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New caryophyllene-type sesquiterpene and flavonol tetraglycoside with sixteen known compounds from sword bean (*Canavalia gladiata*)

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Abstract Eighteen compounds including new caryophyllene-type sesquiterpene and flavonol tetraglycoside were purified and isolated from sword beans (*Canavalia gladiata*). Two new compounds, (*Z*,1*R*,7*S*,9*S*)-7-hydroxy-11,11-dimethyl-8-methylenebicyclo[7.2.0]undec-4-ene-4-carboxylic acid (**2**) and kaempferol-7-*O*-α-L-dirhamnopyranosyl(1 → 2;1 → 6)-*O*-β-D-glucopyranosyl(1 → 2)-*O*-α-L-rhamnopyranoside (**9**), were identified. Other known compounds including methyl gallate (**1**), (2*S*,3*S*,4*E*,8*E*)-2-aminooctadeca-4,8-diene-1,3-diol 1-*O*-β-D-glucopyranoside (**3**), (2*S*,3*S*,4*E*,8*Z*)-2-aminooctadeca-4,8-diene-1,3-diol 1-*O*-β-D-glucopyranoside (**4**), lupeol (**5**), trilinolein (**6**), 1,6-di-*O*-galloyl β-D-glucopyranoside (**7**), *N*-(2-methoxybenzoyl)homoserine (**8**), dihydrophaseic acid (**10**), dillenetin (**11**), kaempferol-7-*O*-[2-*O*-β-D-glucopyranosyl-6-*O*-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**12**), canavalioside (**13**), kaempferol-3-*O*-[2-*O*-β-D-glucopyranosyl-6-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**14**), kaempferol-3-*O*-(2,6-*O*-α-L-dirhamnopyranosyl)-β-D-glucopyranoside (**15**), kaempferol-3-*O*-rutinoside (**16**), gladiatoside A_1 (**17**), and gladiatoside B_1 (**18**) were identified. The chemical structures of these compounds were determined by ESI–MS and NMR analyses.

Keywords *Canavalia galidata* · Sword bean · Flavonoid tetraglycoside · Sesquiterpenoic acid · Cerebioside

Introduction

Sword bean (*Canavalia gladiata*) belongs to the legume family, and it is widely cultivated in Asia and Africa (Lee and Jeong, 2005). The green fruit of sword bean is generally used as a domestic vegetable, and a ripe seed is consumed as cooked beans (Chang et al., 2011; Ekanayake et al., 2007). The bean has been used in traditional Chinese medicine for the treatment of purulent inflammation, such as sinusitis, hemorrhoids, and boils (Kim et al., 2012). Sword bean has been demonstrated to have several biological effects, including anti-oxidant (Gan et al., 2016), anti-bacterial (Lee and Jeong, 2005; Cho et al., 2000), anti-gastric inflammatory (Kim et al., 2013a, b), attenuation of bowel disease (Ji et al., 2018), and antiangiogenic activity (Jeon et al., 2005). Interestingly, sword bean does not contain isoflavones, such as daidzin, genistin, daidzein, and genistein, which are common bioactive compounds in soybean and black bean (Cho et al., 1999). Moreover, sword bean has high total phenolic and flavonoid contents, which are similar to those of soybean and black bean (Gan et al., 2018). Understanding the chemical constituents of sword bean is very important to assess its quality as a food material. Bioactive compounds including gallic acid derivative, ent-kaurane-type diterpene glycoside

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(canavalioside), and various flavonol glycosides (gladiatosides) have been found in sword beans (Kim et al., 2013a, b; Lee and Jeong, 2005; Murakami et al., 2000). However, information on the chemical constituents of the sword bean is limited. We purified and isolated 18 compounds, including new caryophyllene-type sesquiterpene and flavonol tetraglycoside from sword bean. In this study, we demonstrated the structural determination of new caryophyllene-type sesquiterpene and flavonol tetraglycoside found in sword beans.

Materials and methods

General experimental procedures

Nuclear magnetic resonance (NMR) spectra were recorded using ^{unit}INOVA 500 and 600 spectrometers (Varian, Walnut Creek, CA). Deuterated methanol (CD_3OD), deuterated chloroform ($CDCl_3$), and deuterated pyridine (Acros Organics, Morris Plains, NJ, USA) containing tetramethylsilane as an analytical solvent was used. The mass spectra were acquired on a Synapt G2 HDMS electrospray ion source (ESI) quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, Milford, MA). UV spectra were obtained by a Shimadzu Mini-1240 UV/VIS spectrometer (Shimadzu, Kyoto, Japan). The optical rotations were measured by a A.KRÜSS Optronic P8000 Automatic Polarimeter (A.KRÜSS, Berlin, Germany). Silica gel (Kieselgel 60, 70-230 mesh, Merck, Darmstadt, Germany), Amberlite XAD-2 (70-230 mesh, Merck), and ODS (70-230 mesh, Merck) for column chromatography were used to purify the compounds from the sword beans. The compounds were purified and isolated using a high-performance liquid chromatography (HPLC) instrument (LC-20A, Shimadzu, Kyoto, Japan) equipped with Silica-60 (5 μm, 7.8 × 250 mm, Shimadzu), Shim-pack Prep-ODS (H) Kit (5 μm, 20 × 250 mm, Shimadzu), Intersil ODS-3 (5 μm, 7.6 × 250 mm, Shimadzu), and ODS-80Ts (TSK-gel, 4.6 × 250 mm, Tosoh, Tokyo, Japan)]. The eluents were monitored at 210 and 254 nm. Thin-layer chromatography (TLC) was carried out using silica gel TLC plates (silica gel 60 F254, 0.25 mm thickness, Merck) and developed using a mixture of EtOAc/$CHCl_3$/acetic acid = 3:1:2 (v/v). The spots were spayed by a 200 μM DPPH ethanol solution to screen antioxidative compounds. The methanol (MeOH), acetonitrile (MeCN), *n*-hexane, 2-propanol, and ethanol (EtOH) solutions used for analyses were of HPLC grade and were purchased from Fisher Scientific Korea Ltd. (Seoul, Korea). Acetic acid (AcOH) and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA).

Plant material

Sword beans (*Canavalia gladiata*, Jacq. DC) were harvested in September, in Gwang-ju, South Korea. This bean was identified by Prof. Sang-Hyun Lee, Laboratory of Pomology, College of Agriculture and Life Science, Chonnam National University. A voucher sample (no. JNU CG 20150931) was deposited in the laboratory herbarium. The fresh white sword beans were separated from the pod and stored at –70°C before using.

Extraction and solvent fractionation

The fresh sword bean (5 kg) was homogenized with MeOH (20 L) using a homogenizer (Ultra-Turrax T50, IKA, Seoul, Korea). After extraction for 1 day at room temperature, the mixture was filtered under a vacuum using No. 2 filter paper (Whatman, Maidstone, England). The residue was repeatedly extracted by adding MeOH (15 L). The MeOH extracts were combined and concentrated using a vacuum evaporator at 37 °C. The MeOH extracts (206.5 g) were suspended in 2 L of distilled water and solvent fractionated with *n*-hexane (2 L, 3 times), followed by $CHCl_3$ (2 L, 3 times), EtOAc (2 L, 3 times), and water-saturated *n*-butanol (BuOH) (2 L, 3 times).

Purification of 1–6 from *n*-hexane and $CHCl_3$ fraction

The combined *n*-hexane and $CHCl_3$ fraction (21.7 g) was fractionated by silica-gel column (5 × 82 cm) chromatography and eluted with *n*-hexane/EtOAc/MeOH (10:0:0, 8:2:0, 6:4:0, 4:6:0, 2:8:0, 0:10:0, 0:8:2, 0:6:4, 0:4:6, 0:2:8, 0:0:10, v/v, step-wise system, each 2.6 L). Forty fractions (H1 ∼ H40) were grouped by the TLC analysis. Fraction H25 (424.5 mg) was subjected to ODS-HPLC [column, Shim-pack Prep-ODS (H) KIT; flow rate, 9.0 mL/min; linear gradient elution of 20% MeOH (pH 3.0) containing TFA (A) and 100% MeOH (B), 0% B for initial → 50% B for 25 min → 50% B for 40 min; detection, 254 nm] to afford two compounds (**1**, t_R 18.43 min, 23.3 mg; **2**, t_R 37.89 min, 1.6 mg) and the mixture of **3** and **4**. Fraction H3 (6.0 g) was injected onto normal phase-HPLC [column, silica-60; flow rate, 5.0 mL/min; linear gradient elution of *n*-hexane (C) and *n*-hexane/2-propanol/EtOH = 8:4:1 v/v (D), initial 0% D → 100% D for 25 min; detection, 210 nm] to obtain the mixture of **5** and **6** (t_R 15.3 min, 60.2 mg).

