Antioxidant xanthones from *Swertia mussotii*, a high altitude plant

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1. Introduction

*Swertia mussotii* (SM), which belongs to the family of *Swertia* in Gentianaceae, is a traditional Tibetan folk medicine called “sang di” or “zang yin chen”. It is used to treat the febrile diseases in the liver and gallbladder [1]. Naturally, it exists strictly on the high alpine lands of the Qinghai–Tibet Plateau at altitudes ranging between 3200 and 3800 m [2]. Because of the evident effect, scientists have paid much attention to this plant for a long time. So far, there have been a variety of studies on the chemical constituents and biological functions of SM. Chemical analysis disclosed that SM contains xanthones and flavonoids, together with minor constituents like iridoids, terpenes, et al. [3–8]. Investigations showed that xanthones exhibit various biological and pharmacological properties such as anti-depressant, anti-leukaemic, anti-tumor, anti-tubercular, choleretic, diuretic, anti-microbial, anti-fungal, anti-inflammatory, anti-viral, cardiotonic, and hypoglycaemic activity [9–16]. However, pharmacodynamic constituents of SM are far from clear.

As an on-going course of searching lead compounds from traditional Chinese medicine (TCM), a systematic study was launched to investigate chemical constituents focused especially on xanthones in the ethanol aqueous extract of *S. mussotii*. As a high altitude plant, SM needs mechanism to defense against free radicals. Judged from their general structures, xanthones may probably play the role as an antioxidant in SM. We believe it is intriguing to study the antioxidative properties of xanthones systematically. Therefore, in the current paper, we describe the isolation and characterization of four new xanthones (1–4), together with twenty-one known xanthones. Antioxidant activity of all the isolated xanthones using oxygen radical absorbance capacity with fluorescence assay (ORACFr) [17,18] is also included.

2. Experimental

2.1. General experimental procedures

UV spectra were obtained using a JASCO V-550 spectrometer, whereas FT-IR spectra were acquired on a JASCO FI/IR-480 Plus Fourier Transform (using KBr disk method). The ESI–MS spectra were acquired on a Finnigan LCQ...
Advantage MAX spectrometer. The HRESIMS spectra were obtained on an Agilent QTOF spectrometer. NMR spectra were recorded on Bruker AV-300 or Bruker AV-400 NMR spectrometers using TMS as an internal standard. Silica gel (200–300 mesh) for column chromatography (CC) was purchased from Yantai Chemical Factory (Yantai, China) and ODS (50 µm) was purchased from YMC Co. Ltd. (Kyoto, Japan). Pre-coated thin-layer chromatography (TLC) plates (Institute of Yantai Chemical Industry, China) were used for TLC. Spots on TLC plates were detected by either a ZF-7A portable UV detector or spraying KMnO4 solution followed by subsequent heating. The Sephadex LH-20 was purchased from GE Healthcare. The RP-HPLC analysis and preparation were conducted using a P680 pumping system equipped with a PDA-100 photodiode array detector and Cosmols columns (C18; 4.6 × 250 mm for analysis and 20 × 250 mm for preparation).

2.2. Chemicals and apparatus

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Aldrich (Milwaukee, WI), 2,2’-Azobisis (2-aminodipropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Fluorescein (FL) (Na salt) was obtained from Aldrich (Milwaukee, WI). Fluorescence determination was pursued on a FL600 enzyme-labeled meter (Bio-Tek, USA). Fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 527 nm were used. The 96-well FLUOTRAC 200 black microplates (part # 655076) were purchased from Greiner America, Inc. (Lake Mary, FL), and 48 well microplates (Falcon microplates (part # 3230) were purchased from VWR (St. Louis, MO). Clear polystyrene 96-well plates (Nunc) were purchased from Fisher Scientific, Atlanta, GA.

2.3. Plant material

Plant material was collected from Qinghai province (China) in October 2010 and was identified as whole grass of *S. mussotii* by Dr. Jing Sun from Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China.

2.4. Extraction and isolation

Dried powders of *S. mussotii* (5.0 kg) was extracted three times (2 h, 2 h, 1 h respectively) with 75% ethanol aqueous under condition of reflux. The extracts were concentrated in vacuo to yield a brown-black gum (1.1 kg).

