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Full Length Research Paper

Isolation and structural elucidation of chemical constituents of *Mussaenda hainanensis* Merr.

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To study the chemical constituents of *Mussaenda hainanensis* Merr. and also researched the EtOAc, *n*-BuOH and H_2O extract of 70% EtOH extract on DPPH radicals scavenging effect. The compounds were isolated by Diaion HP-20, Sephadex LH-20, Toyopearl HW-40 and Si gel column chromatography methods. Their structures were identified by physicochemical properties and spectroscopic analysis. Twelve compounds were elucidated as pinostrobin(1), quercetin(2), 8-O-acetyl-shanzhiside methyl ester(3), 8[E]-N-[2'-hydroxyl-tetracosan- cosoyl]-1-O-*β*- D-glucopyranosy1-8-en-octadecas-phingenine(4), caffeic acid (5), quercetin-3-O-*β*-D-glucosid(6), quercetin-7-O-*β*-D- glucoside(7), 3*β*-O-*β*-D-gluco-pyranosyl pomolic acid 28-O-*β*-D-glucopyranosyl ester(8), 3,4-di-*o*-caffeoylquinic acid(9), chlorogenic acid(10), lamalbid(11), shanzhiside methyl ester(12). The compounds were isolated from the plant for the first time. All the parts of the 70% EtOH extract and compounds 1 ~ 3, 5~7, 9~12 have the ability to scavenge DPPH free radical. The results provide evidence for the further research of its efficacy and its further development and utilization.

Key words: *Mussaenda hainanensis* Merr., chemical constituents, DPPH, antioxidant activity.

INTRODUCTION

Mussaenda hainanensis Merr. (Rubiaceae) is a kind of climbing shrub with hairy branches that has attractive flowers, distributed in shady hillside, valley and shrub jungle. It grows in Hainan Province of China only. *Mussaenda pubescens* Ait.f is a liana-like shrub, distributed in east, south and southwest China, such as Fujian, Guangdong, Guangxi, Yunnan, Sichuan, Guizhou and other provinces, rattan and root for medicine, can be collected throughout the year, fresh or dry cleaned, chopped standby. *M. pubescens* Ait.f. is a Chinese folk medicine commonly used removal of heat, removal of moisture, detoxify mushroom poisoning, activating the blood (Xu et al., 1992).

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In the present investigation, Ren-sheng XU researcher and other people from Shanghai Institute of Materia Medica Chinese Academy of Sciences have reported the isolation and structural determination of several saponins and iridoids (Zhao et al., 1995, 1996, 1997) to *M. pubescens* Ait.f (Rubiaceae) collected from Zhaoqing city of Guangdong Province, China, and also contains phenolics, amino acids, organic acids, polysaccharides and so on. However, *M. hainanensis* Merr. of Hainan unique plants, there is no reported on its chemical composition.

In order to search for the material basis of the effective and better clarify the effects of medicinal, for the rational development and utilization of resources of medicinal plants to provide a scientific basis, in this paper, 70% ethanol extract of *M. hainanensis* Merr. were isolated, and determined the structure of twelve compounds, take

turns as pinostrobin (1), quercetin (2), 8-O-acetyl-shanzhiside methyl ester (3), 8[E]-N-[2'-hydroxyl-tetracosan-cosoyl]-1-O-*β*-D-glucopyranosy1-8-en-octadecas-phingenine(4),Caffeicacid(5),quercetin-3-O-*β*-D-glucoside(6),quercetin-7-O-*β*-D-glucoside(7),3*β*-O-*β*-D-gluco-pyranosyl pomolic acid 28-O-*β*-D-glucopyranosyl ester (8), 3,4-di-*o*-caffeoylquinicacid (9), chlorogenic acid(10) , lamalbid (11) , shanzhiside methyl ester (12). The compounds 1~12 were isolated from *M. hainanensis* Merr. for the first time.

