Natural Product Sciences
27(4) : 234-239 (2021)
https://doi.org/10.20307/nps.2021.27.4.234

Xanthone and Flavonoid Derivatives from the Leaves of *Maclura tricuspidata* with Antioxidant and Anti-tyrosinase Activity

Yang Hee Jo, Solip Lee, Se Hwan Ryu, Sang Won Yeon, Ayman Turk, Bang Yeon Hwang, and Mi Kyeong Lee*

College of Pharmacy, Chungbuk National University, Cheongju 28160, Republic of Korea

Abstract – *Masclura tricuspidata,* also called as *Cudrania tricuspidata,* is one of the most common Moraceae family plants in East Asia. Its trivial name follows mulberry due to the similar morphology. Investigation of the bioactive constituents of *M. tricuspidata* leaves yielded a new xanthone derivative along with twenty known compounds through various chromatographic techniques. A new compound was defined as mascluraxanthone (**3**), a prenylated xanthone glucoside on the basis of 1D and 2D NMR and MS data. Twenty known compounds were identified as four xanthone derivatives (**1-2** and **4-5**), two flavans (**6-7**), six flavanol derivatives (**8-13**), a flavonone (**14**) and seven flavonol derivatives (**15-21**). Among the isolated compounds, flavanol and flavonoid derivatives with 3′,4′-OH groups showed antioxidant and anti-tyrosinase activities. Conclusively, the leaves of *M. tricuspidata* are rich in aromatic compounds including xanthones and flavonoids. In addition, these constituents showed antioxidant and anti-tyrosinase potentials, which might be useful for oxidative stress related diseases.
Keywords – *Masclura tricuspidata*, xanthone, flavonoid, masclurxanthone, antioxidant, anti-tyrosinase

Introduction

Masclura tricuspidata (also called as *Cudrania tricuspidata*) is one of the most common plants of Moraceae family in East Asia. All parts of this plant such as roots, fruits, stems and leaves, have been used in traditional medicines due to its diverse beneficial effects. Recent studies also reported anti-inflammatory, anti-cancer, anti-diabetic and hepatoprotective activities.[1-4] Among the parts of *M. tricuspidata*, the leaves have been used as tea and added to functional food ingredients.[5-6]

Due to its beneficial effects of *M. tricuspidata* leaves, we continued to carry out investigations to characterize the bioactive constituents of *M. tricuspidata* leaves. We previously reported the new prenylated isoflavonoids with anti-diabetic activities.[7] Further investigation on *M. tricuspidata* leaves afforded to the isolation of 21 compounds including a new compound through various chromatographic techniques. Using spectral analysis, the structures of the isolated compounds were identified as xanthones and flavonoid derivatives with different moieties. The antioxidant and anti-tyrosinase effects of the isolated compounds were evaluated by measuring the DPPH radical scavenging and inhibitory effect on tyrosinase. In this study, isolation and structure elucidation of compounds were described. In addition, antioxidant and anti-tyrosinase activity were also discussed briefly.

Experimental

General – A Jasco UV-550 and Perkin-Elmer model LE599 spectrometer were used respectively, for the measurement of UV and IR spectra. NMR spectra were recorded on AVANCE 400, 500 and 800 MHz spectrometer using acetone-d_6 and methanol-d_4 as solvents. ESIMS data was obtained on VG Autospec Ultima Mass spectrometers. Semi-preparative HPLC was performed using a Waters 515 HPLC pump with a 996 photodiode array detector, and Waters Empower software using a Gemini-NX ODS-column (150 × 10.0 mm and 150 × 21.2 mm). Column chromatography procedures were performed using silica gel (200-400 mesh, Fisher Scientific) and Sephadex LH-20 (25-100 µm, Pharmacia Fine Chemical Industries Co.). Thin-layer chromatography (TLC) was performed using aluminum plates precoated with Kieselgel 60 F_{254} (0.25 mm, Merck, Germany). After spraying with a color reagent (10% vanillin-H_2SO_4 and 10% H_2SO_4 in EtOH), heating revealed the spots.

Dedicated to Prof. Jinwoong Kim of the Seoul National University for his pioneering works on Pharmacognosy.

*Author for correspondence
Prof. Mi Kyeong Lee Ph.D, College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 28644, Korea.
Tel: +82-43-261-2818; E-mail: mklee@chungbuk.ac.kr

Plant material – The dried leaves of *M. tricuspidata* were purchased from local herbal market, Chungbuk, Korea, in October 2013. A voucher specimen was deposited at the herbarium of College of Pharmacy, Chungbuk National University, Korea (CBNU201310-CTL).

Extraction and isolation of compounds – The dried leaves of *M. tricuspidata* (1.8 kg) were chopped in 100% MeOH (2 × 1 L), and the concentrated extract (102.4 g) was partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH.