500 g of the gum was then suspended in H2O and partitioned successively with petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EE), and n-butanol (NB) to yield PE (35 g), DCM (92 g), EE (42 g), NB (170 g), and water (171 g) fractions respectively. The DCM fraction was then submitted to silica gel CC using a gradient system of petroleum ether-ethyl acetate (30:1–0:1). The collected fractions were combined based on their TLC characteristics to offer eight sub-fractions (F1–F8). F1 sub-fraction was separated further by silica gel CC using a gradient system of petroleum ether-ethyl acetate (15:1–0:1) and by recrystallization to yield pure compounds (13–15). F2 was subjected to Sephadex LH-20 CC eluted with CHCl3–MeOH (1:1) and resulted in compound 11. Compounds 16 and 17 were isolated from F3 by repeated silica gel CC with PE-acetone and were further separated by HPLC on a preparative Cosmols column (20 × 250 mm) applying MeOH–H2O as the eluant. F4 was purified by Sephadex LH-20 CC eluted with CHCl3–MeOH (1:1) to produce compound 19. Compound 20 was isolated from F5 by Sephadex LH-20 CC eluted with CHCl3–MeOH (1:1), and further purified by preparative HPLC. Compounds 12, 17 and 18 were isolated from F6 by repeated silica gel CC with PE-acetone and then were further purified by preparative HPLC as usual. F7 was purified by Sephadex LH-20 CC eluted with CHCl3–MeOH (1:1) to produce compounds 20 and 21. Compounds 22–25 were isolated from F6 by Sephadex LH-20 CC eluted with CHCl3–MeOH (1:1), and that further purified by preparative HPLC as usual.

The n-butanol fraction was first separated by a silica gel CC using a gradient system of CHCl3–MeOH (15:1–0:1). The collected fractions were combined based on their TLC characteristics to yield ten sub-fractions (P1–P10). Compounds 5, 8, and 10 were isolated from P1 by preparative HPLC as described above. Compounds 1, 3, 4, and 7 were isolated from P4 by reversed-phase ODS CC eluted with a MeOH–H2O system and were further submitted to Sephadex LH-20 CC eluted with CHCl3–MeOH (1:1). Compounds 2, 6, and 9 were isolated from P5 by Sephadex LH-20 CC eluted with CHCl3–MeOH (1:1), and were further purified by preparative HPLC as usual.

The amounts of each compounds obtained (from 1 to 25) were 20, 30, 11.5, 13, 12.3, 15.1, 21.3, 12.7, 8.5, 31.2, 9.3, 23.5, 17.2, 16.6, 19.1, 10.5, 8.9, 7.5, 22.6, 20.7, 16.8, 13.5, 12.6, 11.4, and 9.5 mg respectively.

3,5,6,8-tetrahydroxynanthone-1-C-β-D-glucoside (1). Yellow amorphous powder, 20 mg, [α]D25 +25.6 (c 0.1, DMSO), UV (MeOH) λmax (logε): 240(4.25), 258(4.30), 316(3.98), 369(3.91) nm; IR νmax (KBr): 3397, 1644, 1603, 1508 cm−1; ESIMS (negative): m/z 421.3 [M−H]− (calcd. for C19H18O11−H, 421.1); HRESIMS (positive): m/z 445.0741 [M + Na]+ (calcd. for C19H18O11 + Na, 445.0747); for 1H and 13C NMR (300 MHz, DMSO-d6) spectroscopic data, see Table 1.

7-hydroxy-3,4,8-trimethoxyxanthone-1-O-(β-D-glucoside) (2). Yellow amorphous powder, 30 mg, [α]D25 +20.3 (c 0.1, DMSO), UV (MeOH) λmax (logε): 241(4.33), 257(4.32), 311(3.87), 367(3.48) nm; IR νmax (KBr): 3386, 1652, 1619, 1508, 1458 cm−1; ESIMS (negative): m/z 479.3 [M−H]− (calcd. for C22H22O12−H, 479.1); HRESIMS (positive): m/z 503.1169 [M + Na]+ (calcd. for C22H22O11 + Na, 503.1165); for 1H and 13C NMR (300 MHz, DMSO-d6) spectroscopic data, see Table 1.

6-hydroxy-3,5-dimethoxynanthone-1-O-(β-D-glucoside) (3). Pale yellow powder, 11.5 mg, [α]D25 +18.5 (c 0.1, DMSO), UV (MeOH) λmax (logε): 238(4.28), 286(3.90), 321(3.90) nm; IR νmax (KBr): 3408, 1652, 1623, 1458 cm−1; ESIMS (negative): m/z 449.3 [M−H]− (calcd. for C22H22O12−H, 449.1); HRESIMS (positive): m/z 473.1060 [M + Na]+ (calcd. for C22H22O11 + Na, 473.1060); for 1H and 13C NMR (300 MHz, DMSO-d6) spectroscopic data, see Table 1.

