliminary bioassay, the crude extracts, Fr.A, Fr.C and Fr.D all exhibited significantly inhibitory activity against α-glucosidase at concentrations (> 20 μg/mL). These inhibitory effects were dose-dependent (Fig. 3). Their IC$_{50}$ values were measured as 21.0, 36.7, 37.2, and 20.3 μg/mL, respectively (Table 3). Bioactivity-guided isolation further afforded 25 compounds, while compounds 1–17 and 21–25 were measured their α-glucosidase inhibitory activity. Compound 11 possessed the most significant activity with an IC$_{50}$ values of 83.4 μM, while compounds 3 and 4 showed moderate inhibitory activity against α-glucosidase with IC$_{50}$ values of 516.0 and 919.0 μM, respectively (Table 3). Other compounds had no significant inhibitory activity.

Comparing the chemical structures and activity of these compounds, it can be found that triterpenoids (2 and 21), jasmonoid glucosides (16 and 17) and N-containing compounds (22–25) were not responsible for the α-glucosidase inhibitory activity of the extracts from M. haplocalyx (M. canadensis). Among the monoterpenoid glucosides (1 and 3–10), only compounds 3 and 4 manifested moderate inhibitory activity against α-glucosidase, while 5 and 6 did not.
Thus, it might be assumed that the absence of cyclic double bond in 3 and 4 markedly decreased their inhibitory activity.

### 3 Experimental

#### 3.1 General Experimental Instruments and Procedures

LC–MS analyses were performed on a UFLC/MS-IT-TOF apparatus and the analytical conditions were set as previously reported [31]. Mass spectra were measured through a Waters AutoSpec Premier P776 (Waters, USA) mass spectrometer. Optical rotations were measured through a Jasco model 1020 digital polarimeter (Horiba, Tokyo, Japan). UV and IR (KBr) spectra were recorded on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan) and a Bio-Rad FTS-135 spectrometer (Hercules, California, USA), respectively. NMR spectra were recorded on the DRX-500 or AdvanceIII-600 NMR (Bruker, Bremerhaven, Germany) spectrometers with TMS as an internal standard. Thin-layer chromatography (TLC) analyses were carried out with silica gel GF254 (Merck, Chemical Co. Ltd., Shanghai, China), and spots were detected under UV light or by heating after spraying with 10% H₂SO₄ in C₂H₅OH (v/v). Preparative TLC (PTLC) was purchased from Yantai Jiangyou silicon development Company (Yantai, China). Silica gel (200–300 mesh, Linyi Haixiang Co., Ltd; Linyi, China), Sephadex LH-20 (Amersham Bioscience, Sweden), and D101 macroporous adsorption resin (Tianjin guangfu fine materials Co., Ltd., Tianjin, China) were used for column chromatography.

#### Table 2 ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) data for compound 2

| No. | δ_H  | δ_C  | No. | δ_H  | δ_C  |
|-----|------|------|-----|------|------|
| 1a  | 1.96 m | 39.1 t | 20  |      | 31.1 s |
| 1b  | 1.32 m | 21a 1.32 m | 34.9 t |
| 2a  | 1.62 m | 23.8 t | 21b | 1.10 m |
| 2b  | 1.23 m | 22a 1.44 m | 37.0 t |
| 3   | 4.51 dd (7.8, 9.0) | 80.4 d | 22b | 1.23 m |
| 4   | 38.0 s | 23 | 0.85 s | 28.2 q |
| 5   | 0.88 m | 55.3 d | 24 | 0.90 s | 16.8 q |
| 6a  | 1.52 m | 18.3 t | 25 | 1.07 s | 16.8 q |
| 6b  | 1.26 m | 26 | 1.00 s | 18.2 q |
| 7a  | 1.50 m | 33.2 t | 27 | 1.21 s | 25.1 q |
| 7b  | 1.30 m | 28 | 0.83 s | 28.5 q |
| 8   | 43.1 s | 29 | 0.89 s | 33.2 q |
| 9   | 1.73 m | 51.1 d | 30 | 0.88 s | 23.6 q |
| 10  | 38.1 s | OCH₃ | 3.25 s | 53.4 q |
| 11  | 3.89 dd (3.0, 9.0) | 75.8 d | 1’ | 173.7 s |
| 12  | 5.32 d (3.0) | 121.8 d | 2’ | 2.29 t (7.8) | 34.7 t |
| 13  | 149.7 s | 3’ | 1.64 m | 25.2 t |
| 14  | 41.7 s | 4’ | 29.2 t |
| 15a | 2.04 m | 26.3 t | 5’ | 29.3 t |
| 15b | 0.84 m | 6’ | 29.4 t |
| 16a | 1.65 m | 26.8 t | 7’~13’ | 29.6~29.7 t |
| 16b | 1.00 m | 14’ | 1.26 m | 31.9 t |
| 17  | 32.3 s | 15’ | 1.27 m | 22.7 t |
| 18  | 2.01 dd (13.8, 4.2) | 47.0 d | 16’ | 0.88 t (7.8) | 14.1 q |
| 19a | 1.66 dd (13.8, 4.2) | 46.5 t |
| 19b | 1.08 m |