MATERIALS AND METHODS

General experimental procedures

Melting points were determined with a WRS-1B melting point apparatus and uncorrected. 1D, 2D NMR spectra data were measured on a Bruker DPX 400 NMR spectrometer with TMS used as internal Standard (400 MHz for ^{1}HNMR and 100 MHz for ^{13}CNMR), the chemical shift values are reported in ppm (δ) units and the scalar coupling constants (*J*) are in Hz. ESI-MS were obtained using Bruker Daltonics mass spectrometer, respectively. Concentration device of vacuum thin film (Yuan et al., 2005). Rotary evaporator (Shanghai Yarong biology technology Ltd).The silica gel GF_{254} were used for thin layer chromatography (TLC) and silica gel G (200-300 mesh) were used for column chromatography (Qingdao Marine Chemical Inc., China). Sephadex LH-20 were used for column chromatography (Pharmacia Bioteck Inc.), Diaion HP-20, MCI gel CHP-20P and Toyopearl HW-40 were used for column chromatography (Mitsubishi Chemical Industry Ltd). Infinite M 200 Enzyme Microplate Reader (Switzerland Tecan), UV-2102 PCS (Unico, Shanghai Instrument Co., Ltd), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 6-hydroxy-2,5,7,8- tetramethychroman-2-carboxylic (Trolox) (purchased in sigma Co.), all the other reagent are analytical grade reagents.

Plant material

The air-dried parts of *M. hainanensis* Merr. were collected from Sanya city, Hainan province, China, in September, 2007, and identified by Prof. Shi-man HUANG of Hainan University. A voucher specimen of this plant has been deposited in our laboratory.

Extraction and isolation

The air-dried and powdered of the plant (10 kg) were extracted eight times with 70% EtOH at room temperature for 5 days by percolation. The extract was concentrated by vacuum thin film below 50℃. Then extract after concentration were dissolved in H_2O and extracted with petroleum ether, EtOAc and *n*-BuOH, successively, the petroleum ether, EtOAc, *n*-BuOH and H_2O layer was concentrated by vacuum thin film below 50℃, respectively.

The condensate of EtOAc layer was dried to give residue (80 g), which of 40 g was subjected on Si gel column chromatography, eluting with petroleum ether-EtOAc and EtOAc-MeOH of a volume ratio gradient, 21 fractions were collected. The fraction 2 was further subjected to Si gel column chromatography, eluting with petroleum ether-EtOAc of a volume ratio gradient, eventually yielded compounds 1 (86 mg), compounds 2 (56 mg). The fraction 16 was further subjected to Sephadex LH-20 column chromatography, eluting with $CHCl_3$-MeOH of a volume ratio gradient, eventually yielded compounds 3 (18 mg), compounds 4 (20 mg) and compounds 5 (26 mg).

The condensate of *n*-BuOH layer was dried to give residue (110 g), which of 60 g was subjected on Diaion HP-20 resin by column chromatography with a successive elution system of H_2O, 10% MeOH, 20% MeOH, 40% MeOH, 60%MeOH and 70% Me_2CO.

The fraction of 10% MeOH from resin were subjected on MCI gel CHP-20P and Toyopearl HW-40 column chromatography respectively, eluting with H_2O, H_2O-MeOH of a volume ratio gradient, Eventually yielded compounds 7 (16 mg), compounds 8 (150 mg), compounds 11 (136 mg) and compounds 12 (126 mg).

The fraction of 40% MeOH from resin were subjected on MCI gel CHP-20P and Toyopearl HW-40 column chromatography respectively, eluting with H_2O, H_2O-MeOH of a volume ratio gradient, Eventually yielded compounds 6 (25 mg), compounds 9 (16 mg) and compounds 10 (35 mg) Figure 1.

Determination of scavenging activity on DPPH free radical

Take EtOAc, *n*-BuOH and H_2O extract of 70% EtOH extract and compounds 1~3, 5~7, 9~12 respectively, dilute them with anhydrous ethanol and settle it to the volumetric flask of 25 ml. Precisely weigh DPPH and dissolve it by anhydrous ethanol. The constant volume is 100 ml precisely weigh Trolox and dissolve it by anhydrous ethanol. The constant volume is 25 ml.

DPPH assay (Contelle et al., 1996) is widely used in the study of anti-oxidative activity, the principle of which is according to the combination of the DPPH free radical with a electron provided by antioxidant, the characteristic color of DPPH disappeared. Comparing the variety of absorbency at 517 nm, it can be used to evaluate the free radical scavenging capacity of antioxidants.

The determination method is 96-hole micro-plate combined with enzyme micro-plate reader (micro-plate quantification method, MQ) (Peng et al., 2000). Add 100 mg sample of different concentration and 200 μl DPPH solution to the micro-plate, and then shake the plate 20 s, keep it under the temperature of 35℃ for 20 min, at last determine the ultraviolet absorption (Ap). Meanwhile, determine the absorption of the sample without adding DPPH (Ac), the DPPH with 100 μl anhydrous ethanol (Amax). Free radical scavenging activity using the following formula: 1- (Ap-Ac)/Amax x100%. Use the sample concentration(X) as the abscissa axis and free radical scavenging activity (Y) as the vertical axis to draw a calibration curve (Table 1). Calculate the IC_{50} of the sample from the calibration curve.