3,4,7,8-tetramethoxynanthone-1-O-(β-D-glucoside) (4). Pale yellow powder, 13 mg, [α]D25 +12.5 (c 0.1, DMSO), UV (MeOH) λmax (logε): 238(4.36), 257(4.33), 311(3.91) nm; IR νmax (KBr): 3397, 1644, 1603, 1508 cm−1; ESIMS (negative):
The detail procedure is described below. To a 96-wells plate, 20 μL of sample solution, 20 μL of buffer, and 20 μL of FL were added to each well. For each tested sample at a scheduled concentration, three parallel wells were set: concurrently, AAPH absorption well (phosphate buffer 40 μL and FL 20 μL), fluorescence control well (phosphate buffer solution 180 μL and FL 20 μL), and Trolox control well (Trolox standard solution 40 μL, FL 20 μL). The plate contents were mixed with shaking for 8 s following each injection. Then, the plate was preset at 37 °C for 5 min. Afterwards, additions of AAPH 140 μL were added to each well except fluorescence control well immediately via a multichannel pipettor. The plate was then set inside a FL600 enzyme-labeled meter (Bio-Tek, USA) in which the temperature was preset at 37 °C. Fluorescence intensity was read at a 2-minute interval for a 2-hour course, in which the excitation wavelength was set at 485 nm and emission wavelength at 527 nm.

The decay curve of fluorescence of each sample was set up by Microsoft Excel (Microsoft, Roselle, IL). The area under curve (AUC) was calculated approximatively as \( \Delta t = 2 \text{ min} \)

\[
AUC = 2 \times (f_0 + f_1 + \ldots + f_{n-1} + f_n) - f_0 - f_n, \tag{1}
\]

The net AUC was obtained by subtracting the AUC of the blank from that of a sample. The data were analyzed by Microsoft Excel (Microsoft, Roselle, IL), apply Eq. (1), to calculate the AUC.

The final ORACFL values were calculated by using a regression equation \( Y = a + bX \) (linear; or \( Y = a + bX + cX^2 \), quadratic) between Trolox concentration \( Y \) (μM) and the net area under the FL decay curve \( X \). Linear regression was used in the range of 6.25–50 μM Trolox. Data are expressed as micromoles of Trolox equivalents (TE) per liter or per gram of FL.
sample (μmol TE/g or μmol TE/L). When the unit of ORACFL is defined as U/mL, then the ORACFL value of tested sample is defined as,

\[
\text{ORACFL} = 5 \times \frac{\text{AUC}_{\text{sample}} - \text{AUC}_{\text{AAPH}}}{\text{AUC}_{\text{Trolox}} - \text{AUC}_{\text{AAPH}}} \times \frac{M_{\text{Trolox}}}{M_{\text{sample}}} \tag{2}
\]

where M is the molarity of either sample or Trolox.

3. Results and discussion

3.1. Isolation and characterization of xanthones

The ethanol aqueous extract of the whole S. mussotii plant dry powder is partitioned successively with petroleum ether, dichloromethane (DCM), ethyl acetate, and n-butanol. Both DCM and n-butanol fractions were subjected to repeated separation on silica gel column chromatography as well as reversed-phase chromatography with an ODS column, Sephadex LH-20 and preparative HPLC, affording 4 new xanthones (1–4) (Chart 1) and 21 known xanthones (5–25) (Chart 2). 1 and 3 belong to tetraoxygenated xanthones. Interestingly, compounds 1 is a C-glycoside, a kind of compound which has been extensively studied during the last three decades. Compounds 2 and 4 are pentaoyxgenated xanthones.