Purification of 7–18 from BuOH fraction

The BuOH fraction (13.0 g) was separated by Amberlite XAD-2 column (5 × 82 cm) chromatography and eluted

with H_2O/MeOH (10:0, 8:2, 6:4, 4:6, 2:8, 0:10, v/v, step-wise system, each 2.4 L). Nineteen fractions (BA-BP) were grouped by the TLC analysis. Fraction BI (424.5 mg) was separated by ODS column chromatography (3.2 × 95 cm) and eluted with H_2O/MeOH (8:2, 6:4, 4:6, 2:8, 0:10, v/v, step-wise system, each 1.2 L) to obtain 24 subfractions (BI1-BI24). Compound **7** (t_R 20.0 min, 1.0 g) was isolated from subfraction BI6 (1.13 g) by ODS-HPLC [column, Intersil ODS-3; flow rate, 1.0 mL/min; linear gradient elution of 20% MeOH (E) and 40% MeOH (F), initial 0% F → 30% F for 20 min → 100% F for 40 min; detection, 210 and 280 nm]. The subfractions, BI9, BI11, BI14, BI17, and BI19, were purified by ODS-HPLC [column, Intersil ODS-3; flow rate, 5.0 mL/min; detection, 210 nm] with their different elutions. Compounds **8** (t_R 8.1 min, 3.0 mg) and **9** (t_R 21.4 min, 19.4 mg) were separated from subfraction BI9 (182.6 mg) by ODS-HPLC [linear gradient elution of 20% MeOH (G) and 35% MeOH (H), initial 0% H → 30% G for 25 min → 100% G for 40 min]. Compounds **10** (t_R 13.9 min, 1.0 mg), **11** (t_R 23.2 min, 3.5 mg), and **12** (t_R 28.8 min, 12.0 mg) were separated from subfraction BI11 (29.2 mg) by ODS-HPLC [linear gradient elution of 20% MeOH (I) and 30% MeOH (J), initial 0% J → 70% J for 25 min → 70% J for 35 min]. The mixture of **13** and **14** (t_R 11.0 min, 12.0 mg) was separated from subfraction BI14 (56.4 mg) by ODS-HPLC [linear gradient elution of 20% MeOH (K) and 50% MeOH (L), initial 0% L → 70% L for 25 min → 70% L for 35 min]. Compound **15** (t_R 12.5 min, 6.0 mg) was separated from subfraction BI17 (71.4 mg) by ODS-HPLC [linear gradient elution of 35% MeOH (M) and 40% MeOH (N), initial 0% N → 100% N for 30 min → 100% L for 40 min]. Compounds **16** (t_R 14.2 min, 5.0 mg), **17** (t_R 19.7 min, 3.0 mg), and **18** (t_R 21.8 min, 4.0 mg) were separated from subfraction BI19 (20.9 mg) by ODS-HPLC [linear gradient elution of 35% MeOH (O) and 60% MeOH (P), initial 0% P → 100% N for 40 min → 100% P for 50 min].

Compound **1**: white powder; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.04 (2H, s, H-2 and H-6), 3.81 (3H, s, -OCH_3); ESI–MS (negative) *m/z* 183.0 $[M - H]^-$.

Compound **2**: white powder; ^{1}H- and ^{13}C-NMR data are shown in Table 1. HR–ESI–MS *m/z* 249.1493 $[M - H]^-$ (calculated for $C_{15}H_{21}O_3$, *m/z* 249.1493, +0.2 mDa).

Compound **3**: white powder; ^{1}H-NMR (500 MHz, pyridine-d_5, TMS) δ 8.40 (1H, d, *J* = 8.5 Hz, NH), 7.59 (1H, br. s, C-2′-OH), 6.90 (1H, br. s, C-3-OH), 6.00 (1H, dd, *J* = 15.5, 6.0 Hz, H-4), 5.96 (1H, dt, *J* = 15.5, 6.0 Hz, H-5), 5.50 (2H, m, H-8 and H-9), 4.91 (1H, d, *J* = 7.7 Hz, H-1″), 4.82 (1H, m, H-2), 4.70 (1H, dd, *J* = 10.5, 5.5 Hz, H-1a), 4.53 (1H, m, H-1b), 4.52 (1H, dd, *J* = 11.5, 3.0 Hz, H-2′), 4.50 (1H, dd, *J* = 11.8, 4.0 Hz, H-6″a), 4.40 (1H, dd, *J* = 11.8, 5.5 Hz, H-6″b), 4.21 (2H, s, H-3″ and H-4″), 4.01 (1H, t, *J* = 7.5 Hz, H-2″), 3.90 (1H, m, H-5″), 2.20 (2H, m, H-6a), 2.09 (2H, m, H-9), 1.86 (1H, m, H-3′a), 1.80 (2H, m, H-10), 1.74 (1H, m, H-4′a), 1.32–1.45 (36H, m, CH_2), 0.88 (3H, t, *J* = 7.5 Hz, H-18, CH_3); ^{13}C-NMR (125 MHz, pyridine-d_5, TMS) δ 176.0 (C-1′), 132.5 (C-5), 132.4 (C-4), 131.0 (C-9), 129.7 (C-8), 106.0 (C-1″), 78.9 (C-5″), 78.8 (C-3″), 75.5 (C-2″), 72.8 (C-2′), 72.6 (C-3), 71.8 (C-4″), 70.5 (C-1), 63.0 (C-6″), 54.9 (C-2), 36.0 (C-3′), 33.2 (C-6), 32.5 (C-16), 30.0–30.4 (C-11–C-15), 27.9 (C-10), 27.8 (C-7), 26.3 (C-4′), 23.3 (C-17), 23.3–32.5 (C-5′–C-17′), 14.6 (C-18); ESI–MS (negative) *m/z* 712.60 $[M - H]^-$.

Table 1 ^{1}H- and ^{13}C- NMR spectral data of **2** in CD_3OD

Carbon	δ_H (*int., mult., J* in Hz)	δ_C
1	1.75 (1H, m)	55.1
2a	1.81 (1H, m)	28.1
2b	1.66 (1H, m)	
3a	2.57 (1H, m)	24.3
3b	2.33 (1H, m)	
4	–	135.5
5	6.69 (1H, dd, 9.0, 5.5)	139.5
6a	2.72–2.66 (1H, m)	36.0
6b	2.53 (1H, m)	
7	4.40 (1H, dd, 7.5, 4.5)	76.2
8	–	159.0
9	2.77 (1H, td, 7.0, 7.5)	33.6
10a	1.69 (1H, m)	41.9
10b	1.80 (1H, m)	
11	–	33.8
12	–	171.7
13a	5.02 (1H, br. s)	113.0
13b	5.07 (1H, br. s)	
14	1.00 (3H, s)	24.3
15	1.03 (3H, s)	30.8

Compound **4**: white powder; ^{1}H-NMR (500 MHz, pyridine-d_5, TMS) δ 8.40 (1H, d, *J* = 8.7 Hz, NH), 7.59 (1H, br. s, C-2′-OH), 6.90 (1H, br. s, C-3-OH), 6.00 (1H, dd, *J* = 15.0, 6.0 Hz, H-4), 5.96 (1H, dt, *J* = 15.0, 6.0 Hz, H-5), 5.50 (2H, m, H-8 and H-9), 4.91 (1H, d, *J* = 7.7 Hz, H-1″), 4.82 (1H, m, H-2), 4.70 (1H, dd, *J* = 10.0, 6.0 Hz, H-1a), 4.53 (1H, m, H-1b), 4.52 (1H, dd, *J* = 11.5, 2.5 Hz, H-2′), 4.50 (1H, dd, *J* = 11.8, 2.5 Hz, H-6″a), 4.40 (1H, dd, *J* = 11.8, 5.5 Hz, H-6″b), 4.21 (2H, s, H-3″ and H-4″), 4.01 (1H, t, *J* = 7.5 Hz, H-2″), 3.90 (1H, m, H-5″), 2.20 (3H, m, H-6a and H-7), 2.04 (1H, m, H-3′a), 1.80 (2H, m, H-10), 1.74 (1H, m, H-4′a), 1.32–1.45 (36H, m, CH_2), 0.88 (3H, t, *J* = 15.0 Hz, H-18); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 176.0 (C-1′), 132.5 (C-5), 132.4 (C-4), 131.0 (C-9), 129.7 (C-8), 106.0 (C-1″), 78.9 (C-5″), 78.8 (C-3″), 75.5 (C-2″), 72.8 (C-2′), 72.6 (C-3), 71.8 (C-4″), 70.5 (C-

1), 63.0 (C-6″), 54.9 (C-2), 36.0 (C-3′), 33.2 (C-6), 32.5 (C-16), 30.0–30.4 (C-11–C-15), 27.9 (C-10), 27.8 (C-7), 26.3 (C-4′), 23.3 (C-17), 23.3–32.5 (C-5′–C-17′), 14.6 (C-18 and C-18′); ESI–MS (negative) *m/z* 712.60 $[M - H]^-$.