Dilute the prepared Trolox solution with anhydrous ethanol to different concentration solutions. Calculate the free radical scavenging activity of Trolox to DPPH. And then, use the Trolox concentration as the abscissa axis and free radical scavenging activity as the vertical axis to draw a calibration curve (Table 1). Calculate the IC_{50} of the Trolox from the calibration curve.

RESULTS AND DISCUSSION

Pinostrobin (1)

Compound 1 (86 mg), was obtained as a colorless crystalloid ($CHCl_3$),mp: 98~100℃, it can be dissolved easily in the $CHCl_3$ and EtOAc. The compound reveal fluorescence of amaranth in the UV_{254} and reveal blue with $FeCl_3$-K_3[Fe(CN) $_6$] reagent. ^{1}H-NMR(400 MHz, $CDCl_3$) δ: 7.43(2H, dd, *J* = 7.8, 2.4 Hz, H-2′,6′),7.47(2H, dd, *J* = 8.8, 2.4 Hz, H-3′,5′), 7.40 (1H, dd, *J* = 8.8, 2.4 Hz, H-4′),6.08(1H, d, *J* = 2.4 Hz, H-8),6.07 (1H, d, *J* = 2.4 Hz, H-6),5.43 (1H, dd, *J* = 13.0, 3.0 Hz, H-2),3.10(1H, dd, *J* =16.0, 12.0 Hz, 3α-H),2.82 (1H, dd, *J* =17.0, 3.0 Hz,

