Chemical Constituents from *Trichosanthis pericarpium*

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Received: 5 August 2013;

Accepted: 10 December 2013;

Published online: 16 July 2014;

AJC-15539

Seventeen compounds were isolated from the *Trichosanthis pericarpium* using silica gel, MCI, Sephadex, reversed-phase C₁₈ column chromatographies and preparative high-performance liquid chromatography (pre-HPLC). Their chemical structures were elucidated on the basis of detailed spectroscopic analysis, including NMR spectroscopy and ESI-MS analysis. Among which, eight compounds were reported from the genus of *Trichosanthes* for the first time.

Keywords: *Trichosanthis pericarpium*, Chemical constituents, Preparative HPLC, NMR.

INTRODUCTION

Trichosanthis pericarpium, known as the peel of Gua Lou, is an important traditional Chinese medicinal herb¹ and grown widely in China, such as Shandong province, Anhui province and other places. It has various therapeutic functions, such as the activities of dilating coronary artery, increasing blood flow, improving the ability of hypoxia, antibacterial and anticancer. In the meantime, whether used separately or together with other herbs, *Trichosanthis pericarpium* has been used for the therapies of cardiovascular diseases effectively^{2,3}.

At home and abroad, the chemical research of Gua Lou mainly focused on the entire fruits and seeds⁴⁻⁷ and there were few investigations related to the systematic study of the peel of Gua Lou, namely *Trichosanthis pericarpium*. Various biological activities led us to investigate the constituents of this plant. Here, we reported the isolation and structural determination of 17 compounds named as: β -sitosterol (1), stigmaterol (2), 4-methoxy-3-hydroxy-benzoic acid (3), quercetin-3-O- α -D-nucleoside (4), vanillic acid-4-O- β -D-glucoside (5), quercetin-3-O- β -D-glucoside (6), chrysosioin-7-O- β -D-glucoside (7), luteolin-7-O- β -D-glucoside (8), adenosine (9), quercetin-3-O-rutinoside (10), vanillic acid (11), luteolin (12), caffeic acid (13), quercetin (14), apigenin (15), chlorogenic acid (16), kaempferol (17). Among which, compounds 3, 4, 5, 6, 12, 13, 15 and 16 were obtained from the genus of *Trichosanthes* for the first time (Fig. 1).

EXPERIMENTAL

Fruits of Gua Lou were collected from Shandong province, China and identified by Professor Zhou Fengqin, Shandong University of Traditional Chinese medicine. After removing pulp and seeds, drying in the shade, the materials were crushed at 60 mesh.

The ¹H and ¹³C NMR spectra were recorded on a Varian 600 MHz NMR spectrometer (Varian, Palo Alto, USA). The ESI-MS data were measured on an Agilent 1100/MSG1946 (Agilent, CA, USA). HPLC was carried out on a Waters Empower system (Milford, MA, USA) coupled with a Model 600 pump, a Model 600 multi-solvent delivery system, a Model 996 diode-array detector (DAD) and an Empower workstation.

Column chromatography was carried out on silica gel 100-200, 200-300 mesh (Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), ODS (Fuji Silysia Chemical Ltd, Fuji Japan) and Sephadex LH-20 (GE Healthcare). Organic solvents including ethanol, petroleum ether, ethyl acetate, chloroform and methanol were all of analytical grade (Guangcheng Chemical Factory, Tianjin, China). Methanol used for HPLC analysis was of chromatographic grade (Tedia Company Inc, Fairfield, USA).

Extraction and separation: The powder of *Trichosanthis pericarpium* (23 Kg) were extracted repeatedly ($\times 3$) with 95 % ethanol. After combined extract concentrated under vacuum, the resulting ethanol extract was suspended in distilled water and extracted with petroleum ether, ethyl acetate and *n*-butanol successively.

Fig. 1. Chemical structures of seventeen compounds from *Trichosanthis pericarpium*

The petroleum ether extract was subjected to silica gel column chromatography with petro-EtOAc as eluent to afford 2 fractions (A, B). Further separation of the two fractions-gave compounds **1** (30.2 mg) and **2** (21.1 mg), respectively.