Compound 1 was isolated as a yellow powder. Its HRESIMS spectrum exhibited a quasi-molecular ion peak at m/z 503.1169 ([M + Na]⁺). The UV spectrum displayed absorption bands at λ_{max} 241, 257, 311, and 367 nm and implied a xanthone derivative [19]. The 1H NMR spectrum showed signals of three aromatic protons, in which two of them were doublets (\(J = 6\) Hz, H-5); another was a singlet at δ_H 7.24 (1H, d, J = 9 Hz, H-6) implying the existence of an intramolecular hydrogen bond, along with meta-coupled aromatic protons at δ_H 13.81 (1H, s, OH-8) implying the existence of an intramolecular hydrogen bond, along with meta-coupled aromatic protons at δ_H 6.39 (1H, d, J = 1.8 Hz, H-2) and δ_H 6.90 (1H, d, J = 1.8 Hz, H-4), and an isolated aromatic proton at δ_H 7.41 (1H, s, H-7). The signals of the anomeric proton are at δ_H 4.65 (1H, d, J = 9 Hz). Analysis of the 13C NMR spectrum (Table 1) indicated thirteen carbon signals at low field, including one carbonyl at δ_C 180.4 and showed that only one hydroxyl is located at either C-1 or C-8 of compound 1. Carbon signals at δ_C 74.4, 72.0, 80.3, 71.6, 82.9 and 62.8 indicated a β-glucopyranosylxy unit in the molecule. Furthermore, the carbon signal at δ_C 74.4 (Glc-1) is correlated with the anomeric proton (δ_H 4.65) in the HSQC spectrum. This is a clear proof of 1-C-glucoside. The detailed structure of compound 1 was deduced further from its HMBC spectrum (Fig. 1). In its HMBC spectrum, the singlet aromatic proton at δ_H 7.41 (H-7) was found correlated with δ_C 180.4 (C=O), 155.6 (C-8), 112.9 (C-8a), 145.1 (C-5), and 152.2 (C-6). In which the correlations of H-7 with C-8a and C-5 are strong, and the others are from mild to weak. This evidence confirmed its location at C-7. The anomeric proton (δ_H 4.65) was found correlated with C-1, C-2, C-3, C-4a, and C-8b. All these evidences suggest that the glucosyl moiety is located at C-1 of the xanthone. Therefore, the structure of 1 was concluded to be 3,5,6,8-tetrahydroxy-xanthone-1-C-β-o-glucoside.

Compound 2 was an amorphous yellow solid. Its molecular formula, C_{22}H_{24}O_{12}, was deduced from HRESIMS, which exhibited a quasi-molecular ion peak at m/z 503.1169 ([M + Na]⁺). The UV spectrum displayed absorption bands at λ_{max} 241, 257, 311, and 367 nm and implied a xanthone derivative [19]. The 1H NMR spectrum showed signals of three aromatic protons, in which two of them were doublets at δ_H 7.33 (1H, d, J = 9 Hz, H-6) and δ_H 7.24 (1H, d, J = 9 Hz, H-5); another was a singlet at δ_H 7.03 (1H, s, H-2) (Table 1). It also indicated signals for three methoxy groups at δ_H 3.92, 3.81, and 3.80 and a non-chelated hydroxyl peak at δ_H 9.49 (7-OH). The signals of the anomeric proton are at δ_H 4.85 (1H, d, J = 6 Hz). Analysis of the 13C NMR spectrum (Table 1) indicated thirteen carbon signals at low field, including one carbonyl at δ_C 180.4 and showed that only one hydroxyl is located at either C-1 or C-8 of compound 1. Carbon signals at δ_C 74.4, 72.0, 80.3, 71.6, 82.9 and 62.8 indicated a β-glucopyranosyloxy unit in the molecule. Furthermore, the carbon signal at δ_C 74.4 (Glc-1) is correlated with the anomeric proton (δ_H 4.65) in the HSQC spectrum. This is a clear proof of 1-C-glucoside. The detailed structure of compound 1 was deduced further from its HMBC spectrum (Fig. 1). In its HMBC spectrum, the singlet aromatic proton at δ_H 7.41 (H-7) was found correlated with δ_C 180.4 (C=O), 155.6 (C-8), 112.9 (C-8a), 145.1 (C-5), and 152.2 (C-6). In which the correlations of H-7 with C-8a and C-5 are strong, and the others are from mild to weak. This evidence confirmed its location at C-7. The anomeric proton (δ_H 4.65) was found correlated with C-1, C-2, C-3, C-4a, and C-8b. All these evidences suggest that the glucosyl moiety is located at C-1 of the xanthone. Therefore, the structure of 1 was concluded to be 3,5,6,8-tetrahydroxy-xanthone-1-C-β-o-glucoside.