The CH_2Cl_2 fraction (15.2 g) was chromatographed over silica gel, having gradient elution of CH_2Cl_2 in MeOH (100 - 0%), that gave 10 fractions (C1 - C10). Fraction C4 was subjected to Sephadex LH-20 with a mixture of CH_2Cl_2-MeOH (1:1) give 5 subfractions (C4A - C4E). Subfraction C4D was subjected to Sephadex LH-20 with a mixture of *n*-hexane-CH_2Cl_2-MeOH (10:10:1) to yield 12 subfractions (C4D1 - C4D12). Compounds **2, 4** and **5** were purified from C4D11 and C4D12, respectively, by semi-preparative HPLC eluting with MeCN-H_2O (55:45).

The EtOAc fraction (4.7 g) was chromatographed over silica gel with the gradient elution of CH_2Cl_2 in MeOH (100 - 0%) which gave 9 fractions (E1 – E9). Fraction E3 was subjected to Sephadex LH-20 with a mixture of CH_2Cl_2-MeOH (1:1) give 6 subfractions (E3A – E3F). Compounds **6**, **8**, **14** and **15** were purified from E3D by semi-preparative HPLC eluting with MeCN-H_2O (25:75). Fraction E4 was subjected to Sephadex LH-20 eluting with CH_2Cl_2-MeOH (1:1) to give 8 subfractions (E4A – E4H). Compounds **1**, **11** and **16** were purified from E4G by semi-preparative HPLC eluting with MeCN-H_2O (25:75). Compound **13** was isolated from E5 by Sephadex LH-20 eluting with CH_2Cl_2-MeOH (1:1) followed by semi-preparative HPLC eluting with MeCN-H_2O (30:70). Compound **3** was purified from E7 by Sephadex LH-20 eluting with CH_2Cl_2-MeOH (1:1) followed by semi-preparative HPLC eluting with MeCN-H_2O (28:72). Sephadex LH-20 chromatography of E8 with CH_2Cl_2-MeOH (1:1) followed by semi-preparative HPLC eluting with MeCN-H_2O (20:80) yielded compounds **7**, **18** and **20.** Fraction E9 was subjected to Sephadex LH-20 with a mixture of CH_2Cl_2-MeOH (1:1) give 4 subfractions (E9A – E9D). Compounds **9**, **10**, **12, 17, 19** and **21** were purified from E9D by semi-preparative HPLC eluting with MeCN-H_2O (20:80).

1,3,5,6-Tetrahydroxyxanthone (1) – brown amorphous powder; UV (MeOH) λ_{max} : 249, 281, 319 nm; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.59 (1H, d, J = 9.0 Hz, H-8), 6.90 (1H, d, J = 8.5 Hz, H-7), 6.45 (1H, brs, H-4), 6.19 (1H, brs, H-2) ppm.

Table 1. ^{1}H NMR and ^{13}C NMR data of compound **3**

Carbon NO.	Compound **3**	
	δ_H [a]	δ_C [b]
1	-	161.0
2	6.28 (1H, s)	97.3
3	-	164.0
4	-	107.3
4a	-	154.5
5	-	146.0
6	7.64 (1H, dd, J = 8.0, 1.6 Hz)	120.5
7	7.34 (1H, t, J = 8.0 Hz)	123.2
8	7.85 (1H, dd, J = 8.0, 1.6 Hz)	117.8
8a	-	121.1
9	-	180.7
9a	-	102.4
10a	-	146.9
11	3.71 (1H, d, J = 5.6 Hz) 3.47 (1H, d, J = 5.6 Hz)	21.1
12	5.41 (1H, t, J = 5.6 Hz)	122.3
13	-	131.1
14	1.90 (3H, s)	17.1
15	1.69 (3H, s)	24.6
Glc-1	5.23 (1H, d, J = 8.0 Hz)	100.9
Glc-2	3.67 (1H, m)	73.6
Glc-3	3.51 (1H, m)	77.0
Glc-4	3.44 (1H, m)	69.9
Glc-5	3.54 (1H, m)	77.0
Glc-6	3.92 (1H, m) 3.73 (1H, m)	61.1

Recorded at [a] 800 MHz, [b] 201 MHz in methanol-d_4.

1,3,5-Trihydroxy-4-prenylxanthone (2) – yellow powder; UV (MeOH) λ_{max} : 214, 243, 322, 363 nm; ^{1}H-NMR (methanol-d_4, 400 MHz) δ_H 7.62 (1H, dd, J = 8.0, 2.0 Hz, H-8), 7.24 (1H, dd, J = 7.6, 1.6 Hz, H-6), 7.18 (1H, t, J = 7.6 Hz, H-7), 6.25 (1H, s, H-2), 5.35 (1H, t, J = 7.6 Hz, H-12), 3.55 (2H, d, J = 7.2 Hz, H-11), 1.88 (3H, s, H-14), 1.68 (3H, s, H-15) ppm.

Mascluraxathone (3) – Yellow syrup; $[\alpha]_D^{25}$ -34.4 (c 0.01, MeOH); UV (MeOH) λ_{max} 239, 258, 317, 359 nm; IR_{max} 3372, 1647, 1425 cm^{-1}; ^{1}H NMR (800 MHz, methanol-d_4) and ^{13}C NMR (201 MHz, methanol-d_4) see Table 1; ESI-MS (positive mode) m/z : 497 $[M+Na]^+$, HREIMS (positive mode) m/z: 497.1418 $[M+Na]^+$ (Calcd for $C_{24}H_{26}NaO_{16}^+$ 497.1424).