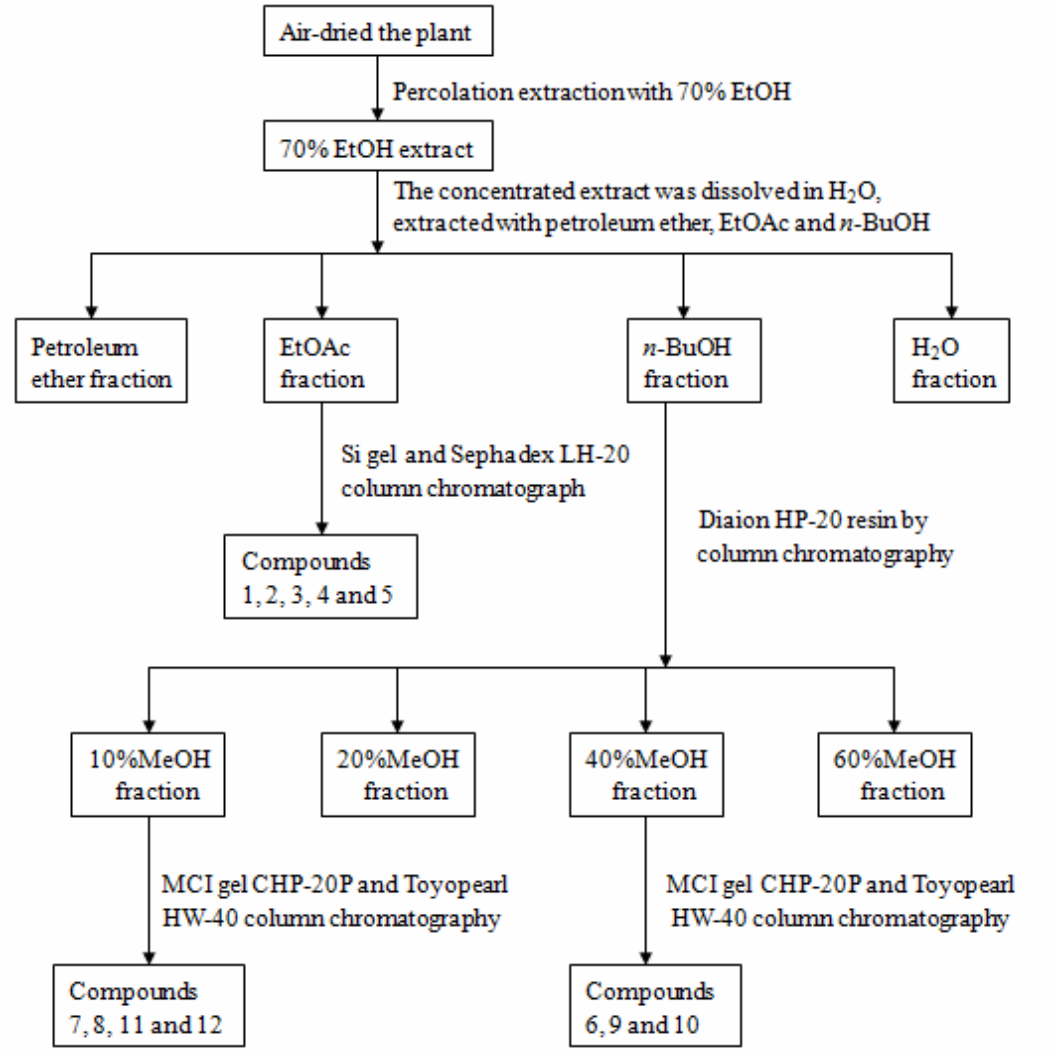

Figure 1. The chart of extraction process.

Table 1. DPPH free radical scavenging ability of extract sample as IC_{50} values and regression equations.

Sample of extract	Regression equations	R^2	IC_{50} (mg /ml)	IC_{50} rate of Trolox and sample (times)
EtOAc extract	y = 1.1185x + 0.3928	0.9730	0.0958	3.832
n-BuOH extract	y = 0.5383x + 0.2584	0.9874	0.4488	17.95
H_2O extract	y =0.1800x + 0.3228	0.9837	0.9844	39.38
Trolox	y = 26.284x + 0.2437	0.9935	0.0250	

3β-H), 3.82(3H, s, 7-OCH_3). ^{13}C-NMR(100 MHz, $CDCl_3$) δ: 79.2(C-2), 43.5(C-3),195.3(C-4), 164.1(C-5),95.6(C-6),168.5(C-7),94.3(C-8),162.6(C 9), 103.9 (C-10), 138.5(C-1′), 126.4(C-2′,6′), 128.9(C-3′,5′), 128.3(C-4′, 55.8(7-OCH_3). The spectral data showed basically agreement with the literature (Zhou et al., 1999), the compounds is Pinostrobin.

Quercetin (2)

Compound 2 (56 mg), was obtained as yellow amorphous powder, mp: 312~314℃. It can be dissolved easily in the $CHCl_3$ and MeOH. The compounds reveal fluorescence of amaranth in the UV_{254} and reveal blue with $FeCl_3$-K_3[Fe(CN) $_6$] reagent. HCl-Mg reactions reveal

carnation. ^{1}H-NMR(400 MHz, MeOD) δ: 7.80(1H, d, *J* = 1.6 Hz, H-2′) ,7.58(1H, dd, *J* = 8.0 Hz,1.6 Hz, H-6′),6.88 (1H, d, *J* = 8.0 Hz, H-5′) , 6.44(1H, d, *J* = 2.0 Hz, H-8), 6.23(1H, d, *J* = 2.0 Hz, H-6) . ^{13}C-NMR(100 MHz, MeOD) δ: 146.2(C-2), 138.6(C-3), 176.5(C-4), 155.0(C-5), 99.9(C-6), 163.8(C-7), 95.5(C-8), 160.4(C-9), 103.8(C-10), 121.4(C-1′), 114.6(C-2′), 144.9(C-3′), 146.1(C-4′), 116.4(C-5′), 118.3(C-6′). The spectral data showed basically agreement with the literature (Deng et al., 2008).The melting point of the mixture of sample and the standard substance does not drop, the sample and the standard substance have the same Rf. value, the compounds is Quercetin.

8-O-acetyl-shanzhiside methyl ester (3)

Compound 3 (18 mg), was obtained as white amorphous powder, it can be dissolved easily in the MeOH and H_2O. The compounds reveal fluorescence of brown in the UV_{254} and reveal amaranth with 10% H_2SO_4-EtOH reagent. ^{1}H NMR(400 MHz, MeOD) δ: 5.90 (1H, d, *J* =2.0 Hz, H-1),7.43(1H, s, H-3), 4.32 (1H,m,H-6),2.17 (1H, brd, *J* = 13.2 Hz, H-7a), 2.03(1H, m, H-7b), 3.71 (3H, s, $COOCH_3$), 3.03(2H, m, H-5 and H-9), 1.50(3H, s, CH_3), 2.00(3H, s, $OCOCH_3$), 4.63 (1H, d, *J* =7.6 Hz, glc- H-1′), 3.16-3.89 (glc H-2′~6′). ^{13}C NMR(100 MHz, MeOD) δ: δ 95.7(C-1), 153.7(C-3), 109.8(C-4), 42.3(C-5), 76.0(C-6), 47.6(C-7), 89.8(C-8), 50.0(C-9), 22.2(C-CH_3), 169.1(C-$COOCH_3$), 53.5(C-$COOCH_3$), 173.1(C-$OCOCH_3$), 100.4(C-1′), 74.7(C-2′), 78.3 C-3′), 71.6(C-4′), 87.0(C-5′), 63.0(C-6′). The spectral data showed basically agreement with the literature (Yu et al., 2006), the compounds is 8-O-acetyl- shanzhiside methyl ester.