The ethyl acetate extract was subjected to silica gel column chromatography with petro-EtOAc-MeOH as eluent to afford

3 fractions (C-H). As a result, the subfraction D was further separated by silica gel column chromatography (Petro-EtOAc) and pre-HPLC to afford compound **3** (20.1 mg). Fraction E was subjected to MCI (MeOH-H₂O), purified by Sephadex LH-20 (MeOH) to afford compound **4** (15.2 mg). Fraction F was further subjected to afford compounds **5-9** (16.8, 19.5, 22.1, 17.7,

23.1 mg), by reversed-phase C₁₈, MCI (MeOH-H₂O) and Sephadex LH-20 column chromatography (MeOH).

The *n*-butanol extract was suspended in distilled water and subjected to macroporous adsorption resin (D101) column chromatography with ethanol-water gradient elution to afford 3 fractions (I, J, K). Fraction I was further subjected to reversed-phase C₁₈ (MeOH-H₂O gradient elution) to give 4 fractions (H1-H4). These four fractions were further purified by pre-HPLC (MeOH:H₂O 4:6) to afford compounds **5** (25.5 mg), **9** (15.3 mg), **10** (21.4 mg). Fraction J was repeatedly subjected to silica gel and polyamide column chromatography (CHCl₃: MeOH 100:1, 80:1, 50:1, 30:1 10:1, 5:1, 1:1) to give 7 fractions (J1-J7). All of these fractions were further purified to afford compounds **11-14** (15.2, 16.4, 15.3, and 17.1 mg) by silica gel, reversed-phase C₁₈, MCI, Sephadex LH-20 column chromatography and preparative HPLC. Fraction K was further subjected to afford compounds **15-17** (23.2 mg, 18.2 mg, 19.5 mg) by the aforementioned methods.

Compound 1 (β-sitosterol): White needle crystal, ESI-MS (*m/z*): 415.1 [M + H]⁺, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 5.37 (1H, m, H-6), 3.48 (1H, m, H-3), 1.02 (3H, s, 19-CH₃), 0.68 (3H, s, 18-CH₃), 0.94 (3H, d, *J* = 6.6 Hz, 21-CH₃). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 36.51 (C-1), 29.72 (C-2), 72.60 (C-3), 40.79 (C-4), 140.89 (C-5), 122.62 (C-6), 32.31 (C-7), 32.38 (C-8), 50.72 (C-9), 36.79 (C-10), 20.41 (C-11), 37.87 (C-12), 42.91 (C-13), 58.13 (C-14), 23.53 (C-15), 26.62 (C-16), 56.73 (C-17), 12.84 (C-18), 19.48 (C-19), 34.55 (C-20), 19.21 (C-21), 32.55 (C-22), 24.96 (C-23), 46.20 (C-24), 28.83 (C-25), 19.84 (C-26), 19.83 (C-27), 21.72 (C-28), 12.61 (C-29).

Compound 2 (stigmasterol): White needle crystal, ESI-MS (*m/z*): 411.4 [M-H]⁻, ¹H NMR (600 MHz, CDCl₃) δ: 0.68 (3H, s, 18-CH₃), 0.77 (3H, d, *J* = 7.2 Hz, 27-CH₃), 0.83 (3H, d, *J* = 5.6 Hz, 26-CH₃), 0.78 (3H, s, 19-CH₃), 0.81 (3H, m, 29-CH₃), 1.01 (3H, d, *J* = 7.4 Hz, H-21), 3.50 (1H, m, H-3α), 4.98 (1H, dd, *J* = 8.5, 15.2 Hz, H-23), 5.13 (1H, m, H-22), 5.30 (1H, d, *J* = 4.5 Hz, H-6). ¹³C NMR (150 MHz, CDCl₃) δ: 37.2 (C-1), 31.8 (C-2), 71.8 (C-3), 41.3 (C-4), 140.7 (C-5), 121.6 (C-6), 31.8 (C-7), 31.9 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.5 (C-12), 42.3 (C-13), 56.4 (C-14), 24.3 (C-15), 28.9 (C-16), 56.4 (C-17), 12.1 (C-18), 19.5 (C-19), 40.6 (C-20), 21.2 (C-21), 138.3 (C-22), 129.3 (C-23), 51.3 (C-24), 31.8 (C-25), 19.0 (C-26), 21.3 (C-27), 25.5 (C-28), 12.2 (C-29).