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![Fig. 1. Key HMBC correlations of 1–4 (recorded on Bruker AV-400).](image-url)
proton (δH 4.85) with δC 103.7 in the HSQC spectrum substantiated that 2 was a β-O-glucoside. And the correlation between the anomeric proton (δH 4.86) and the carbon at δC 105.4 (C-1) found in the HMBC suggested that the glucosyl moiety is linked to C-1. In addition, carbon signals at δC 56.2, 60.9, and 61.0 indicated three methoxyl groups. Correlations of δH 3.92 with C-3 (δC 156.7), δH 3.81 with C-4 (δC 130.9), and δH 3.80 with C-8 (δC 145.2) in the HMBC made the locations of three methoxyl groups very clear. The doublet at δH 7.24 was found correlated with C-4b, C-8a, C-6, and C-8, in which the correlation with C-8a was found strong and the others were mild; while another doublet at δH 7.33 was found correlated with C-5, C-7, and C-8, in which the correlation with C-8 was strong and the others were mild. The hydroxyl proton at δH 9.45 was also confirmed at C-1. This is the evidence that the glucosyl moiety is linked to C-1. Hence, the structure of compound 2 was identified as 6-hydroxy-3,5-dimethoxy-xanthone-1-O-β-D-glucoside.

Compound 3 was obtained as a pale yellow powder. Its molecular formula C23H26O12 was deduced from the HRESIMS on the basis of quasi-molecular ion peak at m/z 473.1053 ([M + Na]+). Its UV spectrum with absorption bands at λmax 321 nm was suggestive of a xanthone derivative [19]. The 1H NMR spectrum (Table 1) displayed a set of meta-coupled aromatic protons at δH 6.78 (1H, d, J = 3.0 Hz, H-4) and δH 6.73 (1H, d, J = 3.0 Hz, H-2); two ortho-coupled aromatic protons at δH 7.31 (1H, d, J = 9 Hz, H-7) and δH 7.17 (1H, d, J = 9 Hz, H-8), together with an anomeric proton at δH 4.90 (1H, d, J = 7 Hz). The spectrum also showed signals for two methoxy groups (δH 3.88 and 3.80). Analysis of the 13C NMR spectrum (Table 1) indicated thirteen carbon signals at low field, including one carbonyl at δC 175.2 and disclosed that there is no free hydroxy group located at either C-1 or C-8. A set of six carbon signals at high field, which are δC 103.6, 74.0, 76.4, 70.3, 78.1, and 61.4, implied a β-glucosyl group in the molecule. The structure of compound 3 was further confirmed by the key HMBC cross-peak of H-8 with C-9, which implied that there are substitutions at both C-5 and C-6. This deduction is in agreement with the evidences that δH 6.95 (6-OH) is correlated with C-5 (δC 145.6) and C-7 (δC 123.5) as well as δH 3.80 (5-OCH3) is correlated with C-5 (Fig. 1). Therefore, the free hydroxyl at δH 9.45 was assigned to C-6; while the methoxyl at δH 3.80 was assigned to C-5. The correlation of δH 3.88 with C-3 supported that the location of another methoxyl (δC 56.43) is at C-3. In its HMBC, it was observed that the anomeric proton (δH 4.90) is correlated with the carbon at δC 159.6 (C-1). In HSQC spectrum, δH 4.90 was also confirmed at C-1. This is the evidence that the glucosyl moiety is linked to C-1. Hence, the structure of 3 was determined as 6-hydroxy-3,5-dimethoxy-xanthone-1-O-β-D-glucoside.

Compound 4 was isolated as a yellow powder. Its HRESIMS spectrum exhibited a quasi-molecular ion peak at m/z 495.1499 ([M + H]⁺), which implied a molecular formula of C23H22O12. The UV spectrum with absorption bands at λmax 257, 311 nm was suggestive of a xanthone derivative [19]. The 1H NMR spectrum of 4 (Table 1) exhibited signals of three aromatic protons, in which two of them are doublets at δH 7.56 (1H, d, J = 9 Hz, H-6) and δH 7.36 (1H, d, J = 9 Hz, H-5); while another was a singlet at δH 7.01 (1H, s, H-2) (Table 1). The spectrum also showed signal for four methoxy groups (δH 3.92, 3.86, 3.82, 3.81). The signals of the anomeric proton δH 4.90 (1H, d, J = 6 Hz) substantiated that 4 was a β-O-glucoside. Analysis of the 13C NMR spectrum (Table 1) exhibited thirteen carbon signals at low field, including one carbonyl at δC 175.1, indicating that there is a hydroxyl group located on either C-1 or C-8. A set of six carbon signals at high field, which are δC 103.7, 73.5, 76.2, 70.2, 77.8, and 60.9, implied a β-glucosyl group in the molecule.