8[E]-N-[2'-hydroxyl-tetracosan-cosoyl]-1-O-*β*-D-glucopyranosy1-8-en-octadecas-phingenin (4)

Compound 4 (20 mg), was obtained as white amorphous powder, it can be dissolved easily in the $CHCl_3$, the compound reveal amaranth with 10% H_2SO_4-EtOH reagent. It's molecular formula was assigned as $C_{48}H_{93}NO_{10}$ and molecular weight was assigned as 843 on the basis of the ESI-MS (*m/z* 867 $[M+Na+H]^+$). ^{1}H-NMR(400 MHz, $CDCl_3$) δ: 4.05(1H, dd, *J* =10.5, 6.3 Hz, 1α-H) 3.80(1H, dd, *J* =10.4, 3.6 Hz, 1β-H), 5.41(1H, m, H-8), 5.40(1H, m, H-9), 4.02(1H, m, H-2′), 4.28(1H, d, *J* =7.8 Hz, glc H-1), 3.17(1H, m, glc H-2), 5.34(1H, m, glc H-3), 3.26(1H, m, glc H-4), 3.25 (1H, m, glc H-5), 3.86(1H, d, *J* =11.7 Hz, glc 6α-H), 3.66(1H, m, glc 6β-H). ^{13}C-NMR(100 MHz, $CDCl_3$) δ: 69.9(C-1), 51.7(C-2), 75.6(C-3), 72.9(C-4), 32.8(C-5), 27.2(C-6), 33.8(C-7), 131.6(C-8), 131.4(C-9), 33.7(C-10), 30.3(C-11), 30.2(C-12), 30.5(C -13), 30.6(C-14), 30.8(C-15), 33.1(C-16), 23.7(C-17), 14.4(C-18), 177.1(C-1′), 73.0(C-2′), 35.7(C-3′), 26.1(C-4′), 30.3~30. 8(C-5~21′), 33.1(C-22′), 23.7(C-23′), 14.4(C-24′), 104.7(glc C-1), 75.0(glc C-2), 77.9(glc C-3), 71.6 (glc C-4),78.0(glc C-5),62.7(glc C-6).The spectral data showed basically agreement with the literature (Xiao et al., 2000). The compound is 8[E]-N-[2'-hydroxyl -tetracosan-cosoyl]-1-O-*β*-D-glucopyranosy1-8- en-octadecas- phingenin.

Caffeic acid (5)

Compound 5 (26 mg), was obtained as a colorless crystalloid, it can be dissolved easily in the Me_2CO and MeOH, the compound reveal fluorescence of amaranth in the UV_{254} and reveal blue with $FeCl_3$-$K_3[Fe(CN)_6]$ reagent. the ESI-MS *m/z*: 179 $[M-1]^-$. ^{1}H-NMR(400 MHz, Me_2CO-d_6) δ: 6.33(1H, d, *J* = 16.0 Hz, H-8), 7.61(1H, d, *J* = 16.0 Hz, H-7),7.17(1H, d, *J* = 2.0 Hz, H-2), 7.04(1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.88(1H, d, *J* = 8.0 Hz, H-5). ^{13}C-NMR(100 MHz, Me_2CO-d_6)δ: 127.3(C-1), 116.2(C-2), 146.7(C-3), 148.6(C-4), 122.5(C-5), 116.6(C-6), 146.1(C-7), 115.6(C-8), 168.8(C-9, C=O). The spectral data showed basically agreement with the literature (Carnat et al., 2000), the compounds is caffeic acid.