Compound **5**: white powder; ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 4.69 (1H, s, H-29a), 4.56 (1H, s, H-29b), 3.18 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 1.68 (3H, s, H-30), 1.02 (3H, s, H-26), 0.96 (3H, s, H-23), 0.94 (3H, s, H-27), 0.82 (3H, s, H-25), 0.78 (1H, s, H-28), 0.76 (3H, s, H-24); ^{13}C-NMR (125 MHz, $CDCl_3$, TMS) δ 150.96 (C-20), 109.31 (C-29), 78.99 (C-3), 55.30 (C-4), 50.44 (C-9), 48.30 (C-18), 47.98 (C-19), 43.00 (C-17), 42.83 (C-14), 40.83 (C-8), 40.00 (C-22), 38.86 (C-4), 38.71 (C-1), 38.05 (C-13), 37.17 (C-10), 35.58 (C-16), 34.28 (C-7), 29.85 (C-21), 27.98 (C-23), 27.44 (C-2), 27.30 (C-15), 25.14 (C-12), 20.93 (C-11), 18.32 (C-6), 19.30 (C-30), 18.00 (C-28), 16.11 (C-25), 15.97 (C-26), 15.36 (C-24), 14.54 (C-27); ESI–MS (negative) *m/z* 425.30 $[M - H]^-$.

Compound **6**: white powder; ^{13}C-NMR (125 MHz, $CDCl_3$, TMS) δ 173.27 (C-1′ and C-1‴), 172.82 (C-1″), 130.21 (C-9″ and C-13″), 130.19 (C-9′ and C-13′), 130.00 (C-9‴ and C-13‴), 128.07 (C-10′ and C-12′), 128.05 (C-10″ and C-12″), 127.88 (C-10‴ and C-12‴), 68.87 (C-1 and C-3), 62.09 (C-2), 34.19 (C-2″), 34.02 (C-2′ and C-2‴), 31.52 (C-16′, C-16″ and C-16‴), 29.65 (C-4′ and C-7′), 29.62 (C-4″, C-4‴, C-7″, and C-7‴), 29.36, (C-15′, C-15″, and C-15‴), 29.27 (C-5′), 29.20 (C-5″ and C-5‴), 29.19 (C-8′, C-8″, and C-8‴), 29.18 (C-6′), 29.11 (C-6″ and C-6‴), 29.08 (C-4′), 29.05 (C-4″ and C-4‴), 27.19 (C-14′, C-14″, and C-14‴), 25.63 (C-11′, C-11″, and C-11‴), 24.88 (C-3′), 24.86 (C-3″ and C-3‴), 22.57 (C-17′, C-17″, and C-17‴), 14.07 (C-18′, C-18″, and C-18‴); ESI–MS (positive) *m/z* 879.35 $[M + H]^+$.

Compound **7**: white powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 7.14 (2H, s, H-2″ and H-6″), 7.09 (2H, s, H-2′ and H-6′), 5.68 (1H, d, *J* = 8.0 Hz, H-1), 4.55 (1H, dd, *J* = 12.0 2.0 Hz, H-6a), 4.40 (1H, dd, *J* = 12.0, 5.0 Hz, H-6b), 3.73 (1H, m, H-5), 3.49–3.53 (3H, m, H-2, H-3, and H-4); ESI–MS (positive) *m/z* 507.08 $[M + Na]^+$.

Compound **8**: white powder; $[a]_D^{25}$ −1.9 (*c* 0.006, MeOH); UV (CH_3OH) λ_{max} (nm) (log ε): 285 (4.49); ^{1}H- and ^{13}C-NMR data are shown in Table 2.; HR–ESI–MS *m/z* 254.0968 $[M + H]^+$ (calculated for $C_{12}H_{16}N_1O_5$, *m/z* 254.0950, +1.8 mDa).

Compound **9**: white powder; $[a]_D^{25}$ −3.7 (*c* 0.02, MeOH); UV (CH_3OH) λ_{max} (nm) (log ε): 265 (4.50) and 348 (4.33); ^{1}H- and ^{13}C-NMR data are shown in Table 3; ESI–MS (positive) *m/z* 925.2595 $[M + Na]^+$ (calculated for $C_{39}H_{50}O_{24}Na$, *m/z* 925.2590, −0.5 mDa).

Compound **10**: white powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.01 (1H, d, *J* = 16.0 Hz, H-2′), 6.55 (1H, d, *J* = 16.0 Hz, H-1′), 5.77 (2H, s, H-4′), 4.12 (1H, m, H-4), 3.81 (1H, dd, *J* = 7.3, 2.5 Hz, H-8a), 3.72 (1H, br. d, *J* = 7.3 Hz, H-8b), 2.09 (3H, s, H-6′), 2.05 (2H, br. d, *J* = 10.3 Hz, H-5a), 1.86 (1H, ddd, *J* = 13.4, 7.0, 1.7 Hz, H-3a), 1.75 (1H, dd, *J* = 13.8, 10.3 Hz, H-5b), 1.68 (1H, ddd, *J* = 13.4, 11.0, 1.7 Hz, H-3b), 1.16 (3H, s, H-9), 0.94 (3H, s, H-7); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 168.16 (C-5′), 150.11 (C-3′), 133.78 (C-1′), 130.37 (C-2′), 117.77 (C-4′), 86.39 (C-6), 81.83 (C-1), 75.85 (C-8), 64.59 (C-4), 48.21 (C-2), 44.58 (C-5), 43.12 (C-3), 19.83 (C-6′), 18.23 (C-9), 14.93 (C-7); HR–ESI–MS *m/z* 305.1382 $[M + Na]^-$ (calculated for $C_{15}H_{22}O_5Na$, *m/z* 305.1365, +1.7 mDa).

Compound **11**: yellow powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.11 (1H, br. s, H-2′), 8.10 (1H, br. d, *J* = 9.0 Hz, H-6′), 6.91 (1H, d, *J* = 9.0 Hz, H-5′), 6.80 (1H, d, *J* = 2.5 Hz, H-8), 6.74 (1H, d, *J* = 2.5 Hz, H-6), 3.82 (3H, s, H-7′), 3.81 (3H, s, H-8′); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 182.83 (C-4), 163.90 (C-5), 157.95 (C-7), 157.79 (C-9), 156.56 (C-2), 151.23 (C-3′), 147.54 (C-4′), 128.93 (C-3), 122.84 (C-1′), 121.38 (C-6′), 116.37 (C-2′), 110.41 (C-5′), 105.11 (C-10), 103.59 (C-6), 99.41 (C-8); ESI–MS (positive) *m/z* 353.27 $[M + Na]^+$.