Compound 3 (4-methoxy-3-hydroxy-benzoic acid): White flake crystal. ESI-MS (*m/z*): 167.1 [M-H]⁻, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 7.56 (2H, brs, H-2, H-6), 6.85 (1H, d, *J* = 7.2 Hz, H-5), 3.89 (3H, s, -OCH₃). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 168.70 (-COOH), 151.18 (C-4), 147.20 (C-3), 123.82 (C-6), 121.74 (C-1), 114.38 (C-2), 112.33 (C-5), 54.95 (-OCH₃).

Compound 4 (quercetin-3-O-α-D-nucleoside): Yellow powder. ESI-MS (*m/z*): 433.4 [M-H]⁻, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 12.65 (1H, s, H-5'), 7.67 (1H, d, *J* = 7.8 Hz, H-2'), 7.52 (1H, brs, H-6'), 6.85 (1H, d, *J* = 7.8 Hz, H-5'), 6.39 (1H, brs, H-8), 6.18 (1H, s, H-6), 5.28 (1H, d, *J* = 5.4 Hz, H-1''). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 177.85 (C-4), 156.54 (C-2), 134.07 (C-3), 161.61 (C-5), 99.32 (C-6), 165.49 (C-7), 94.04 (C-8), 156.54 (C-9), 104.02 (C-10), 121.24 (C-1'), 115.83 (C-2'), 145.51 (C-3'), 149.20 (C-4'), 116.16 (C-5'),

122.42 (C-6'), 101.86 (C-1''), 72.11 (C-2''), 71.15 (C-3''), 66.55 (C-4''), 64.75 (C-5'').

Compound 5 (vanillic acid-4-O-β-D-glucoside): White amorphous powder. ESI-MS (*m/z*): 329.2 [M-H]⁻, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 7.64 (1H, d, *J* = 8.4 Hz, H-6), 7.60 (1H, s, H-2), 7.20 (1H, d, *J* = 8.4 Hz, H-5), 3.89 (3H, s, -OCH₃), 5.04 (1H, d, *J* = 7.2 Hz, H-1''); ¹³C NMR (150 MHz, CD₃OD) δ: 124.67 (C-1), 112.88 (C-2), 148.86 (C-3), 150.41 (C-4), 114.90 (C-5), 123.32 (C-6), 168.18 (C-7), 55.21 (-OCH₃), 100.49 (C-1'), 76.85 (C-2'), 76.40 (C-3'), 73.32 (C-4'), 69.80 (C-5'), 60.99 (C-6').

Compound 6 (quercetin-3-O-β-D-glucoside): Light yellow powder. ESI-MS (*m/z*): 463.5 [M-H]⁻, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 12.65 (1H, s, H-5), 7.59 (1H, brs, H-6'), 7.58 (1H, brs, H-2'), 6.86 (1H, d, *J* = 6.6 Hz, H-5'), 6.39 (1H, brs, H-8), 6.19 (1H, brs, H-6), 5.48 (1H, *J* = 7.2 Hz, H-1'). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 156.46 (C-2), 133.66 (C-3), 177.74 (C-4), 161.62 (s, C-5), 99.32 (C-6), 165.46 (C-7), 94.04 (C-8), 156.81 (C-9), 104.07 (C-10), 121.99 (C-1'), 115.66 (C-2'), 145.31 (C-3'), 149.03 (C-4'), 116.60 (C-5'), 121.52 (C-6'), 104.07 (C-1''), 74.52 (C-2''), 76.93 (C-3''), 77.98 (C-4''), 70.34 (C-5''), 61.4 (C-6'').