Quercetin-3-O-*β*-D-glucoside (6)

Compound 6 (25 mg), was obtained as yellow amorphous powder, mp: 228~230℃, it can be dissolved easily in the MeOH and H_2O. The compound reveal fluorescence of amaranth in the UV_{254} and reveal blue with $FeCl_3$-$K_3[Fe(CN)_6]$ reagent. HCl-Mg reactions reveal carnation. ^{1}H NMR(400 MHz, MeOD) δ: 6.35 (1H, d, *J* = 1.8 Hz, H-6), 6.54 (1H, d, *J* = 1.8 Hz),6.81(2H, d, *J* = 8.2 Hz),7.68 (1H, dd, *J* = 7.8, 1.6 Hz, H-6′), 7.78 (1H, d, *J* = 1.6 Hz, H-2′),6.78 (1H, d, *J* = 7.8 Hz, H-5′),3.43 (1H, m),3.35 (1H, m), 3.31(1H, m),3.37 (1H, m),3.68 (2H, m)。 ^{13}C-NMR(100 MHz, MeOD) δ: 158.1(C-2), 135.2(C-3), 180.2(C-4), 162.8(C-5), 100.3(C-6), 164.6(C-7), 96.7(C-8), 160. 4(C-9), 105.4(C-10), 122.2(C-1′), 115.4(C-2′), 146.6(C-3′), 149.5(C-4′), 121.8(C-5′), 120.9(C-6′), 104.6(C-1″), 75.6(C-2″), 76.9(C-3″), 71.3(C-4″), 76.7(C-5″), 62.5(C-6″). The spectral data showed basically agreement with the literature (Hou et al., 2008), the compounds is Quercetin-3-O-*β*-D-glucoside.

Quercetin-7-O-*β*-D-glucoside (7)

Compound 7 (16 mg), was obtained as yellow amorphous powder, it can be dissolved easily in the MeOH and H_2O. The compound reveal fluorescence of amaranth in the UV_{254} and reveal blue with $FeCl_3$-$K_3[Fe(CN)_6]$ reagent. Molish reactions reveal masculine, acid hydrolysis gave glucose and apiose, identified by TLC and comparison with authentic sample. ^{1}H NMR(400 MHz, in MeOD) δ: 6.40 (1H, d, *J* = 2.0 Hz, H-6), 6.20 (1H, d, *J* = 1.8 Hz, H-8), 7.63 (1H, dd, *J* = 8.4, 2.1 Hz, H-6′),

7.66 (1H, d, *J* = 2.1 Hz, H-2′), 6.86(1H, d, *J* =8.4 Hz, H-5′). ^{13}C-NMR(100 MHz, MeOD) δ: 148.8(C-2), 135.6(C-3), 177.6(C-4), 158.5(C-5), 96.7(C-6), 165.0(C-7), 96.3(C-8), 160.2(C-9), 105.6(C-10), 122.1(C-1′), 117.7(C-2′), 148.3(C-3′), 145.9(C-4′), 116.0(C-5′), 122.5(C-6′), 104.2(C-1″), 75.1(C-2″), 78.2(C-3″), 75.2(C-4″), 73.9(C-5″), 62.1(C-6″). The spectral data showed basically agreement with the literature (Wang et al., 2006), the compounds is Quercetin-7-O-*β*-D- glucoside.

3*β*-O-*β*-D-gluco-pyranosyl pomolic acid 28-O-*β*-D-glucopyranosyl ester (8)

Compound 8 (150 mg), was obtained as yellow amorphous powder, it can be dissolved easily in the MeOH and H_2O. The compounds reveal amaranth with 10% H_2SO_4-EtOH reagent. ^{1}H NMR (400 MHz, MeOD) δ: 5.30(1H, m, H-12), 4.31(1H, d, *J* =8.0 Hz, Glu H-1), 1.32(3H, s, H-29), 1.19(3H, s), 1.05(3H, s), 0.93(3H, s), 0.85(3H, s), 0.77(3H, s). ^{13}C NMR(100MHz, in MeOD) δ: 38.3(C-1), 27.1(C-2), 90.9(C-3), 39.9(C-4), 57.0(C-5), 19.4(C-6), 34.2(C-7), 41.0(C-8), 47.1(C-9), 35.9(C-10), 24.6(C-11), 129.7(C-12), 139.5(C-13), 42.8(C-14), 29.5(C-15), 26.5(C-16), 48.6(C-17), 54.9(C-18), 73.6(C-19), 41.3(C-20), 27.2(C-21), 37.8(C-22), 28.5(C-23), 16.0(C-24), 17.6(C-25), 16.6(C-26), 24.9(C-27), 178.5(C-28), 27.1(C-29), 17.0(C-30), 106.7(C-1′), 75.6(C-2′), 78.5(C-3′), 71.7(C-4′), 77.7(C-5′), 62.8(C-6′), 95.8(C-1″), 73.9(C-2″) 78.3(C-3″),71.1(C-4″), 78.3(C-5″), 62.4(C-6″).The spectral data showed basically agreement with the literature (Zhao et al., 1995), the compound is 3*β*-O-*β*-D-gluco-pyranosyl pomolic acid 28-O-*β*-D-glucopyranosyl ester.