Compound **12**: yellow powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.13 (2H, br. d, *J* = 9.0 Hz, H-2′ and H-6′), 6.91 (2H, br. d, *J* = 9.0 Hz, H-3′ and H-5′), 6.74 (1H, d, *J* = 2.3 Hz, H-8), 6.47 (1H, d, *J* = 2.3 Hz, H- 6), 5.64 (1H, d, *J* = 8.0 Hz, H-1‴), 5.57 (1H, d, *J* = 1.5 Hz, H-1″), 4.52 (1H, d, *J* = 1.5 Hz, H-1⁗), 4.06 (1H, m, H-5‴), 4.03 (1H, dd, *J* = 5.0, 1.5 Hz, H-2″), 3.95–3.27 (H-2″–H-5″, H-2‴–H-6‴, and H-2⁗–H-5⁗), 1.27 (3H, d, *J* = 6.0 Hz, H-6″), 1.18 (3H, d, *J* = 6.0 Hz, H-6⁗); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 178.12 (C-4), 161.98 (C-7), 161.52 (C-5), 160.12 (C-4′), 157.79 (C-2), 156.52 (C-9), 133.31 (C-3), 130.93 (C-2′ and C-6′) 121.36 (C-1′),

Table 2 ^{1}H- and ^{13}C- NMR spectral data of **8** in CD_3OD

Carbon	δ_H (*int., mult., J* in Hz)	δ_C
1	–	120.9
2	–	158.1
3	7.16 (1H, br. d, 8.0)	111.6
4	7.51 (1H, dt, 8.0, 2.0)	133.0
5	7.06 (1H, dt, 8.0, 1.0)	120.6
6	8.02 (1H, dd, 8.0, 2.0)	130.9
7	–	165.2
$-OCH_3$	4.01 (3H, s)	55.2
1′		176.9
2′	4.57 (1H, t, 6.0)	52.7
3′	2.19–2.33 (2H, m)	31.2
4′	3.98–4.06 (2H, m)	73.5

Table 3 ^{1}H- and ^{13}C- NMR spectral data of **9** in CD_3OD

Carbon	δ_H (*int., mult., J* in Hz)	δ_C
2	–	157.79
3	–	133.31
4	–	178.12
5	–	161.52
6	6.47 (1H, d, 2.0)	98.99
7	–	161.98
8	6.73 (1H, d, 2.0)	94.19
9	–	156.52
10	–	106.07
1′	–	121.36
2′, 6′	8.10 (2H, d, 9.0)	130.93
3′, 5′	6.91 (2H, d, 9.0)	114.83
4′	–	160.12
1″	5.57 (1H, d, 1.5)	98.49
2″	4.03 (1H, dd, 3.5, 1.5)	70.29
3″	3.84 (1H, dd, 9.0, 3.5)	70.83
4″	3.49 (1H, dt, 9.0, 7.0)	72.20
5″	3.61 (1H, m)	69.85
6″	1.27 (1H, d, 6.0)	16.69
1‴	5.65 (1H, d, 7.5)	99.42
2‴	3.95 (1H, dd, 9.5, 7.5)	76.15
3‴	3.71 (1H, dd, 9.5, 8.0)	74.31
4‴	3.79 (1H, dd, 8.0, 8.0)	69.29
5‴	3.66 (1H, m)	73.96
6‴a	3.73 (1H, dd, 11.5, 6.0)	65.74
6‴b	3.46 (1H, dd, 11.5, 1.5)	
1⁗	5.23 (1H, d, 1.5)	101.20
2⁗	4.01 (1H, dd, 3.5, 1.5)	71.00
3⁗	3.80 (1H, dd, 9.5, 3.5)	70.92
4⁗	3.27 (1H, dd, 9.5, 9.5)	72.47
5⁗	3.47 (1H, m)	68.27
6⁗	1.18 (1H, d, 6.0)	16.54
1⁗′	4.53 (1H, d, 1.5)	100.42
2⁗′	3.55 (1H, dd, 3.5, 1.5)	70.66
3⁗′	3.48 (1H, dd, 9.5, 3.5)	70.83
4⁗′	3.35 (1H, dd, 9.5, 9.5)	72.64
5⁗′	4.06 (1H, m)	68.42
6⁗′	0.98 (1H, d, 6.5)	16.13

114.83 (C-3′ and C-5′), 106.07 (C-10), 101.20 (C-1⁗), 99.42 (C-1‴), 98.99 (C-6), 98.49 (C-1″), 94.19 (C-8), 76.15 (C-2‴), 74.31 (C-3‴), 73.96 (C-5‴), 72.64 (C-4⁗), 72.20 (C-4″), 71.00 (C-2⁗), 70.92 (C-3⁗), 70.66 (C-3″), 70.29 (C-2″), 69.85 (C-5″), 69.29 (C-4‴), 68.42 (5⁗), 65.74 (C-6‴), 16.69 (6″), 16.13 (6⁗); ESI–MS (positive) *m/z* 741.20 $[M + H]^+$.

Compound **13**: white powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 4.31 (1H, d, *J* = 7.0 Hz, H-1′), 4.26 (1H, dd, *J* = 11.5, 5.5 Hz, H-6), 4.11 (1H, dd, *J* = 8.0, 6.0 Hz, H-3′), 3.82 (1H, dd, *J* = 10.0, 2.5 Hz, H-6′b), 3.71 (1H, dd, *J* = 10.0, 5.5 Hz, H-6′a), 3.37 (1H, m, H-5′), 3.24 (1H, dd, *J* = 8.0, 6.0 Hz, H-2′), 4.10 (1H, d, *J* = 5.5 Hz, H-7), 3.61 (1H, d, *J* = 11.0 Hz, H-17b), 3.50 (1H, d, *J* = 5.0 Hz, H-17a), 3.49 (1H, dd, *J* = 8.0, 5.6 Hz, H-4′), 3.39 (1H, d, *J* = 2.0 Hz, H-7), 2.12 (1H, d, *J* = 12.5 Hz, H-15b), 2.11 (1H, s, H-13), 2.10 (1H, m, H-2b), 1.93 (1H, m, H-3b), 1.88 (1H, m, H-12b), 1.87 (1H, m, H-5), 1.86 (1H, m, H-11b), 1.85 (1H, d, *J* = 12.5 Hz, H-15a), 1.84 (1H, m, H-14b), 1.83 (1H, m, H-1b), 1.76 (1H, m, H-3a), 1,73 (1H, m, H-14a), 1,67 (1H, m, H-11a), 1,65 (1H, m, H-12a), 1,47 (1H, m, H-9), 1,39 (1H, m, H-2a), 0.88 (1H, m, H-1a), 1.45 (3H, s, H-18), 1.00 (3H, s, H-20); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 179.7 (C-19), 104.6 (C-1′), 81.2 (C-7), 80.6 (C-13), 79.8 (C-3′ and C-5′), 77.4 (C-16), 77.3 (C-17), 74.7 (C-2′), 72.4 (C-6), 71.0 (C-4′), 63.5 (C-6′), 50.8 (C-5), 49.1 (C-15), 48.6 (C-9), 45.3 (C-8), 43.9 (C-4,14), 42.4 (C-10), 40.7 (C-1), 40.5 (C-3), 34.7 (C-12), 32.4 (C-18), 19.7 (C-2, 11), 16.9 (C-20); *m/z* 547.28 $[M + H]^+$.

Compound **14**: yellow powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.09 (2H, br. d, *J* = 9.0 Hz, H-2′ and H-6′), 6.91 (2H, br.d, *J* = 9.0 Hz, H-3′ and H-5′), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.25 (1H, d, *J* = 7.5 Hz, H-1″), 4.77 (1H, d, *J* = 7.8 Hz, H-1‴), 4.50 (1H, d, *J* = 1.5 Hz, H-1⁗), 4.28–3.27 (H-2″–H-6″, H-2″–H-6‴, and H-2⁗–H-5⁗), 1.18 (3H, d, *J* = 6.5 Hz, H-6⁗); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 180.60 (C-4), 164.50 (C-7), 161.61 (C-5), 160.18 (C-4′), 157.79 (C-2), 157.06 (C-9), 133.47 (C-3), 131.08 (C-2′ and C-6′) 121.26 (C-1′), 114.90 (C-3′ and C-5′), 104.25 (C-10), 103.24 (C-1‴), 100.43 (C-1″), 100.37 (C-1⁗), 98.54 (C-6), 93.44 (C-8), 78.41 (C-2″), 76.61 (C-5‴), 76.42 (C-2‴), 73.91 (C-3″), 73.73 (C-5″), 73.72 (C-3‴), 72.44 (C-4⁗), 71.06 (C-4‴), 70.65 (C-2⁗), 70.13 (C-4″), 69.84 (3⁗), 68.29 (C-5⁗), 65.72 (C-6″), 61.18 (6‴), 16.54 (6⁗); ESI–MS (positive) *m/z* 779.20 $[M + Na]^+$.