Fig. 3 α-Glucosidase inhibitory activities of crude extract, Fr.A, Fr.C and Fr.D at concentrations of 160, 80, 40, 20, 10 and 5 μg/mL, respectively. Values are presented as inhibitory rate compared to the blank control. Means significantly lower than the controls are indicated with one asterisk (*) (Dunnett’s one-sided t test; p < 0.05) or two asterisks (**) (p < 0.01). Error bars are one standard error of the mean. N = 5
Table 3 α-Glucosidase inhibitory activities of extracts and compounds from M. haplocalyx

| Extracts/a     | IC$_{50}$$\pm$ SD | Compounds/b | IC$_{50}$$±$ SD |
|---------------|---------------------|-------------|-----------------|
| Crude extract | 21.0 ± 1.9          | 3           | 516.0 ± 2.1     |
| Fr.A          | 36.7 ± 2.6          | 4           | 919.0 ± 37.3    |
| Fr.C          | 37.2 ± 5.5          | 11          | 83.4 ± 1.3      |
| Fr.D          | 20.3 ± 2.8          |             |                 |
| Acarbosec     | 25.8 ± 3.3          |             |                 |

aIC$_{50}$ values in μg/mL are mean ± SD from three independent experiments
bIC$_{50}$ values in μM are mean ± SD from three independent experiments
cIC$_{50}$ values in nM are mean ± SD from three independent experiments

chemical co. LTD, Tianjin, China) were used for column chromatography. MPLC separations were conducted on a Dr-Flash II apparatus using a MCI gel CHP 20P (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) column. Semipreparative HPLC purifications were performed on a Shimadzu LC-CBM-20 system (Shimadzu, Kyoto, Japan) with the XDB-C18 column.

3.2 Plant Material

The aerial parts of fresh M. haplocalyx Briq. (Mentha canadensis L.) were bought from market, Kunming, Yunnan Province, China, in March 2013, and were identified by Prof. Li-gong Lei, Kunming Institute of Botany. A voucher specimen (No. 2013032401) was deposited at the Laboratory of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, CAS.

3.3 Extraction and Isolation

The aerial parts of fresh M. haplocalyx (M. canadensis) (20 kg) were powdered and extracted with 50% ethanol for three times at room temperature (each 200 L). The combined extraction was concentrated in vacuo to yield a residue. The residue was then suspended in water and extracted with ethyl acetate (Fr.A, 130 g). The aqueous phase was subjected to D101 macroporous adsorption resin using a step gradient elution with C$_2$H$_5$OH–H$_2$O (0:100, 50:50 and 90:10, v/v) as the mobile phase to give three fractions (Fr.B–Fr.D).

The Fr.A (130 g) and Fr.D (10 g) were combined (Fr.E, 140 g). Fr.E (140 g) was fractionated on silica gel CC using a step gradient elution with MeOH–CHCl$_3$ (2:98, 5:95, 10:90, v/v) and H$_2$O–MeOH–CHCl$_3$ (2:20:80, 3:30:70, 4:40:60, v/v) as the mobile phase, and following washed with MeOH to give seven fractions (Fr.E-1–Fr.E-7). Fr.E-3 (34 g) was subjected to silica gel CC eluted with EtOAc-petroleum ether (from 6:94 to 50:50) to give five fractions (Fr.E-3-1–Fr.E-3-5). Fr.E-3-1 (8.8 g) was subjected to silica gel CC eluted with Me$_2$CO-petroleum ether (5:95), then applied to Sephadex LH-20 CC eluted with CHCl$_3$–MeOH (50:50), and finally purified by silica gel CC with EtOAc-petroleum ether (40:60) to yield 2 (10 mg), 19 (13 mg) and 20 (12 mg). Compound 21 (2 g) was got from Fr.E-3-2 (9.1 g) that was separately separated on MCI gel CHP 20P CC (MeOH–H$_2$O, 30:70, 70:30, 90:10) and Sephadex LH-20 CC (MeOH–CHCl$_3$, 50:50). Fr.E-3-5 (5.6 g) was subjected on MCI gel CHP 20P CC using MeOH–H$_2$O (60:40, 90:10), subsequently compound 24 (10 mg) was yielded from Sephadex LH-20 CC (MeOH–CHCl$_3$, 50:50). Fr.E-5 (5.8 g) was subjected to MCI gel CHP 20P CC using a step gradient elution with MeOH–H$_2$O (40:60, 80:20) to give two fractions (Fr.A-5-1 and Fr.A-5-2). Fr.A-5-1 (2.7 g) was fractionated on silica gel CC (MeOH–CHCl$_3$, 10:90) to give three fractions (from Fr.E-5-1-1 to Fr.E-5-1-3). Fr.E-5-1-1 (327 mg) was preferred on Sephadex LH-20 eluted with MeOH–CHCl$_3$ (50:50), and following purified by semipreparative HPLC (MeCN–H$_2$O, 25:75, v/v, 3.0 mL/min) over an XDB-C$_{18}$ column (9.4 × 250 mm, 5 μm) to yield compounds 3 (5 mg), 5 (4 mg), 6 (5 mg) and 25 (8 mg). Fr.E-5-1-2 (1.3 g) was separated on Sephadex LH-20 CC (MeOH–CHCl$_3$, 50:50), and later fractionated on silica gel CC (MeOH–CHCl$_3$, 90:10), therefore, compounds 1 (4 mg), 7 (14 mg), 10 (2 mg), the mixture of 12 and 13 (8 mg), the mixture of 14 and 15 (6 mg) were obtained by semipreparative HPLC (MeCN–H$_2$O, 40:60, v/v, 3.0 mL/min) over an XDB-C$_{18}$ column (9.4 × 250 mm, 5 μm).