Morusignin D (4) – colorless syrup; UV (MeOH) λ_{max} : 243, 319 nm; ^{1}H-NMR (methanol-d_4, 400 MHz) δ_H 7.82 (1H, d, J = 8.8 Hz, H-8), 6.92 (1H, d, J = 8.8 Hz, H-7), 6.45 (1H, s, H-4), 5.27 (1H, t, J = 7.2 Hz, H-12), 3.98

(3H, s, 5-OCH_3), 1.81 (3H, s, H-14), 1.69 (3H, s, H-15) ppm.

6-Deoxyisojacareubin (5) – brown syrup; UV (MeOH) λ_{max} : 251, 269, 329, 378 nm; ESI-MS *m/z* 311 $[M+H]^+$; ^{1}H-NMR (acetone-d_6, 400 MHz) δ_H 13.11 (1H, s, 1-OH), 7.70 (1H, d, J = 8.0 Hz, H-8), 7.41 (1H, d, J = 6.8 Hz, H-6), 7.31 (1H, t, J = 8.0 Hz, H-7), 7.08 (1H, d, J = 10.4 Hz, H-11), 6.22 (1H, s, H-2), 5.78 (1H, d, J = 10.0 Hz, H-12), 1.50 (6H, s, H-14, 15) ppm.

Naringenin (6) – yellow amorphous powder; $[\alpha]_D^{25}$ +54.8 (*c* 0.01, MeOH); UV (MeOH) λ_{max} : 286 nm; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.34 (2H, dd, J = 8.5, 1.5 Hz, H-2', 6'), 6.84 (2H, dd, J = 8.5, 2.0 Hz, H-3', 5'), 5.92 (1H, d, J = 2.5 Hz, H-8), 5.90 (1H, d, J = 2.0 Hz, H-6), 5.37 (1H, dd, J = 13.5, 3.0 Hz, H-2), 3.14 (1H, dd, J = 17.0, 8.0 Hz, H-3a), 2.72 (1H, dd, J = 17.0, 3.0 Hz, H-3b) ppm.

Prunin (7) – light brown syrup; $[\alpha]_D^{25}$ +68.0 (*c* 0.01, MeOH); UV (MeOH) λ_{max} : 212, 282, 322 nm; ^{1}H-NMR (methanol-d_4, 400 MHz) δ_H 7.32 (2H, d, J = 8.8 Hz, H-2', 6'), 6.81 (2H, d, J = 8.8 Hz, H-3', 5'), 6.21 (1H, d, J = 2.0 Hz, H-8), 6.18 (1H, d, J = 2.0 Hz, H-6), 5.38 (1H, dd, J = 12.8, 2.4 Hz, H-2), 4.96 (1H, d, J = 7.2 Hz, Glc-1), 3.88~3.37 (6H, m, Glc-2-6), 3.17 (1H, dd, J = 17.2, 12.8 Hz, H-3a), 2.74 (1H, dd, J = 17.2, 2.8 Hz, H-3b) ppm.

Aromadendrin (8) – yellow powder; UV (MeOH) λ_{max} : 212, 290 nm; ESI-MS *m/z* 287 $[M-H]^-$; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.38 (2H, dd, J = 8.5, 2.0 Hz, H-2', 6'), 6.85 (2H, dd, J = 8.5, 2.0 Hz, H-3', 5'), 5.95 (1H, d, J = 2.5 Hz, H-8), 5.90 (1H, d, J = 2.0 Hz, H-6), 5.00 (1H, d, J = 11.5 Hz, H-2), 4.57 (1H, d, J = 11.5 Hz, H-3) ppm.

(–)-Sinensin (9) – brown syrup; $[\alpha]_D^{25}$ -10.8 (*c* 0.03, MeOH); UV (MeOH) λ_{max} : 215, 284 nm; ESI-MS *m/z* 449 $[M-H]^-$; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.38 (2H, d, J = 8.5 Hz, H-2', 6'), 6.85 (2H, d, J = 8.5 Hz, H-3', 5'), 6.25 (1H, d, J = 2.0 Hz, H-8), 6.22 (1H, d, J = 2.0 Hz, H-6), 5.04 (1H, d, J = 11.5 Hz, H-2), 4.99 (1H, d, J = 7.5 Hz, Glc-1), 4.62 (1H, d, J = 11.5 Hz, H-3), 3.90~3.38 (6H, m, Glc-2-6) ppm.