Compound **15**: yellow powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.07 (2H, br. d, *J* = 9.0 Hz, H-2′ and H-6′), 6.90 (2H, br. d, *J* = 9.0 Hz, H-3′ and H-5′), 6.39 (1H, d, *J* = 2.3 Hz, H-8), 6.19 (1H, d, *J* = 2.3 Hz, H- 6), 5.62 (1H, d, *J* = 8.0 Hz, H-1″), 5.22 (1H, d, *J* = 1.5 Hz, H-1‴), 4.53 (1H, d, *J* = 1.5 Hz, H-1⁗), 4.07–3.32 (H-2″–H-6″, H-2‴–H-5‴, and H-2⁗–H-5⁗), 1.18 (3H, d, *J* = 6.0 Hz, H-6⁗), 0.98 (3H, d, *J* = 6.0 Hz, H-6‴); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 177.99 (C-4), 164.33 (C-7), 161.72 (C-5), 159.87 (C-4′), 157.24 (C-2), 157.00 (C-9), 133.02 (C-3), 130.79 (C-2′ and C-6′) 121.62 (C-1′), 114.76 (C-3′ and C-5′), 104.42 (C-10), 101.19 (C-1′), 100.42 (C-1⁗), 99.44 (C-1″), 98.43 (C-6), 93.27 (C-8), 76.13 (C-2″), 74.30 (C-3″), 73.87 (C-5″), 72.66 (C-4‴),

72.66 (C-4‴), 72.46 (C-4⁗), 71.00 (C-2‴), 70.92 (C-3‴), 70.86 (C-3⁗), 70.67 (C-2⁗), 69.29 (C-4″), 68.42 (5‴), 68.28 (C-5⁗), 65.69 (C-6″), 16.54 (C-6⁗), 16.12 (C-6‴); ESI–MS (negative) *m/z* 739.30 $[M–H]^-$.

Compound **16**: yellow powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.11 (2H, br. d, *J* = 9.0 Hz, H-2′ and H-6′), 6.90 (2H, br. d, *J* = 9.0 Hz, H-3′ and H-5′), 6.40 (1H, d, *J* = 2.3 Hz, H-8), 6.23 (1H, d, *J* = 2.3 Hz, H-6), 5.05 (1H, d, *J* = 8.0 Hz, H-1″), 4.52 (1H, d, *J* = 1.5 Hz, H-1‴), 3.87–3.28 (H-2″–H-6″ and H-2‴–H-5‴), 1.19 (3H, d, *J* = 6.1 Hz, H-6‴); ESI–MS (negative) *m/z* 593.20 $[M–H]^-$.

Compound **17**: yellow powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.11 (2H, br. d, *J* = 9.0 Hz, H-2′ and H-6′), 7.83 (1H, dd, *J* = 7.5, 1.7 Hz, H-6⁗⁗), 7.55 (1H, ddd, *J* = 8.5, 7.0, 1.7 Hz, H-4⁗⁗), 7.15 (1H, dd, *J* = 7.3, 1.7 Hz, H-3⁗⁗), 7.03 (1H, ddd, *J* = 8.5, 7.3, 1.7 Hz, H-5⁗⁗), 6.91 (2H, br.d, *J* = 9.0 Hz, H-3′ and H-5′), 6.73 (1H, d, *J* = 2.0 Hz, H-8), 6.48 (1H, d, *J* = 2.5 Hz, H-6), 5.66 (1H, d, *J* = 1.5 Hz, H-1⁗), 5.57 (1H, d, *J* = 1.5 Hz, H-1⁗), 5.25 (1H, d, *J* = 7.5 Hz, H-1″), 4.77 (1H, d, *J* = 7.8 Hz, H-1‴), 4.50 (1H, d, *J* = 1.5 Hz, H-1⁗), 4.28–3.27 (H-2″–H-6″, H-2‴–H-6‴, H-2⁗–H-5⁗, and H-2⁗–H-5⁗), 1.27 (3H, d, *J* = 6.0 Hz, H-6⁗), 1.18 (3H, d, *J* = 6.5 Hz, H-6⁗); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 178.0 (C-4), 165.0 (C-7⁗⁗), 161.7 (C-7), 160.6 (C-4′), 158.2 (C-1⁗⁗), 156.8 (C-2), 156.3 (C-9), 134.1 (C-5⁗⁗), 133.6 (C-3), 131.7 (C-2′ and C-6′), 131.6 (C-3⁗⁗), 121.1 (C-1⁗⁗), 120.6 (C-1′), 120.3 (C-4⁗⁗), 115.7 (C-3′ and C-5′), 113.0 (C-6⁗⁗), 106.0 (C-10), 104.6 (C-1‴ and C-1⁗), 99.8 (C-6), 98.9 (C-1″), 98.7 (C-1⁗), 95.2 (C-8), 80.6 (C-2″), 77.0 (C-5‴), 76.7 (C-3‴), 74.7 (C-2‴), 74.3 (C-4⁗), 74.1 (C-5″), 73.6 (C-3″), 72.4 (C-4⁗), 70.8 (C-2⁗), 70.7 (C-3⁗), 70.1 (C-2⁗), 69.2 (C-4″), 68.6 (C-5⁗), 68.1 (C-3⁗), 67.8 (C-4‴ and C-5⁗), 65.1 (C-6″), 61.2 (C-6‴), 56.2 (-OCH_3) 18.4 (C-6⁗ and C-6⁗); ESI–MS (positive) *m/z* 1059.30 $[M + Na]^+$.

Compound **18**: yellow powder; ^{1}H-NMR (500 MHz, CD_3OD, TMS) δ 8.11 (2H, br. d, *J* = 9.0 Hz, H-2′ and H-6′), 7.83 (1H, dd, *J* = 7.5, 1.7 Hz, H-6⁗⁗), 7.55 (1H, *J* = ddd, 8.5, 7.5, 1.7 Hz, H-4⁗⁗), 7.15 (1H, dd, *J* = 8.0, 2.0 Hz, H-3⁗⁗), 7.03 (1H, ddd, *J* = 8.5, 7.5, 1.7 Hz, H-5⁗⁗), 6.91 (2H, br. d, *J* = 9.0 Hz, H-3′ and H-5′), 6.73 (1H, d, *J* = 2.3 Hz, H-8), 6.48 (1H, d, *J* = 2.3 Hz, H-6), 5.62 (1H, d, *J* = 8.0 Hz, H-1″), 5.57 (1H, d, *J* = 1.5 Hz, H-1⁗), 5.22 (1H, d, *J* = 1.5 Hz, H-1‴), 4.53 (1H, d, *J* = 1.5 Hz, H-1⁗), 4.07 (1H, m, H-5‴), 4.03–3.32 (H-2″–H-6″, H-2‴–H-5‴, H-2⁗–H-5⁗, and H-2⁗–H-5⁗), 1.27 (3H, d, *J* = 6.0 Hz, H-6⁗), 1.18 (3H, d, *J* = 6.0 Hz, H-6⁗), 0.98 (3H, d, *J* = 6.0 Hz, H-6‴); ^{13}C-NMR (125 MHz, CD_3OD, TMS) δ 177.9 (C-4), 165.0 (C-7⁗⁗), 161.4 (C-7), 158.9 (C-4′), 158.2 (C-2⁗⁗), 156.4 (C-2), 155.9 (C-9), 134.1 (C-5⁗⁗), 133.4 (C-3), 131.4 (C-2′ and C-6′), 131.6 (C-3⁗⁗), 121.1 (C-1⁗⁗), 120.6 (C-1′), 120.3 (C-4⁗⁗), 115.6 (C-3′ and C-5′), 113.0 (C-6⁗⁗), 106.2 (C-10), 100.4 (C-1‴), 100.0 (C-1⁗), 99.4 (C-6), 98.7 (C-1″), 98.1 (C-1⁗), 95.3 (C-8), 75.3 (C-2″), 74.2 (C-4⁗⁗), 73.8 (C-5″), 73.7 (C-4⁗⁗), 71.9 (C-4⁗), 70.5 (C-2⁗), 70.3 (C-3⁗), 70.6 (C-2‴ and C-3‴), 70.1 (C-2⁗⁗), 69.2 (C-4″), 68.7 (C-5⁗), 68.2 (C-5‴), 68.1 (C-3⁗⁗), 67.8 (C-4‴ and C-5⁗⁗), 65.1 (C-6″), 61.2 (C-6‴), 56.2 (-OCH_3), 18.4 (C-6⁗ and C-6⁗⁗); ESI–MS (positive) *m/z* 1043.32 $[M + Na]^+$.