3,4-di-*o*-caffeoylquinicacid (9)

Compound 9 (16 mg), was obtained as white amorphous powder, it can be dissolved easily in the MeOH and H_2O, the compound reveal blue with $FeCl_3$-K_3[Fe(CN) $_6$] reagent. It's molecular formula was assigned as $C_{25}H_{24}O_{12}$ on the basis of the ESI-MS (*m/z* 515 [M-H]$^-$). ^{1}HNMR(400 MHz, D_2O) δ: 7.22(1H, d, *J* = 2.0 Hz, H-5′), 7.17(1H, dd, *J* = 8.0, 2.0 Hz, H-9′), 6.99(1H, d, *J* = 8.0 Hz, H-8′), 7.21(1H, d, *J* = 2.0 Hz, H-5″), 6.717.18(1H, dd, *J* = 8.0, 2.0 Hz, H-9″),6.98(1H, d, *J* = 8.0 Hz, H-8″), 6.47(1H, d, *J* = 16 Hz, H-2′), 7.70(1H, d, *J* = 16 Hz, H-3′), 6.49(1H, d, *J* = 15.6 Hz, H-2″), 7.67(1H, d, *J* = 15.6 Hz, H-3″), 2.20(1H, d, *J* = 11.8 Hz, H-2),1.80(1H, d, *J* = 12.2 Hz, H-2), 5.46(1H, m, H-3),4.99(1H, , H-4),4.25(1H, d, *J* = 12.6 Hz, H-5), 2.30(1H, m, H-6), 2.10(1H, m, H-6). ^{13}CNMR(100 MHz, D_2O) δ: 76.2(C-1), 39.9(C-2), 69.6(C-3), 75.4(C-4), 70.7(C-5), 38.3(C-6), 183.8(C-7), 171.6(C-1′), 116.8(C-2′), 148.9(C-3′), 129.6(C-4′), 117.7(C-5′),146.6(C-6′), 149.6(C-7′), 118.7(C-8′), 125.3(C 9′), 171.4(C-1″), 116.9(C-2″), 148.6(C-3″), 129.5(C-4″), 117.4(C-5″), 146.7(C-6″), 149.5(C-7″), 118.7(C-8″), 125.2(C-9″). The spectral data showed basically agreement with the literature (Yuan et al., 2008), the compound is 3,4-di-*o*-caffeoylquinicacid.

Chlorogenic acid (10)

Compound 10 (35 mg), was obtained as white amorphous powder, mp 205~207℃, it can be dissolved easily in the MeOH and reveal blue with $FeCl_3$-K_3[Fe(CN) $_6$] reagent. ESI-MS *m/z:* [M-H]$^-$ 353. ^{1}H-NMR(400 MHz, MeOD) δ: 6.84(1H, d, *J* =16.0 Hz, H-2′),7.63(1H, d, *J* = 16.0 Hz, H-3′), 6.79(1H, d, *J* = 8.0 Hz, H-8′), δ6.92(1H, dd, *J* = 1.8, 8.0 Hz, H-9′),δ7.14(1H, d, *J* = 1.8 Hz, H-5′), 2.18(2H, m, H-2), 5.45(1H, t, *J* = 4.2 Hz, H-3), 3.72(1H, m, H-4), 4.22(1H, m, H-5),2.18(1H, q, H-6), 2.26(1H, m, H-6). ^{13}C-NMR(100 MHz, MeOD) δ:170.8(C-1′), 115.6C-2′),148.1(C-3′), 127.4(C-4′), 117.3(C-5′), 151.1(C-6′), 148.1(C-7′), 116.3(C-8′), 124.4(C-9′), 76.3(C-1),37.1(C-2),74.7(C-3), 75.2(C-4), 68.9(C-5), 43.3(C-6), 178.2(C-7). The spectral data showed basically agreement with the literature (Yuan et al., 2006). The melting point of the mixture of sample and the standard substance does not drop. The sample and the standard substance have the same Rf value. The compound is Quercetin.