(+)-Sinensin (10) – brown syrup; $[\alpha]_D^{25}$ +17.7 (*c* 0.04, MeOH); UV (MeOH) λ_{max} : 213, 286 nm; ESI-MS *m/z* 449 $[M-H]^-$; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.38 (2H, d, J = 9.0 Hz, H-2', 6'), 6.86 (2H, d, J = 9.0 Hz, H-3', 5'), 6.26 (1H, d, J = 2.5 Hz, H-8), 6.22 (1H, d, J = 2.0 Hz, H-6), 5.06 (1H, d, J = 12.0 Hz, H-2), 5.01 (1H, d, J = 7.0 Hz, Glc-1), 4.63 (1H, d, J = 11.5 Hz, H-3), 3.91~3.40 (6H, m, Glc-2-6) ppm.

(+)-Taxifolin (11) – brown amorphous powder; $[\alpha]_D^{25}$ +43.6 (*c* 0.01, MeOH); UV (MeOH) λ_{max} : 216, 288 nm; ESI-MS *m/z* 303 $[M-H]^-$; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 6.99 (1H, d, J = 1.5 Hz, H-2'), 6.87 (1H, dd, J = 8.0, 1.5 Hz, H-6'), 6.82 (1H, d, J = 8.0 Hz, H-5'), 5.94 (1H, d, J = 2.0 Hz, H-8), 5.90 (1H, d, J = 1.5 Hz, H-6), 4.93 (1H, d, J = 12.0 Hz, H-2), 4.52 (1H, d, J = 11.5 Hz, H-3) ppm.

Taxifolin-7-*O*-*β*-glucopyranoside (12) – brown syrup; $[\alpha]_D^{25}$ +12.4 (*c* 0.01, MeOH); UV (MeOH) λ_{max} : 284 nm; ^{1}H-NMR (methanol-d_4, 400 MHz) δ_H 6.96 (1H, d, J = 2.0 Hz, H-2'), 6.85 (1H, dd, J = 8.4, 2.0 Hz, H-6'), 6.80 (1H, d, J = 8.0 Hz, H-5'), 6.22 (1H, d, J = 2.0 Hz, H-8), 6.20 (1H, d, J = 2.4 Hz, H-6), 4.96 (1H, d, J = 11.6 Hz, H-2), 4.97 (1H, d, J = 7.2 Hz, Glc-1), 4.56 (1H, d, J = 11.6 Hz, H-3), 3.89~3.33 (6H, m, Glc-2-6) ppm.

Gericudranin C (13) – brown amorphous powder; $[\alpha]_D^{25}$ +113.6 (*c* 0.01, MeOH); UV (MeOH) λ_{max} : 291 nm; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.12 (2H, d, J = 8.5 Hz, H-3'', 7''), 6.98 (1H, d, J = 2.0 Hz, H-2'), 6.86 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 6.82 (1H, d, J = 8.0 Hz, H-5'), 6.64 (2H, d, J = 8.5 Hz, H-4'', 6''), 5.97 (1H, s, H-8), 4.92 (1H, d, J = 11.5 Hz, H-2), 4.52 (1H, d, J = 11.5 Hz, H-3), 3.78 (2H, s, H-1'') ppm.

Apigenin (14) – white amorphous powder; UV (MeOH) λ_{max} : 266, 336 nm; ^{1}H-NMR (methanol-d_4, 400 MHz) δ_H 7.88 (2H, dd, J = 8.8, 2.0 Hz, H-2', 6'), 6.95 (2H, dd, J = 8.8, 2.0 Hz, H-3', 5'), 6.62 (1H, s, H-3), 6.48 (1H, d, J = 2.4 Hz, H-8), 6.23 (1H, d, J = 2.0 Hz, H-6) ppm.

Kaempferol (15) – yellow powder; UV (MeOH) λ_{max} : 264, 363 nm; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 8.11 (2H, d, J = 9.0 Hz, H-2', 6'), 6.93 (2H, d, J = 8.5 Hz, H-3', 5'), 6.42 (1H, d, J = 2.0 Hz, H-8), 6.20 (1H, d, J = 2.0 Hz, H-6) ppm.

Quercetin (16) – yellow amorphous powder; UV (MeOH) λ_{max} : 253, 362 nm; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.76 (1H, d, J = 1.5 Hz, H-2'), 7.66 (1H, dd, J = 8.5, 1.5 Hz, H-6'), 6.90 (1H, d, J = 8.5 Hz, H-5'), 6.41 (1H, d, J = 1.5 Hz, H-8), 6.20 (1H, d, J = 2.0 Hz, H-6) ppm.

Populnin (17) – light yellow amorphous powder; UV (MeOH) λ_{max} : 264, 358 nm; ESI-MS *m/z* 447 $[M-H]^-$; ^{1}H-NMR (acetone-d_6, 500 MHz) δ_H 8.18 (2H, d, J = 9.0 Hz, H-2', 6'), 7.04 (2H, d, J = 9.0 Hz, H-3', 5'), 6.84 (1H, brs, H-8), 6.46 (1H, d, J = 2.0 Hz, H-6), 5.19 (1H, d, J = 7.5 Hz, Glc-1), 3.95~3.48 (6H, m, Glc-2-6) ppm.