Determination of DPPH radical-scavenging activity

The DPPH free radical-scavenging activities of the isolated 12 compounds except for **3–6**, **13**, and **14**, which were finally purified as mixtures, and ferulic acid, as a positive control, were determined. In brief, 100 μL of 0.5 mM compound (final concentration, 50 μM) in EtOH was mixed to the 900 μL of DPPH radical EtOH solution (final concentration, 100 μM). The mixture was incubated for 20 min in darkness and the absorbance was measured at 517 nm. The DPPH radical-scavenging activities of the isolated 12 compounds and ferulic acid were calculated as the decreased percentage for the absorbance of sample solution to control. The DPPH radical-scavenging activity was expressed as the mean ± standard deviation ($n = 3$) using (SPSS, Chicago, IL, USA) 20.0 package programs. Statistical differences were measured by one-way analysis of variance followed by Duncan's multiple-range test. The significance was considered in $p < 0.05$.

Results and discussion

DPPH free radical-scavenging activities of solvent-fractionated fractions of sword bean MeOH extracts

The MeOH extracts of the fresh sword bean were partitioned to obtain *n*-hexane, $CHCl_3$, EtOAc, and BuOH fractions. These fractions were subjected to TLC analysis and screened as antioxidative compounds by spraying of DPPH solution. Various antioxidative compounds were observed in the *n*-hexane, $CHCl_3$, and BuOH fractions. The *n*-hexane and $CHCl_3$ factions exhibited similar patterns of antioxidative compounds detected on their TLC plates (data not shown).

Structural determination of 1–18 from sword bean MeOH extracts

The chemical constituents were separated from the *n*-hexane/$CHCl_3$ and BuOH fractions of the fresh sword bean MeOH extracts. Briefly, 6 compounds were purified and

isolated from the *n*-hexane/$CHCl_3$ fraction by silica-gel column chromatography and HPLC. Additionally, 12 compounds were purified and isolated from the BuOH fraction by separation tools using Amberlite XAD-2, ODS, and ODS-HPLC.

The structures of the 18 compounds were determined by MS and NMR experiments. Among them, the 15 known compounds were methyl gallate (**1**) (Hussain et al., 1979), (2*S*,3*S*,4*E*,8*E*)-2-aminooctadeca-4,8-diene-1,3-diol 1-*O*-β-D-glucopyranoside (**3**) (Cateni et al., 2008), (2*S*,3*S*,4*E*,8*Z*)-2-aminooctadeca-4,8-diene-1,3-diol 1-*O*-β-D-glucopyranoside (**4**) (Cateni et al., 2008), lupeol (**5**) (Cho et al., 2013), trilinolein (**6**) (Fierro et al., 2012), 1,6-di-*O*-galloyl β-D-glucopyranoside (**7**) (Kim et al., 2013a, b), dihydrophaseic acid (**10**) (Rho and Yoon, 2017), dillenetin (**11**) (Megawai and Fajriah, 2013), kaempferol-7-*O*-[2-*O*-β-D-glucopyranosyl-6-*O*-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**12**) (Okoye et al., 2012), canavalioside (**13**) (Murakami et al., 2000), kaempferol-3-*O*-[2-*O*-β-D-glucopyranosyl-6-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**14**) (Chen et al., 2009), kaempferol-3-*O*-(2,6-*O*-α-L-dirhamnopyranosyl)-β-D-glucopyranoside (**15**) (Clarkson et al., 2005), kaempferol-3-*O*-rutinoside (**16**) (Clarkson et al., 2005), gladiatoside A_1 (**17**) (Murakami et al., 2000), and gladiatoside B_1 (**18**) (Murakami et al., 2000) (Fig. 1). We confirmed that **3** and **4**, **5** and **6**, and **13** and **15** were purified respectively as mixtures, based on their ^{1}H- and ^{13}C-NMR spectra. The structures of cerebrosides (**3** and **4**), terpene derivatives (**10** and **13**), and kaempferol derivative and its glycosides (**11**, **12**, and **14–18**) were determined by ESI–MS, ^{1}H-NMR, ^{13}C-NMR, HSQC, ^{1}H-^{1}H COSY, and HMBC experiments. Compounds **2** and **9** were elucidated as new compounds. Compound **8** was isolated for the first time in nature; consequently, no report on its MS and NMR spectroscopic data exists. Therefore, herein, we described the structural determination of **2**, **8**, and **9**.

The HR-ESI–MS (negative) spectrum of **2** showed a quasi-molecular ion peak at *m/z* 249.1493 $[M - H]^-$ ($C_{15}H_{21}O_3$), indicating that its molecular formula was $C_{15}H_{22}O_3$ (MW 250). The ^{1}H-NMR spectrum exhibited two singlet exomethylene proton signals at δ 5.07 (H-13b) and 5.02 (H-13a), as well as one olefinic double bond proton signal at δ 6.69 (H-5) (Table 1). Additionally, an oxygenated methine proton signal at δ 4.40 (H-7), five methylene proton signals at δ 1.66-2.77 (H-2, H-3, H-6, H-9, and H-10), and two methyl group signals at δ 1.03 (H-

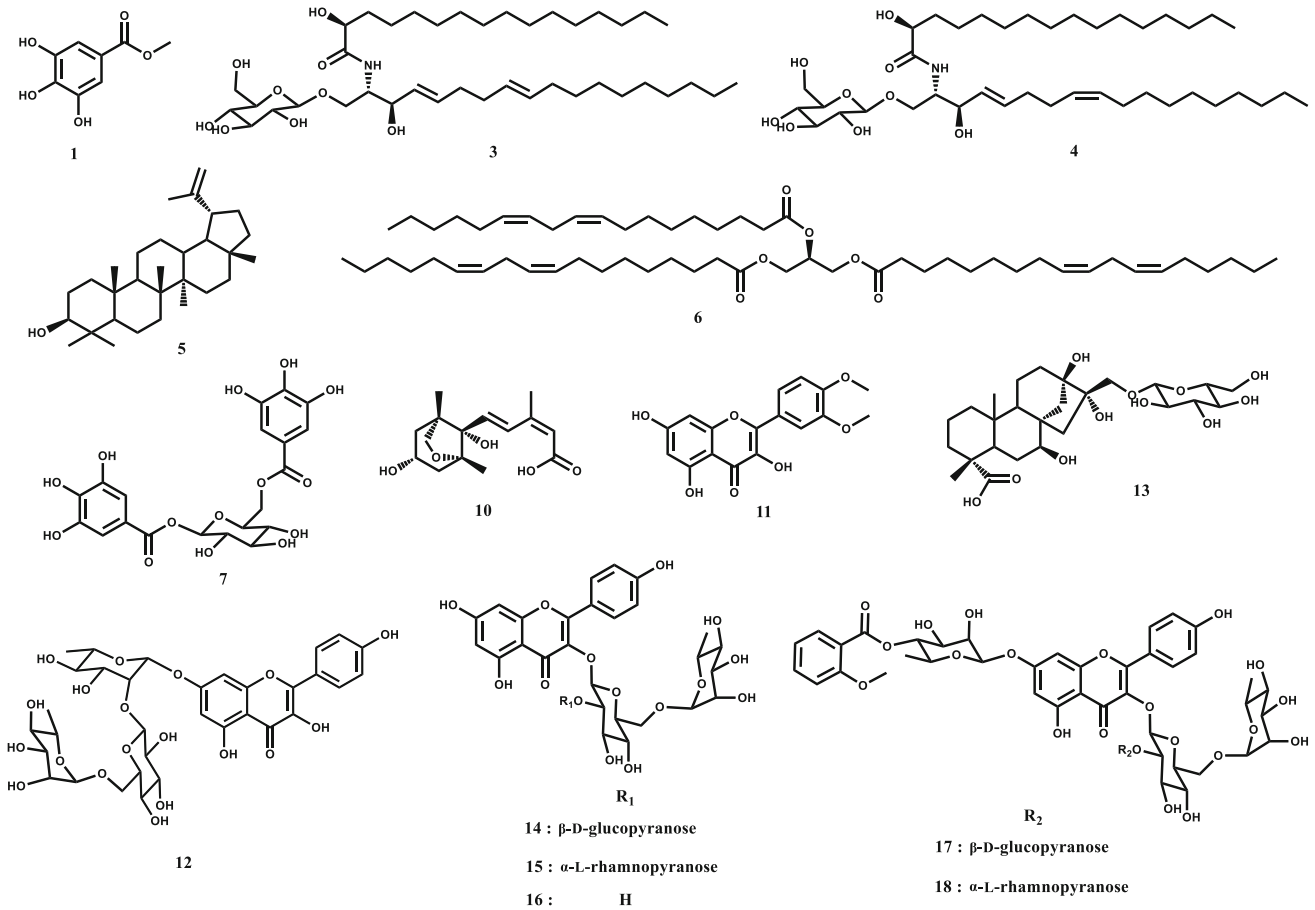

Fig. 1 Structures of the isolated compounds, **1**, **3–7**, and **10–18,** from sword beans