Fr.C (50 g) was subjected to silica gel CC and then Al$_2$O$_3$ CC with H$_2$O–MeOH–CHCl$_3$ (3:30:70) to give four fractions (from Fr.C-1 to Fr.C-4). Fr.C-1 (10.4 g) was fractionated on MCI gel CHP 20P CC with MeOH–H$_2$O (40:60, 80:20), then applied to Sephadex LH-20 CC eluted with MeOH–CHCl$_3$ (50:50), and later purified by silica gel CC eluted with MeOH–EtOAc (2:98) to yield 4 (120 mg), 8 (800 mg), 9 (311 mg), 11 (200 mg) and 16 (20 mg). Compounds 17 (201 mg), 22 (46 mg) and 23 (17 mg) were afforded from Fr.C-2 (20.0 g) by repeated silica gel CC (MeOH–CHCl$_3$, 15:85, 20:80, 25:75) and Sephadex LH-20 (MeOH–CHCl$_3$, 50:50).

3.4 Spectroscopy Data of Compounds

3.4.1 Compound 1

Colorless caramelized solid, C$_{16}$H$_{24}$O$_7$, [α]$_D^{20}$ = −22.0 (c, 0.20, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 230 (3.65) nm; IR (KBr) $\nu_{max}$: 3427, 1635, 1385, 1046, 902 cm$^{-1}$; $^1$H NMR (600 MHz, CD$_3$OD) and $^{13}$C NMR (DEPT, 150 MHz, Springer)
Colorless powder, C_{47}H_{82}O_{3}; {^{1}H} NMR (600 MHz, CDCl_{3}) and {^{13}C} NMR (DEPT, 150 MHz, CDCl_{3}) see Table 2; HREIMS m/z: 694.6258 [M+] (calc. 694.6264 for C_{47}H_{82}O_{3}).

### 3.4.2 Compound 2

Colorless powder, C_{16}H_{24}O_{7}Na; {^{1}H} NMR (600 MHz, CD_{3}OD) see Table 1; HRESIMS m/z: 351.1404 [M+Na]^{+} (calc. 351.1414 for C_{16}H_{24}O_{7}Na).

### 3.5 Inhibitory Assay of α-Glucosidase

The α-glucosidase inhibitory activity was measured in a 96-well microtiter plate based on p-nitrophenyl-α-D-glucopyranoside (PNPG, Yuanye Biosciences Co. Ltd., Shanghai, China) as a substrate following the reported method with slight modifications [32]. In brief, 5.0 mM PNPG (20 μL) and 20 μL tested compounds of dissolved in 10 μL DMSO and 990 μL phosphate buffer (PB, 0.1 M, pH = 6.8) were sequentially added to a 96-well plate to be mixed. The mixture was incubated at 37 °C for 5 min. Reactions were initiated by addition of 0.2 M Na_{2}CO_{3} (40 μL). The absorbance was recorded at 405 nm by a Bio-Rad 680 microplate reader (Hercules, CA, USA). The negative control was set by adding PB instead of the sample using the same procedure for the tests. Acarbose (Bayer) dissolved in PB was utilized as the positive control. The blank was set by adding phosphate buffer instead of the α-glucosidase using the same method. Inhibition rate (%) = [(ODnegative control − ODblank) − (ODtest − ODtest blank)]/(ODnegative control − ODblank) × 100%. All data were subjected to an analysis of variance between the treatment and blank control were calculated using one-way analysis of variance (ANOVA).

### 4 Conclusion

In this study, the extracts of the aerial parts of M. haplocalyx (M. canadensis) were firstly found to exhibit significantly inhibitory activity against α-glucosidase. Two new compounds (1 and 2) and 23 known ones were isolated and identified through bioactivity-guided fractionation. Among them, compounds 3–9 and 24 were reported from M. haplocalyx (M. canadensis) for the first time, while compounds 10–20, 22–23 and 25 were firstly isolated from the genus Mentha. Bioactivity assay further traced the active compounds (3, 4 and 11), whose inhibitory activity against α-glucosidase had not been reported before. It was noted that the monoterpene glucosides and the ionone glycosides endowed this plant with the α-glucosidase inhibitory activity.

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### Compliance with Ethical Standards

Conflict of interests No potential conflict of interest was reported by the authors.

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