Astragalin (18) – light yellow amorphous powder; UV (MeOH) λ_{max} : 263, 340 nm; ESI-MS *m/z* 447 $[M-H]^-$; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 8.07 (2H, d, J = 9.0 Hz, H-2', 6'), 6.91 (2H, d, J = 9.0 Hz, H-3', 5'), 6.41 (1H, brs, H-8), 6.22 (1H, d, J = 1.5 Hz, H-6), 5.27 (1H, d, J = 6.0 Hz, Glc-1), 3.73~3.21 (6H, m, Glc-2-6) ppm.

Quercimeritrin (19) – brown amorphous powder; UV (MeOH) λ_{max} : 253, 370 nm; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.78 (1H, brs, H-2'), 7.68 (1H, d, J = 8.0 Hz, H-6'), 6.91 (1H, d, J = 8.5 Hz, H-5'), 6.76 (1H, d, J = 1.5 Hz, H-8), 6.48 (1H, d, J = 2.0 Hz, H-6), 5.08 (1H, d, J = 7.0 Hz, Glc-1), 3.97~3.43 (6H, m, Glc-2-6) ppm.

Isorhamnetin-7-*O*-*β*-glucoside (20) – light yellow amorphous powder; UV (MeOH) λ_{max} : 253, 366 nm; ^{1}H-NMR (methanol-d_4, 400 MHz) δ_H 7.89 (1H, brs, H-2'), 7.78 (1H, d, J = 8.0 Hz, H-6'), 6.93 (1H, d, J = 8.4 Hz, H-5'), 6.81 (1H, d, J = 2.0 Hz, H-8), 6.47 (1H, d, J = 2.0 Hz, H-6), 5.06 (1H, d, J = 7.6 Hz, Glc-1), 3.94 (3H, s, OCH_3), 3.72~3.39 (6H, m, Glc-2-6) ppm.

Hirsutrin (21) – light yellow amorphous powder; UV (MeOH) λ_{max} : 255, 353 nm; ESI-MS *m/z* 463 [M-H]$^-$; ^{1}H-NMR (methanol-d_4, 500 MHz) δ_H 7.73 (1H, d, J = 2.0 Hz, H-2'), 7.61 (1H, dd, J = 8.5, 2.0 Hz, H-6'), 6.89 (1H, d, J = 8.5 Hz, H-5'), 6.41 (1H, d, J = 1.5 Hz, H-8), 6.22 (1H, d, J = 2.0 Hz, H-6), 5.27 (1H, d, J = 7.5 Hz, Glc-1), 3.75~3.36 (6H, m, Glc-2-6) ppm.

Measurement of DPPH radical scavenging activity – The antioxidant activity was evaluated by measuring the free radical scavenging activity using DPPH as reported previously.[8] In brief, freshly prepared DPPH solution was mixed with the samples. The mixture was reacted at room temperature for 10 minutes, and the absorbance was measured at 550 nm.

Measurement of tyrosinase inhibitory activity – Tyrosinase inhibitory assays were performed using an mushroom tyrosinase.[9] Test samples were mixed with enzyme buffer, and incubated for 5 min at room temperature. Then, tyrosine solution was added and the enzyme reaction was allowed to proceed for 20 min at room temperature. After incubation, the amount of dopachrome formed in the reaction mixture was determined by measuring the absorbance at 490 nm using a microplate reader.

Results and Discussion

Chromatographic separation of CH_2Cl_2 and EtOAc fraction of *M. tricuspidata* resulted in the isolation of a new compounds (**3**) together with twenty known compounds.

Known compounds **1 - 2**, **4 - 21** were identified as four xanthone derivatives, 1,3,5,6-tetrahydroxyxanthone (**1**), 1,3,5-trihydroxy-4-prenylxanthone (**2**), morusignin D (**4**), 6-deoxyisojacareubin (**5**), two flavans, naringenin (**6**),