15) and 1.00 (H-14) were observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of **2** contained 15 carbon signals, including a carbonyl carbon at δ 171.7 (C-12), four olefinic double bond carbons at δ 159.0 (C-8), 139.5 (C-5), 135.5 (C-4), and 113.0 (C-13), one quaternary carbon at 33.8 (C-11), four methylene carbons at δ 41.9 (C-10), 36.0 (C-6), 28.1 (C-2), and 24.3 (C-3), and two methyl carbons at δ 30.8 (C-15) and 24.3 (C-14). From the MS and 1D-NMR results, **2** was suggested to be caryophyllene-type sesquiterpene that is composed of cyclobutane and cyclononene rings. The accurate structure of compound **2** could be determined by ^{1}H-^{1}H COSY, HSQC, and HMBC experiments. The ^{1}H-^{1}H COSY correlations (bold lines) of H-1/H-2, H-1/H-9, H-2/H-3, H-5/H-6, and H-6/H-7 and the HMBC correlations of δ 1.75 (H-1) to 159.0 (C-8), δ 2.53 (H-6b) to 135.5 (C-4) and 159.0 (C-8), and δ 1.81 (H-2a) to 135.5 (C-4) established a connection of the cyclononene ring in **2** (Fig. 2). Additionally, the ^{1}H-^{1}H COSY correlations (bold lines) of H-1/H-9 and H-9/H-10 and the HMBC correlations of δ 1.75 (H-1) to 41.9 (C-10), 24.3 (C-14), and 30.8 (C-15), δ 2.77 (H-9) to 33.8 (C-11), and δ 1.00 (H-14) and 1.03 (H-15) to 55.1 (C-1) indicated that the cyclobutane ring fused to C-1 and C-9 of the cyclononene ring and two methyl groups coupled with C-11 of the cyclobutane ring. The exomethylene group positioned at C-8 (δ 159.0) and the hydroxyl group positioned at C-7 (δ 76.2) were confirmed by the HMBC correlations of δ 5.07 (H-13b) and 5.02 (H-13a) to 76.2 (C-7) and 33.6 (C-9), and δ 2.77 (H-9) and 4.40 (H-7) to 113.0 (C-13). The carboxylic acid group positioned at C-4 (δ 135.5) was also confirmed by the HMBC correlations of δ 2.33 (H-3b) and 6.69 (H-5) to 171.7 (C-12). Compound **2** was suggested to be 7-hydroxy-11,11-dimethyl-8-methylenebicyclo[7.2.0]undec-4-ene-4-carboxylic acid, which is the similar structure of lychnopholic acid identified from Lychnophora martiana (Raffauf et al., 1987; Vichewski et al., 1980). The relative stereochemistry of compound **2** was established, based on the nuclear Overhauser effect (NOE) experiment and comparison of ^{13}C-NMR data reported previously in literature (Raffauf et al., 1987;

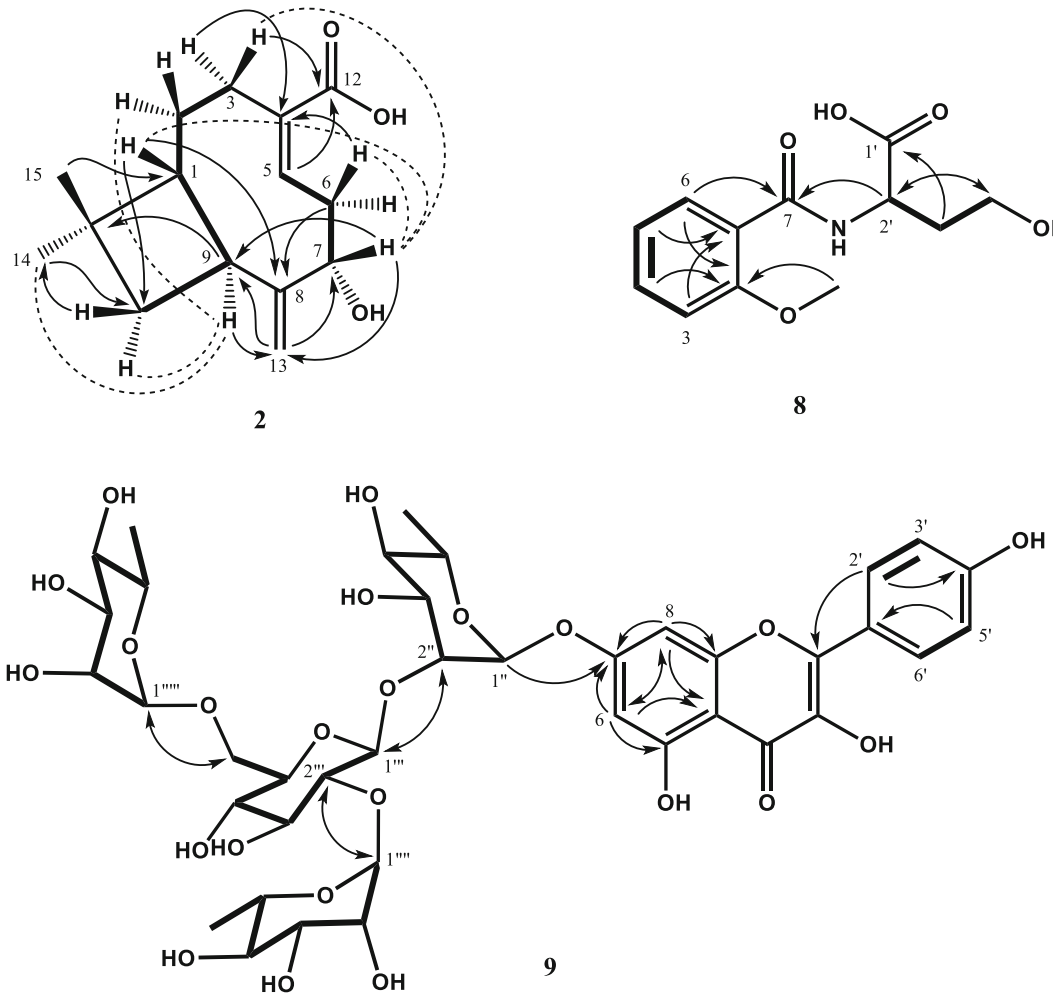

Fig. 2 Structures, ^{1}H-^{1}H COSY (bold line), NOE (dash line), and HMBC (arrows) correlations of **2**, **8**, and **9**

Vichewski et al., 1980). H-1 at δ 1.75, H-3a at δ 2.57, H-5 at δ 6.69, H-13a at 5.02, and H-6a at δ 2.72–2.66 were enhanced by irradiation of H-7 at δ 4.40, suggesting that H-1 and H-7 have α-orientation and hydroxyl group of C-7 has β-orientation (Fig. 2). The β-orientation of H-9 and C-14 was deduced from enhancement of H-14 at δ 1.00, H-10b at δ 1.80, and H-2b at δ 1.66 by irradiation of H-9 at δ 2.77. Therefore, the structure of compound **2** was determined as (1*R*,7*S*,9*S*)-7-hydroxy-11,11-dimethyl-8-methylenebicyclo[7.2.0]undec-4-ene-4-carboxylic acid (caryophyllenic acid) (Fig. 2).