Compound 7 (chrysoeriol-7-O-β-D-glucoside): Light yellow powder. ESI-MS (*m/z*): 461.2 [M-H]⁻, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 7.60 (1H, H-6'), 7.59 (1H, H-5'), 6.95 (1H, d, *J* = 7.8 Hz, H-2'), 6.99 (1H, s, H-3), 6.87 (1H, s, H-6), 6.45 (1H, brs, H-8), 6.87 (1H, brs, H-6), 5.07 (1H, d, *J* = 7.8 Hz, H-1''), 3.90 (3H, s, 3'-OCH₃). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 164.60 (C-2), 105.78 (C-3), 182.48 (C-4), 157.36 (C-5), 99.93 (C-6), 163.41 (C-7), 95.46 (C-8), 161.54 (C-9), 103.82 (C-10), 120.98 (C-1'), 110.73 (C-2'), 151.36 (C-3'), 148.54 (C-4'), 116.25 (C-5'), 121.78 (C-6'), 56.40 (-OCH₃).

Compound 8 (luteolin-7-O-β-D-glucoside): Yellow powder. ESI-MS (*m/z*): 447.5 [M-H]⁻, 471.5 [M + Na]⁺, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 7.44 (1H, d, *J* = 8.4 Hz, H-6'), 7.41 (1H, brs, H-2'), 6.87 (1H, brs, H-5), 6.79 (1H, s, H-8), 6.73 (1H, s, H-3), 6.43 (1H, d, *J* = 1.8 Hz, H-6), 5.09 (1H, d, *J* = 7.2 Hz, H-1''). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 182.21 (C-4), 163.35 (C-2), 103.13 (C-3), 161.55 (C-5), 100.28 (C-6), 165.10 (C-7), 95.09 (C-8), 157.34 (C-9), 105.72 (C-10), 121.43 (C-1'), 113.55 (C-2'), 146.63 (C-3'), 150.10 (C-4'), 116.40 (C-5'), 119.73 (C-6'), 99.89 (C-1''), 73.54 (C-2''), 76.82 (C-3''), 69.96 (C-4''), 77.56 (C-5''), 61.02 (C-6'').

Compound 9 (adenosine): Colourless needle crystal. ESI-MS (*m/z*): 268.2 [M + H]⁺, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 8.35 (1H, s, H-2), 8.14 (1H, s, H-8), 7.35 (2H, s, -NH₂), 5.88 (1H, d, *J* = 6.4 Hz, H-1'), 5.42 (2H, m, H-5'). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 156.33 (C-6), 152.84 (C-2), 149.27 (C-4), 140.51 (C-8), 119.53 (C-5), 88.37 (C-1'), 86.30 (C-4'), 73.91 (C-2'), 71.02 (C-3'), 62.01 (C-5').

Compound 10 (quercetin-3-O-rutinoside): Yellow amorphous powder. ESI-MS (*m/z*): 611.2 [M + H]⁺, ¹H NMR (600 MHz, DMSO-*d*₆) δ: 7.54 (H, d, *J* = 8.4 Hz, H-6'), 7.53 (H, d, *J* = 6.8 Hz, H-2'), 6.85 (1H, d, *J* = 8.4 Hz, H-5'), 6.37 (1H, s, H-8), 6.18 (1H, s, H-6), 5.35 (1H, d, *J* = 7.2 Hz, H-1''), 4.39 (1H, d, *J* = 1.5 Hz, H-1), 1.00 (3H, d, *J* = 6.6 Hz, 5-CH₃). ¹³C NMR (150 MHz, DMSO-*d*₆) δ: 177.67 (C-4), 161.68 (C-5), 156.93 (C-9), 156.89 (C-2), 148.94 (C-3'), 145.21 (C-4'), 133.67 (C-3), 122.00 (C-6'), 121.51 (C-1'), 116.62 (C-5'),

115.64 (C-2'), 104.13 (C-1''), 101.67 (C-1'''), 99.28 (C-6), 94.10 (C-8), 76.86 (C-3''), 76.30 (C-5''), 74.49 (C-2''), 72.25 (C-4'''), 70.96 (C-3'''), 70.78 (C-2'''), 70.39 (C-4''), 68.66 (C-5'''), 67.40 (C-6''), 18.17 (-CH₃).