In its HMBC spectrum, the singlet at δH 7.01 (H-2) was found correlated with C-8b (δC 107.7), C-4 (δC 130.8), C-9 (δC 175.1), and C-1 (δC 154.3) (Fig. 1). It was observed that the
(5) $R_1 = R_6 = \text{-OH}, R_2 = R_3 = \text{-OCH}_3, R_4 = H, R_5 = \text{-Oglc}$
(6) $R_1 = R_6 = \text{-OH}, R_2 = \text{-OCH}_3, R_3 = R_4 = H, R_5 = \text{-Oglc}$
(7) $R_1 = \text{-OH}, R_2 = R_3 = \text{-OCH}_3, R_4 = R_6 = H, R_5 = \text{-Oglc}$
(8) $R_1 = \text{-Ogluc}, R_2 = R_5 = R_6 = \text{-OCH}_3, R_4 = H$

(9)

(10)

(11) $R_1 = R_2 = R_6 = \text{-OCH}_3, R_3 = R_5 = H, R_4 = \text{-OH}$
(12) $R_1 = R_2 = \text{-OCH}_3, R_3 = R_4 = H, R_5 = R_6 = \text{-OH}$
(13) $R_1 = R_5 = \text{-OCH}_3, R_2 = R_3 = R_4 = H, R_6 = \text{-OH}$
(14) $R_1 = R_5 = R_6 = \text{-OCH}_3, R_2 = R_3 = R_4 = H$
(15) $R_1 = R_6 = \text{-OCH}_3, R_2 = R_3 = R_4 = H, R_5 = \text{-OH}$
(16) $R_1 = \text{-OCH}_3, R_2 = R_3 = R_4 = H, R_5 = R_6 = \text{-OH}$
(17) $R_1 = R_3 = R_6 = \text{-OH}, R_2 = R_4 = R_5 = H$
(18) $R_1 = R_2 = R_6 = \text{-OCH}_3, R_3 = R_4 = H, R_5 = \text{-OH}$
(19) $R_1 = R_2 = R_5 = R_6 = \text{-OCH}_3, R_3 = R_4 = H$
(20) $R_1 = R_6 = \text{-OH}, R_2 = R_3 = R_4 = H, R_5 = \text{-OCH}_3$
(21) $R_1 = \text{-OCH}_3, R_2 = R_3 = R_4 = R_6 = H, R_5 = \text{-OH}$
(22) $R_1 = R_5 = \text{-OH}, R_2 = R_3 = R_4 = R_6 = H$
(23) $R_1 = R_5 = R_6 = \text{-OH}, R_2 = R_3 = R_4 = H$
(24) $R_1 = \text{-OH}, R_2 = R_3 = R_4 = H, R_5 = R_6 = \text{-OCH}_3$
(25) $R_1 = R_2 = \text{-OCH}_3, R_3 = R_6 = \text{-OH}, R_4 = R_5 = H$

*Chart 2. Structures of compounds 5–25 from *Swertia mussotii.*
and 1,5,8-trihydroxy-3,4-dimethoxy xanthone (trihydroxyxanthone) were first isolated from its genera; while compounds were first isolated from its family, and compounds and were first isolated from its polyphenol family. Therefore, screening natural antioxidants from xanthones should be an effective pathway to discover lead compounds.

In the present study, antioxidant capacity of all the isolated xanthones (1–25) was evaluated using the radical absorbance capacity assay (ORACFL) [17,18]. The ORACFL method is based on the inhibition of peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds, which was 2,2′-azo-bis(2-amidino-propane) dihydrochloride (AAPH) in the current investigation. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard reference. Data are expressed as micromoles of Trolox equivalents (TE) per microliter of sample (μmol TE/mL or μL).

As shown in Table 2, compound 1 is the most active antioxidant with ORAC value of 75.8 U/mL when tested at the concentration of 6.25 μM. The great radical scavenging activity of this compound is quite self-evident that it has a catechol moiety. The second most active compound is 10, namely mangiferin, with ORAC value of 66.2 U/mL when tested at the same concentration. Mangiferin is a tetroxygenated xanthone with four free hydroxyls located at C-2, C-3, C-6 and C-8, and a 1-glycoside at C-7; while compound 1 is also a tetroxygenated xanthone with four free hydroxyls located at C-3, C-5, C-6 and C-8 and a 1-glycoside at C-1.