Lamalbid (11)

Compound 11 (136 mg), was obtained as white amorphous powder, it can be dissolved easily in the MeOH and H_2O. The compounds reveal fluorescence of brown in the UV_{254} and reveal amaranth with 10% H_2SO_4-EtOH reagent. ^{1}H NMR(400 MHz, MeOD) δ: 5.67(1H, brs, H-1), 7.48 (1H, s, H-3), 2.95(1H, d, *J* =3.6 Hz, H-5), 4.07(1H, t, m, H-6), 3.68(1H, d, *J* = 4.4 Hz, H-7), 2.85 (1H, d, *J* = 11.2 Hz, H-9), 1.24 (3H, s, CH_3), 3.78 (3H, s, COOMe), 4.78 (1H, d, *J* = 8.4 Hz, glc H-1′), 3.25-4.09 (glc H-2′~6′). ^{13}C NMR(100 MHz, MeOD) δ: δ 96.4(C-1),154.3(C-3),112.8(C-4),37.8(C-5),78.8(C-6),80.0(C-7),80.1 (C-8), 49.7(C-9), 23.2(C-CH_3), 171.9(C- $COOCH_3$), 54.4(C-$COOCH_3$), 100.7(C-1′), 75.1(C-2′), 78.11(C-3′), 72.1(C-4′),78.4(C-5′), 63.2(C-6′). The spectral data showed basically agreement with the literature (Li et al., 1999), the compound is Lamalbid.

Shanzhiside methyl ester (12)

Compound 12 (126 mg), was obtained as white amorphous powder, it can be dissolved easily in the MeOH and H_2O. The compounds reveal fluorescence of brown in the UV_{254} and reveal amaranth with 10% H_2SO_4-EtOH reagent. ^{1}H NMR(400 MHz, MeOD) δ: 5.56 (1H, d, *J* = 5.2 Hz, H-1), 7.39(1H, s, H-3), 2.98(1H, dd, *J* =10.0,

Table 2. DPPH free radical scavenging ability of compounds sample as IC_{50} values.

Sample of compounds	IC_{50} (mg /ml)	IC_{50} rate of Trolox and sample(times)	Sample of compounds	IC_{50} (mg /ml)	IC_{50} rate of Trolox and sample(times)
1	0.2974	11.90	7	0.0732	2.93
2	0.0545	2.18	9	0.0925	3.70
3	0.6038	24.15	10	0.1135	4.54
5	0.0836	3.34	11	0.6456	25.82
6	0.0683	2.73	12	0.8247	32.99
Trolox	0.0250		Trolox	0.0250	

3.1 Hz, H-5),4.05 (1H,m,H-6),1.83(1H, m, H-7a),2.01 (1H, m, H-7b), 2.61(1H, dd, *J* = 10.0, 2.5 Hz, H-9), 3.72 (3H, s, $COOCH_3$), 1.24(3H, s, CH_3), 4.61(1H, d, *J* = 8.2 Hz, glc H-1′), 3.15-3.88 (glc H-2′~6′). ^{13}C NMR(100 MHz, MeOD) δ: 94.8(C-1), 152.8(C-3), 111.4(C-4), 41.3(C-5), 77.4(C-6), 49.2(C-7), 78.9(C-8), 51.7(C-9), 24.7(C-CH_3), 169.7(C-$COOCH_3$), 51.9(C-$COOCH_3$), 99.8(C-1′), 74.6(C-2′), 77.9(C-3′), 78.3(C-4′), 71.6(C-5′), 62.8(C-6′). The spectral data showed basically agreement with the literature (Yang et al., 2004), the compound is shanzhiside methyl ester.

DPPH free radical scavenging ability

Antioxidant have the ability of scavenging free radical, so it can protect human body from hurting by free radical and postpone the process of some chronic disease. DPPH assay is widely used in the determination of free radical scavenging ability. The samples and Trolox of synthetic antioxidants were prepared of 5 different concentrations, and the antioxidant activity of DPPH was measured respectively. Use the sample concentration(X) as the abscissa axis and free radical scavenging activity(Y) as the vertical axis to draw a calibration curve, Calculate the IC_{50} of the samples and Trolox from the calibration curve. DPPH free radical scavenging ability and regression equations are as follow Tables 1 and 2. From the Tables 1 and 2, it is obviously seen that EtOAc, *n*-BuOH and H_2O extract of 70% EtOH extract and compounds 1 ~ 3, 5~7, 9~12 have the ability to scavenging DPPH free radical. The scavenging ability of DPPH free radical increases along with the rise of sample concentration. In order to compare the antioxidant capacity of these samples, IC_{50} values are used as indicators of free radical scavenging ability. IC_{50} value is the concentration of the antioxidant when half of the DPPH free radical was scavenged by it. The smaller of the IC_{50} value, the better its free radical scavenging ability, the stronger the antioxidant activity, which shows that EtOAc extract has a strong ability for scavenging DPPH free radicals. The results provide evidence for the further research of its efficacy and its further development and utilization.

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