Compound 11 (vanillic acid): White flake crystal. ESI-MS m/z : 167.1 [M-H]⁻. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 7.45 (1H, s, H-2), 7.44 (1H, s, H-6), 6.85 (1H, d, J = 7.8 Hz, H-5), 3.81 (3H, s, 3-OCH₃). ¹³C NMR (150 MHz, DMSO-*d*₆) δ : 167.64 (-COOH), 150.51 (C-4), 147.63 (C-3), 123.90 (C-6), 122.01 (C-1), 115.44 (C-2), 113.10 (C-5), 55.95 (-OCH₃).

Compound 12 (luteolin): Yellow amorphous powder. ESI-MS m/z : 287.2 [M + H]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 12.98 (1H, s, 5-OH), 7.41 (1H, dd, J = 8.4, 2.4 Hz, H-6'), 7.39 (1H, d, J = 2.4 Hz, H-2'), 6.89 (1H, d, J = 8.4 Hz, H-5'), 6.67 (1H, s, H-3), 6.44 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6). ¹³C NMR (150 MHz, DMSO-*d*₆) δ : 182.09 (C-4), 164.56 (C-7), 164.31 (C-2), 161.90 (C-5), 157.71 (C-9), 150.13 (C-3'), 146.16 (C-4'), 121.91 (C-1'), 119.42 (C-6'), 116.43 (C-2'), 113.79 (C-5'), 104.11 (C-10), 103.29 (C-3), 99.25 (C-6), 94.27 (C-8).

Compound 13 (caffeic acid): Light yellow powder. ESI-MS m/z : 180.8 [M + H]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 12.13 (-COOH), 9.55 (-OH), 9.14 (-OH), 7.43 (1H, d, J = 16.2 Hz, H-7), 7.02 (1H, d, J = 1.8 Hz, H-2), 6.97 (1H, dd, J = 1.8, 7.8 Hz, H-6), 6.76 (1H, d, J = 7.8 Hz, H-5), 6.18 (1H, d, J = 16.2 Hz, H-8). ¹³C NMR (150 MHz, DMSO-*d*₆) δ : 168.0 (C-9), 148.4 (C-4), 145.7 (C-3), 144.9 (C-7), 125.7 (C-1), 121.4 (C-6), 115.8 (C-8), 115.4 (C-5), 115.1 (C-2).

Compound 14 (quercetin): Yellow granular crystal. ESI-MS (m/z): 302 [M]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 12.50 (1H, s, 5-OH), 10.79 (1H, s, 7-OH), 9.60 (1H, s, 4'-OH), 9.37 (1H, s, 3-OH), 9.32 (1H, s, 3'-OH), 6.18 (1H, d, J = 2.4 Hz, H-6), 6.40 (1H, d, J = 1.8 Hz, H-8), 7.67 (1H, d, J = 2.4 Hz, H-2'), 6.89 (1H, d, J = 8.4 Hz, H-5'), 7.54 (1H, dd, J = 8.4, 2.4 Hz, H-6'). ¹³C NMR (150 MHz, DMSO-*d*₆) δ : 156.9 (C-2), 134.1 (C-3), 178.2 (C-4), 162.1 (C-5), 99.8 (C-6), 164.9 (C-7), 93.8 (C-8), 156.6 (C-9), 104.3 (C-10), 120.7 (C-1'), 108.9 (C-2'), 145.7 (C-3'), 137.3 (C-4'), 146.3 (C-5'), 109.2 (C-6'), 101.1 (C-1''), 71.3 (C-2''), 76.9 (C-3''), 70.5 (C-4''), 78.2 (C-5''), 61.3 (C-6'').