Generally, xanthones with higher oxygenation display better activity than those with less oxygenation. Glycosylated xanthones (1–10) are more active than those that are not (5–25), e.g., 1 is more active than 14, and 5 is more active than 25. Antioxidant capacity of xanthones does not benefit from free hydroxyl group(s) located only at either C-1 or C-8 (see compounds 1, 6, 13), whereas 13 shows the least activity (ORAC value of 5.41 U/mL at tested concentration of 50 μM) amongst all the tested samples although it has two free hydroxyl groups at C-1 and C-8. However, free hydroxyl group(s) at C-2, C-3, C-6, and C-7 will increase the antioxidant activity of xanthones. The reason may probably be ascribed to the existence of intramolecular hydrogen bond with carbonyl at C-9 when free hydroxyl group(s) are located at either C-1 or C-8. Effect of methylation(s) to hydroxyl(s) is equivocal (compared 2 with 4, 15 with 16, 12 with 18, respectively). However, either acetylation to all the four free hydroxyl groups or cinnamoylation of hydroxyl at C-7 of mangiferin resulted in definitive decrease in radical scavenging activity [32].

It is reasonable to conclude that xanthones, as the secondary metabolites of S. musotii, respond to the oxidative stress in the climate of Plateau [34,35]. They are capable of defending against free radicals. Usually, higher antioxidant activity means better biological effect to diseases that are related to oxidation process such as low-density lipoprotein oxidation, platelet aggregation, cancer, and hepatocyte damage [13,36,37]. The current results

Table 2: ORAC values of all the isolated xanthones.*

<table>
<thead>
<tr>
<th>Compd.</th>
<th>ORACFL (μM)</th>
<th>Compd.</th>
<th>ORACFL (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25 μM</td>
<td>3.125 μM</td>
<td>6.25 μM</td>
<td>3.125 μM</td>
</tr>
<tr>
<td>1</td>
<td>75.8 ± 0.8</td>
<td>76.3 ± 0.4</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>30.9 ± 0.9</td>
<td>30.2 ± 0.2</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>31.4 ± 0.3</td>
<td>33.1 ± 0.2</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>29.7 ± 0.3</td>
<td>33.2 ± 0.7</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>38.2 ± 0.3</td>
<td>39.5 ± 0.7</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>47.1 ± 0.3</td>
<td>45.1 ± 0.8</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>53.1 ± 0.4</td>
<td>52.8 ± 0.3</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>20.6 ± 0.7</td>
<td>26.2 ± 0.8</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>71.4 ± 0.2</td>
<td>72.9 ± 0.7</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>66.2 ± 0.8</td>
<td>73.6 ± 0.3</td>
<td>20</td>
</tr>
<tr>
<td>21</td>
<td>24.3 ± 0.1</td>
<td>24.6 ± 0.4</td>
<td>22</td>
</tr>
<tr>
<td>23</td>
<td>37.8 ± 0.1</td>
<td>41.4 ± 0.4</td>
<td>24</td>
</tr>
<tr>
<td>25</td>
<td>19.1 ± 0.3</td>
<td>19.3 ± 0.3</td>
<td>26</td>
</tr>
</tbody>
</table>

* Data expressed as means ± SEM of 3 observations per sample.
offer useful information to further structural modification on xanthones for the purpose of searching lead compounds.

In short, four new xanthones, 3,5,6,8-tetrahydroxyxanthone-1-C-β-D-glucoside (1), 7-hydroxy-3,4,8-trimethoxyxanthone-1-O-((β-D-glucoside) (2), 6-hydroxy-3,5-dimethoxyxanthone-1-O-(β-D-glucoside) (3) and 3,4,7,8-tetramethoxyxanthone-1-O-(β-D-glucoside) (4), together with twenty-one known xanthones (5–25) were isolated from the ethanol aqueous extract of *S. mussotii*. Their structures were elucidated via spectroscopic analyses. Oxygen radical absorbance capacity (ORAC) of all the isolated xanthones was evaluated. The data show that all the tested xanthones have moderate to high antioxidant activity.

Conflict of interest

We state that there is no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2013.08.021.

References