The molecular formula ($C_{12}H_{15}N_1O_5$, MW 253) of **8** was established by observation of quasi-molecular ion peak at *m/z* 254.0968 $[M + H]^+$ (calculated for $C_{12}H_{16}N_1O_5$, +1.8 mDa) by the HR–ESI–MS analysis. The ^{1}H-NMR (500 MHz, CD_3OD, TMS) spectrum of **8** showed four proton signals corresponding to *ortho*-substituted benzene ring at δ 8.02 (1H, dt, *J* = 8.0, 2.0 Hz, H-6), 7.51 (1H, dt, *J* = 8.0, 2.0 Hz, H-4), 7.16 (1H, br. d, *J* = 8.0 Hz, H-3), and 7.06 (1H, dt, *J* = 8.0, 1.0 Hz, H-5), and a methoxy proton signal at δ 4.01 (3H, s) (Table 2). Additionally, a nitrogenated methine proton signal was observed at δ 4.57 (1H, t, *J* = 6.0 Hz, H-2′), an oxygenated methylene proton signal at δ 3.98–4.06 (2H, m, H-4′), and a methylene proton signal at δ 2.19–2.33 (2H, m, H-3′) that were confirmed by the ^{13}C-NMR and HSQC spectra. The ^{13}C-NMR (125 MHz, CD_3OD, TMS) spectrum showed 12 carbon signals, including two carbonyl carbons at δ 176.9 (C-1′) and 165.2 (C-7) (Table 2). The presence of 2-methoxybenzoic acid was established by ^{1}H-^{1}H COSY correlations of H-3/H-4, H-4/H-5, and H-5/H-6 and HMBC correlations of δ 8.02 (H-6) to 165.2 (C-7) and δ 7.51 (H-4) and 4.01 (-OCH_3) to 158.1 (C-2) (Fig. 2). Homoserine moiety was confirmed by the ^{1}H-^{1}H COSY correlations of H-2′/H-3′ and H-3′/H-4′ and HMBC correlations of δ 4.57 (H-2′) and 2.19–2.33 (H-3′) to 176.9 (C-1′). In particular, the correlation of δ 4.57 (H-2′) to 165.2 (C-7) was observed in the HMBC experiment, indicating that 2-methoxybenzoic acid was amidified with the C-2 of homoserine. Therefore, the planar structure of **8** was determined as *N*-(2-methoxybenzoyl)homoserine (Fig. 2). Further investigation of the absolute stereochemical structure of **8** is required.

The molecular formula ($C_{39}H_{50}O_{23}$, MW 908) of **9** was determined by analyzing the quasi-molecular ion peak at *m/z* 925.2595 $[M + H]^+$ by the HR–ESI–MS (positive) spectrum. This result was supported by the ^{1}H-NMR, ^{13}C-NMR, and HSQC spectra. From the MS and 1D-NMR spectra, **9** was suggested to be flavonoid tetraglycoside. The presence of a kaempferol moiety was suggested by two *p*-substituted benzene ring proton signals at δ 8.10 (2H, d, *J* = 9.0 Hz, H-2′ and H-6′) and 6.91 (2H, d, *J* = 9.0 Hz, H-3′ and H-5′) and two *meta*-coupling benzene ring proton signals at δ 6.73 (1H, d, *J* = 2.0 Hz, H-8), and 6.47 (1H, d, *J* = 2.0 Hz, H-6) in the ^{1}H-NMR (500 MHz, CD_3OD, TMS) spectrum (Table 4). The presence of the kaempferol moiety was further supported by its related 15 carbon signals, including one carbonyl carbon at δ 178.12 (C-4) and four oxygenated quaternary carbons at δ 161.98–156.52. The presence of one β-D-glucopyranose and three α-L-rhamnopyranose was suggested by four anomeric proton signals at δ 5.65 (1H, d, *J* = 7.5 Hz, H-1‴), 5.57 (1H, d, *J* = 1.5 Hz, H-1″), 5.23 (1H, d, *J* = 1.5 Hz, H-1⁗), and 4.53 (1H, d, *J* = 1.5 Hz, H-1⁗′), and three methyl proton signals at δ 1.27 (3H, d, *J* = 6.0 Hz, H-6″), 1.18 (3H, d, *J* = 6.0 Hz, H-6⁗), and 0.98 (3H, d, *J* = 6.5 Hz, H-6⁗′) observed in the ^{1}H-NMR spectrum of **9**. These glycoses were also confirmed by their ^{1}H-^{1}H COSY correlations and coupling constant values in the ^{1}H-NMR spectrum. From the HMBC spectrum of **9**, it was possible to establish a connection with kaempferol tetraglycoside. In particular, the HMBC correlations of δ 5.57 (H-1″) to 161.98 (C-7) established the C-1 of rhamnose coupled with the C-7 position of kaempferol (Fig. 2). The HMBC correlations of δ 4.03 (H-2″) to 99.42 (C-1‴), and δ 5.65 (H-1‴) to 70.29 (C-2″) established that the C-1 of glucose was connected to the C-2 position of rhamnose in kaempferol 7-*O*-rhamnoside. The HMBC correlations of δ 3.95 (H-2‴) to 101.20 (C-1⁗), δ 5.23 (H-1⁗) to 76.15 (C-2‴), δ 3.73 (H-6‴a) to 100.42 (C-1⁗′), and δ 4.53 (H-1⁗′) to 65.74 (C-6‴) established that two rhamnopyranoses were etherified to the C-2 and C-6 positions of glucose. Therefore, compound **9** was determined to be kaempferol-7-*O*-α-L-dirhamnopyranosyl(1 → 2;1 → 6)-

Table 4 DPPH radical-scavenging activities of the isolated compounds from sword beans

Compounds	DPPH radical-scavenging activities (%)
1	85.6 ± 3.6[a]
2	4.1 ± 1.7[e]
7	90.6 ± 5.5[a]
8	10.2 ± 2.1[d]
9	15.9 ± 2.4[c]
10	10.8 ± 2.2[d]
11	9.3 ± 2.4[d]
12	15.6 ± 3.2[c]
15	14.4 ± 2.6[c]
16	14.1 ± 1.4[c]
17	13.2 ± 2.8[c]
18	13.8 ± 2.5[c]
Ferulic acid	71.3 ± 2.3[b]

Ferulic acid was used as positive control. The isolated compounds except for **3**–**6**, **13**, and **14** were assayed at the concentration of 50 μM. Values are expressed as mean ± SD (n = 3)

[a–e]Results with a different letter differ significantly ($p < 0.05$)

O-β-D-glucopyranosyl(1 → 2)-*O*-α-L-rhamnopyranoside (Fig. 2).

DPPH free radical-scavenging activities of the isolated compounds

In this experiment, compounds **3**–**6**, **13**, and **14** were excluded because these compounds were finally purified as mixtures. The DPPH free radical-scavenging activities of 12 compounds of the isolated 18 compounds and ferulic acid at the same concentration (50 μM) were evaluated (Table 4). As expected, two galloyl derivatives (**1** and **7**) showed the highest DPPH free radical-scavenging activities. These activities were very higher in comparison to that of ferulic acid, which is used as a positive control. However, the free radical-scavenging activities of flavonol glycosides **9** and **12**–**18** were similar and lowered in compared to that of ferulic acid. Other compounds including new compounds **2** and **8** were very low DPPH free radical-scavenging activities, although these activities in TLC analysis during purification and isolation were observed. These observations indicated that two galloyl derivatives (**1** and **7**) including the phenolic compounds identified in this study may be responsible for the antioxidative activity of sword bean.

In this study, we demonstrated the presence of 18 compounds, including two galloyl derivatives (**1** and **7**), trilinolein (**6**), two cerebrosides (**3** and **4**), three terpene derivatives (**2**, **5**, **10,** and **13**), salicyloyl derivative (**8**), and eight kaempferol derivatives and their glycosides (**9, 11**, **12**, and **14**–**18**) in sword beans. Among them, caryophyllene-type sesquiterpene (**2**) and flavonol tetraglycoside (**9**) were elucidated as new compounds. The occurrence of two galloyl derivatives (**1** and **7**), canavalioside (**13**), and salicyloyl kaempferol glycosides (**17** and **18**) in sword bean (Kim et al., 2013a, b; Lee and Jeong, 2005; Murakami et al., 2000) has been previously reported. Contrarily, compounds **3**–**8**, **10**–**12**, **14**, and **15** were newly identified in this plant.

Compound **2** is a caryophyllene-type sesquiterpene like β-caryophyllene, β-caryophyllene oxide, and humulene, which are constituents of essential oil in plants and exert anticancer, antioxidant, and antimicrobial activities (Dahham et al., 2015; Fidyt et al., 2016). Phenolic compounds including two galloyl derivatives (**1** and **7**) and eight kaempferol glycosides (**9, 11**, **12**, and **14**–**18**) are widely distributed in plant kingdoms and are also known as biological active compounds that have anticancer, antioxidant, and anti-inflammatory effects (Kumar and Pandey, 2013; Tanase, 2019). In this study, we confirmed two galloyl derivatives (**1** and **7**) exerting strong DPPH free radical-scavenging activities. Additionally, cerebrosides (**3** and **4**) inhibited the growth of bacteria and *Candida* species (Cateni et al., 2008). Trilinolein (**6**) has been demonstrated to have anti-ischemic, antiarrhythmic, and antioxidant properties (Chan et al., 2005). The chemical constituents found in this study might offer valuable information for evaluating the health benefits and food quality of sword beans and for selecting biomarker compounds in food processing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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