Compound 15 (apigenin): Yellow powder. ESI-MS m/z : 270 [M]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 12.96 (1H, s, 5-OH), 10.82 (1H, s, 7-OH), 10.35 (1H, s, 4'-OH), 7.93 (2H, d, J = 7.8 Hz, H-2', 6'), 6.93 (2H, d, J = 8.4 Hz, H-3', 5'), 6.78 (1H, s, H-3), 6.48 (1H, d, J = 1.8 Hz, H-8), 6.19 (1H, d, J = 1.8 Hz, H-6). ¹³C NMR (150 MHz, DMSO-*d*₆) δ : 182.19 (C-4), 164.56 (C-7), 164.17 (C-2), 161.89 (C-4'), 161.60 (C-5), 157.74 (C-9), 128.92 (C-2', 6'), 121.61 (C-1'), 116.39 (C-3', 5'), 104.13 (C-10), 103.27 (C-3), 99.26 (C-6), 94.39 (C-8).

Compound 16 (chlorogenic acid): White powder. ESI-MS (m/z): 352.9 [M-H]⁻. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 12.40 (-COOH), 9.58, 9.14 (Ar-OH), 7.43 (1H, d, J = 16.2 Hz, H-7'), 7.03 (1H, d, J = 1.8 Hz, H-2'), 6.99 (1H, dd, J = 8.4 Hz, 1.8 Hz, H-6'), 6.77 (1H, d, J = 7.8 Hz, H-5'), 6.16 (1H, d, J = 16.2 Hz, H-8'), 5.53 (1H, m, H-3), 3.92 (1H, m, H-3), 3.56 (1H, m, H-4), 2.02-1.76 (4H, m, H-2, H-6). ¹³C NMR (150 MHz, DMSO-*d*₆) δ : 175.34 (C-7), 166.14 (C-9), 148.76 (C-4'), 145.99 (C-7'), 145.36 (C-3'), 126.01 (C-1'), 121.77 (C-6'), 116.15 (C-5'), 115.20 (C-2'), 114.70 (C-8'), 73.87 (C-3), 37.62 (C-6), 36.61 (C-2).

Compound 17 (kaempferol): Yellow amorphous powder. ESI-MS m/z : 285.2 [M-H]⁻. ¹H NMR (600 MHz, DMSO-*d*₆) δ : 12.48 (1H, s, 5-OH), 10.79 (1H, s, 7-OH), 10.11 (1H, s, 4'-OH), 9.41 (1H, s, 3-OH), 8.05 (2H, d, J = 9.0 Hz, H-2', 6'), 6.93 (2H, d, J = 8.4 Hz, H-3', 5'), 6.44 (1H, d, J = 1.8 Hz, H-8), 6.19 (1H, d, J = 1.8 Hz, H-6). ¹³C NMR (150 MHz, DMSO-*d*₆) δ : 176.34 (C-4), 164.31 (C-7), 161.14 (C-5), 159.63 (C-4'), 156.60 (C-9), 147.24 (C-2), 136.09 (C-3), 129.94 (C-2', 6'), 122.09 (C-1'), 115.87 (C-3', 5'), 103.47 (C-10), 98.63 (C-6), 93.91 (C-8).

RESULTS AND DISCUSSION

Compound **3** was obtained as a white flake crystal. The ESI-MS of **3** had a molecular ion peak at m/z 167.1 [M-H]⁻, consistent with the m.f. C₈H₈O₄. From the ¹H NMR spectral data, δ_{H} 3.89 which was a single peak of three protons, was inferred to be a methoxy proton signal. Combined with ¹³C NMR spectral data⁸, compound **3** was identified as 4-methoxy-3-hydroxy-benzoic acid.