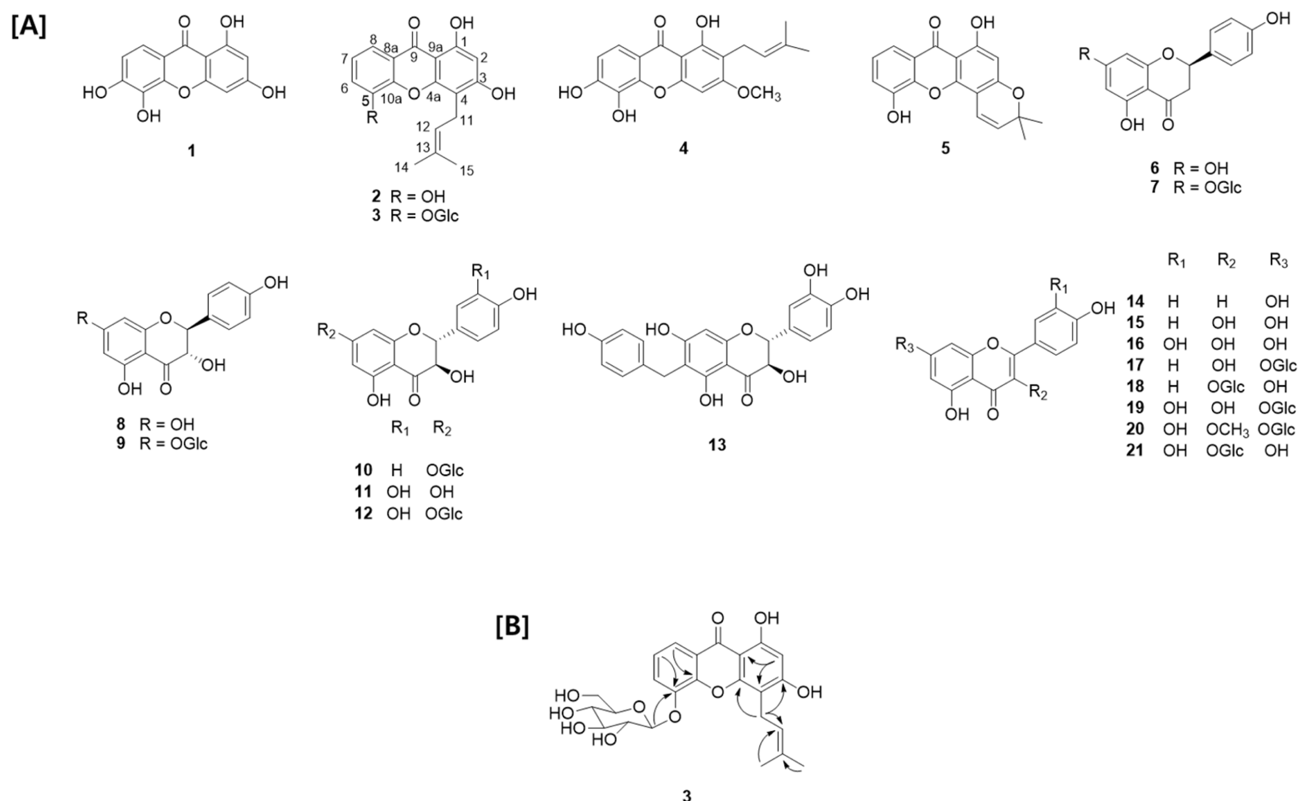

Fig. 1. [A] Chemical structure of compounds **1**-**21** from *M. tricuspidata* leaves and [B] key HMBC correlations of new compound **3**.

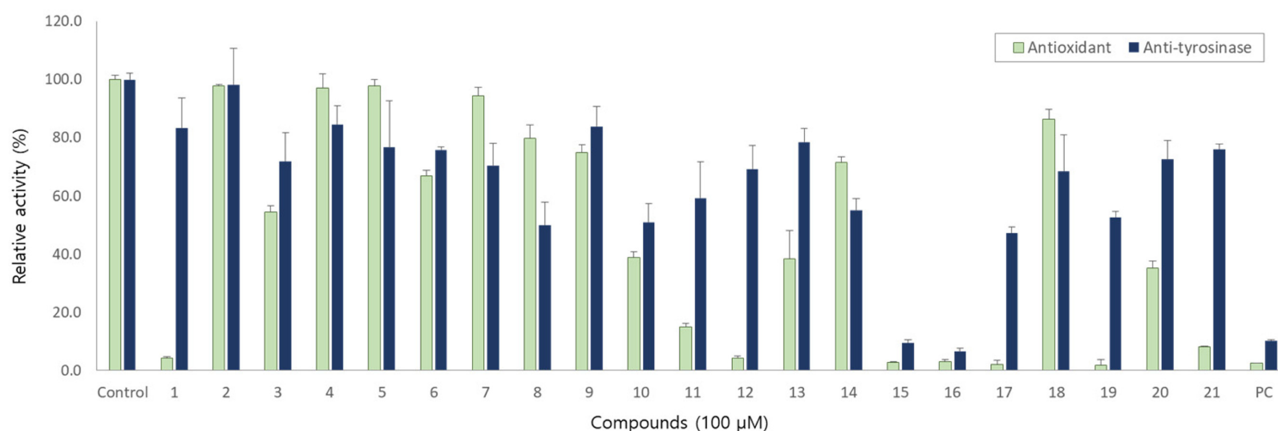

Fig. 2. Antioxidant and anti-tyrosinase activity of the isolated compounds. PC: positive control. Ascorbic acid and arbutin were used as positive controls for antioxidant and anti-tyrosinase, respectively.

prunin (**7**), six flavanols, aromadendrin (**8**), (-)-sinensin (**9**), (+)-sinensin (**10**), (+)-taxifolin (**11**), taxifolin-7-*O*-*β*-glucopyranoside (**12**), gericudranin C (**13**), a flavone, apigenin (**14**), and seven flavonols, kaempferol (**15**), quercetin (**16**), populnin (**17**), astragalin (**18**), quercimeritrin (**19**), isorhamnetin-7-*O*-*β*-glucoside (**20**) and hirsutrin (**21**) (Fig. 1) by the analysis of their spectroscopic data and comparison with literature values.[10-26]