Compound **4** was isolated as a yellow powder. The ESI-MS (m/z) of compound **4** showed a molecular ion at m/z 434 corresponding to a m.f. C₂₀H₁₈O₁₁. The ¹H NMR spectrum of **4** contained a typical ABX aromatic proton system at δ_{H} 7.67 (1H, d, J = 7.8 Hz), 7.52 (1H, brs), 6.85 (1H, d, J = 7.8 Hz), indicating that B ring was substituted at the position of 3', 4' and δ_{H} 6.39, 6.18 were the H-8, H-6 protons signals of A ring respectively. ¹³C NMR signals displayed a five-carbon sugar in the structure. D-ribose was detected after acid hydrolysis. Furthermore, the NMR data can confirm that the D-ribose was linked at the 3rd carbon of aglycone and the D-ribose was determined to be a configuration according to the coupling constant. Compound **4** was established as quercetin-3-O- α -D-ribofuranoside⁹.

Compound **5** was isolated as a white amorphous powder. The ESI-MS of **5** had a molecular ion at m/z 329.2 [M-H]⁻, consistent with the m.f. C₁₄H₁₈O₉. From the ¹H NMR spectral data, δ_{H} 3.89 was methoxy proton signal. In addition, D-glucose was detected after acid hydrolysis. The above ¹H and ¹³C NMR spectral data was consistent with the data of vanillic acid-4-O- β -D-glucoside reported in the literature¹⁰. Thus, Compound **5** was identified as vanillic acid-4-O- β -D-glucoside.

Compound **6** was obtained as a light yellow powder. The ESI-MS of **6** had a molecular ion at m/z 463.5 [M-H]⁻, consistent with the m.f. C₂₁H₂₀O₁₂. Based on the data of ¹H and ¹³C NMR spectral, the structure of aglycone was determined as quercetin. ¹³C NMR signals ascertained that glycosides linked at the 3rd carbon of aglycone. Furthermore, all the data was consistent with the data of quercetin-3-O- β -D-glucopyranoside reported in the literature¹¹. Thus, Compound **6** was elucidated to be quercetin-3-O- β -D-glucopyranoside.

Compound **12** was obtained as a yellow amorphous powder. The ESI-MS of **12** had a molecular ion at m/z 287.2 [M + H]⁺, consistent with the m.f. C₁₅H₁₀O₆. All of the ¹H and ¹³C-NMR spectral data were consistent with the data of luteolin reported in the literature¹². Thus, compound **12** was deduced to be luteolin.

Compound **13** was isolated as a light yellow powder. As shown in mass spectrum, [M + H]⁺ appeared as the most abundant ion at m/z = 181 corresponding to a m.f. C₉H₈O₄. It

was identified as caffeic acid on the basis of its ^1H and ^{13}C NMR spectral data¹³.

Compound **15** was isolated as a yellow powder. The ESI-MS (m/z) of compound **15** showed a molecular ion at m/z 270 corresponding to a m.f. $\text{C}_{15}\text{H}_{10}\text{O}_5$. According to the ^1H NMR spectral data, δ_{H} 7.93 (2H, d, $J = 7.8$ Hz) and 6.93 (2H, d, $J = 8.4$ Hz) indicated that B ring of the para-substituted form and δ_{H} 6.48 (1H, d, $J = 1.8$ Hz), 6.19 (1H, d, $J = 1.8$ Hz) were the H-8, H-6 protons signals of A ring respectively. Compound **15** was identified as apigenin by comparison of its ^1H and ^{13}C NMR spectral data to the literature¹².

Compound **16** was obtained as a white powder. The ESI-MS of **16** had a molecular ion at m/z 352.9 $[\text{M}-\text{H}]^-$, consistent with the m.f. $\text{C}_{16}\text{H}_{18}\text{O}_9$. By means of comparison of its ^1H and ^{13}C NMR spectral data to the literature¹³, compound **16** was deduced to be chlorogenic acid.

ACKNOWLEDGEMENTS

Financial supports from the Impotent National Science and Technology Specific Projects (2011BAI06B06) are gratefully acknowledged.

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