Compound **3** was obtained as yellow syrup and gave HREIMS at 497.1418 (calcd 497.1424), consistent with a molecular formula of $C_{24}H_{26}O_{16}$. The IR spectrum showed the presence of hydroxy (3372 cm^{-1}) and carbonyl (1647 cm^{-1}) groups. The UV spectrum showed characteristic xanthone absorption maxima at 239, 258, 317, 359 nm.[27] The ^{1}H and ^{13}C NMR spectrum showed the presence of a trisubstituted benzene ring at [δ_H 7.64 (1H, dd, J = 8.0, 1.6 Hz, H-6), 7.34 (1H, t, J = 8.0 Hz, H-7), 7.85 (1H, dd, J = 8.0, 1.6 Hz, H-8); δ_C 146.0 (C-5), 120.5 (C-6), 123.2 (C-7), 117.8 (C-8), 121.1 (C-8a), 146.9 (C-10a)] and a pentasubstituted benzene ring at [δ_H 6.28 (1H, s, H-2); δ_C 161.0 (C-1), 97.3 (C-2), 164.0 (C-3), 107.3 (C-4), 154.5 (C-4a), 102.4 (C-9a)]. Additionally, signals for a 3,3-dimethylallyl group at [δ_H 3.71 (1H, d, J = 5.6 Hz, H-11a), 3.47 (1H, d, J = 5.6 Hz, H-11b), 5.41 (1H, t, J = 5.6 Hz, H-12), 1.90 (3H, s, H-14), 1.69 (3H, s, H-15); δ_C 21.1 (C-11), 122.3 (C-12), 131.1 (C-13), 17.1 (C-14), 24.6 (C-15)] and a glucose moiety at [δ_H 5.23 (1H, d, J = 8.0 Hz, Glc-1), 3.67 (1H, m, Glc-2), 3.51 (1H, m, Glc-3), 3.44 (1H, m, Glc-4), 3.54 (1H, m, Glc-5), 3.92 (1H, m, Glc-6), 3.73 (1H, m, Glc-6); δ_C 100.9 (Glc-1), 73.6 (Glc-2), 77.0 (Glc-3), 69.9 (Glc-4), 77.0 (Glc-5), 61.1 (Glc-6)] were observed in the ^{1}H and ^{13}C NMR spectrum together with HSQC spectrum. Besides aforementioned signals, a carbonyl signal was observed at δ_C 180.7 (C-9) in the ^{13}C NMR spectrum. Taken together, compound **3** was suggested to be a xanthone derivative with 3,3-dimethylallyl group and a glucose. The position of the 3,3-dimethylallyl group was determined at C-4 by the HMBC correlations between H-11 and C-3, C-4, C-4a. The HMBC correlations from Glc-1 to C-5 allowed the position of glucose moiety at C-5. On the basis of the obtained data, compound **3** was determined as shown and named mascluraxathone.

The antioxidant and anti-tyrosinase activity of the isolated compounds were evaluated by measuring the DPPH radical scavenging and tyrosinase inhibitory activity (Fig. 2). The isolated compounds can be divided into xanthone derivatives (**1-5**), flavans (**6-7**), five flavanols (**8-13**), a flavone (**14**) and seven flavonols (**15-21**). Overall, the isolated compounds were more efficient for antioxidant than tyrosinase inhibition. Considering the structures, flavonoids were more active than xanthones in our present study. Antioxidant activity of isolated compounds were different depending on the skeletons and its substituents. Consistent with previous study,[28] flavonoids with 3,4-dihydroxy moiety showed excellent antioxidant activity. However, addition of a glucose and methoxyl group reduced the antioxidant activity. Among the isolated compounds, compounds **15** and **16** showed the most potent activity for both antioxidant and anti-tyrosinase inhibition.

Conclusively, 21 compounds including xanthones and flavonoids were isolated from the leaves of *M. tricuspidata*. Among them, a prenylated xanthone glucoside, mascluraxathone (**3**) was not described before. These compounds showed antioxidant and anti-tyrosinase activity. This will provide further insight into the design of anti-

melanogenesis and antioxidant resources from natural products.

Acknowledgement

This work was financially supported by the Research Year of Chungbuk National University in 2020

References

(1) Chang, S. H.; Jung, E. J.; Lim, D. G.; Oyungerel, B.; Lim, K. I.; Her, E.; Choi, W. S.; Jun, M. H.; Choi, K. D.; Han, D. J.; Kim, S. C. *J. Pharm. Pharmacol*. **2008**, *60*, 1221-1226.

(2) Kim, D. H.; Lee, S.; Chung, Y. W.; Kim, B. M.; Kim, H.; Kim, K.; Yang, K. M. *Biomed. Res. Int.* **2016**, *2016*, 8432759.

(3) Lee, B. W.; Lee, J. H.; Lee, S. T.; Lee, H. S.; Lee, W. S.; Jeong, T. S.; Park, K. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5548-5552.

(4) Sunada, Y.; Nakamura, S.; Kamei, C. *Int. Immunopharmacol*. **2008**, *8*, 1761-1766.

(5) Lee, S. B.; Shin, J. S.; Han, H. S.; Lee, H. H.; Park, J. C.; Lee, K. T. *Chem. Biol. Interact*. **2018**, *284*, 101-111.

(6) You, Y.; Min, S.; Lee, Y. H.; Hwang, K.; Jun, W. *Food Chem. Toxicol*. **2017**, *108*, 298-304.

(7) Jo, Y. H.; Lee, S.; Yeon, S. W.; Turk, A.; Lee, J. H.; Hong, S. M.; Han, Y. K.; Lee, K. Y.; Hwang, B. Y.; Kim, S. Y.; Lee, M. K. *Bioorg. Chem*. **2021**, *114*, 105098.

(8) Jiang, D. J.; Hu, G. Y.; Jiang, J. L.; Xiang, H. L.; Deng, H. W.; Li, Y. J. *Bioorg. Med. Chem.* **2003**, *11*, 5171-5177.

(9) Jeong, J. Y.; Liu, Q.; Kim, S. B.; Jo, Y. H.; Mo, E. J.; Yang, H. H.; Song, D. H.; Hwang, B. Y.; Lee, M. K. *Molecules* **2015**, *20*, 8730-8741.

(10) Ahn, J. H.; Jo, Y. H.; Kim, S. B.; Turk, A.; Oh, K. E.; Hwang, B. Y.; Lee, K. Y.; Lee, M. K. *Phytochem. Lett*. **2018**, *26*, 20-24.

(11) Wang, H.; Ye, G.; Ma, C. H.; Tang, Y. H.; Fan, M. S.; Li, Z. X.; Huang, C. G. *J. Pharm. Biomed. Anal.* **2007**, *45*, 793-798.

(12) Fu, M.; Qiu, S. X.; Xu, Y.; Wu, J.; Chen, Y.; Yu, Y.; Xiao, G. *Nat. Prod. Commun.* **2013**, *8*, 1733-1734.

(13) Zhou, G.; Yang, X.; Jiang, Y.; Tu, P. *J. Chin. Pharm. Sci.* **2013**, *22*, 286-288.

(14) Welford, R. W. D.; Clifton, I. J.; Turnbull, J. J.; Wilson, S. C.; Schofield, C. J. *Org. Biomol. Chem.* **2005**, *3*, 3117-3126.

(15) Shimoda, K.; Kubota, N.; Taniuchi, K.; Sato, D.; Nakajima, N.; Hamada, H.; Hamada, H. *Phytochemistry* **2010**, *71*, 201-205.

(16) Jeon, S. H.; Chun, W.; Choi, Y. J.; Kwon, Y. S. *Arch. Pharm. Res.* **2008**, *31*, 978-982.

(17) Pan, H.; Lundgren, L. N. *Phytochemistry* **1996**, *42*, 1185-1189.

(18) Nonaka, G.; Goto, Y.; Kinjo, J.; Nohara, T.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, *35*, 1105-1108.

(19) Foo, L. Y.; Karchesy, J. J. *Phytochemistry* **1989**, *28*, 1237-1240.

(20) Lee, I. K.; Kim, C. J.; Song, K. S.; Kim, H. M.; Koshino, H.; Uramoto, M.; Yoo, I. D. *Phytochemistry* **1996**, *41*, 213-216.

(21) Itoh, T.; Ninomiya, M.; Yasuda, M.; Koshikawa, K.; Deyashiki, Y.; Nozawa, Y.; Akao, Y.; Koketsu, M. *Bioorg. Med. Chem.* **2009**, *17*, 5374-5379.

(22) Xu, J.; Li, X.; Zhang, P.; Li, Z. L.; Wang, Y. *Arch. Pharm. Res.* **2005**, *28*, 395-399.

(23) Lin, S.; Zhu, Q.; Wen, L.; Yang, B.; Jiang, G.; Gao, H.; Chen, F.; Jiang, Y. *Food Chem.* **2014**, *145*, 220-227.

(24) Choi, J. S.; Woo, W. S.; Young, H. S.; Park, J. H. *Arch. Pharm. Res.* **1990**, *13*, 374-378.

(25) El-Ghazooly, M. G.; El-Lakany, A. M.; Abou-Shoer, M. I.; Aly, A. H. *Nat. Prod. Sci.* **2003**, *9*, 213-219.

(26) Wu, T.; Abdulla, R.; Yang, Y.; Aisa, H. A. *Chem. Nat. Compd.* **2008**, *44*, 370-371.

(27) Jo, Y. H.; Shin, B.; Liu, Q.; Lee, K. Y.; Oh, D. C.; Hwang, B. Y.; Lee, M. K. *J. Nat. Prod.* **2014**, *77*, 2361-2366.

(28) Amić, D.; Davidović-Amić, D.; Beslo, D.; Rastija, V.; Lucić, B.; Trinajstić, N. *Curr. Med. Chem*. **2007**, *14*, 827-845.

Received November 23, 2021
Revised December 4, 2021
Accepted December 7, 2021