Supporting Information

Antioxidant and Tyrosinase Inhibitory Effects of Neolignan Glycosides from *Crataegus pinnatifida* Seeds

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1. Antioxidant activity assay

1.1 DPPH radical scavenging activity

Radical scavenging activity of 1-6 and trolox (positive control) was determined using DPPH as a reagent with modification by using 96-well plates. A 0.1 mM solution of DPPH radical in ethanol was prepared, and then 100 µL of this solution was mixed with 100 µL of sample solution. The mixture was incubated for 30 min in a dark room at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm. The percentage of scavenged DPPH was calculated using the following equation: DPPH scavenging activity (%) = \[1-(S-SB)/(C-CB)\]×100% where S, SB, C, and CB are the absorbencies of the sample, the blank sample, the control, and the blank control, respectively [1].

1.2 ABTS radical scavenging activity

The radical scavenging activity of the isolated compounds was carried out using an improved ABTS decolourisation assay with some modification. ABTS radical cation (ABTS\(^{\bullet+}\)) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS\(^{\bullet+}\) solution was diluted with ethanol to an absorbance of 0.7±0.02 at 734 nm. An ethanolic solution (50 µL) of the samples at various concentrations was mixed with 150 µL diluted ABTS\(^{\bullet+}\) solution. After reaction at room temperature for 20 min, the absorbance at 734 nm was measured.
measured. Lower absorbance of the reaction mixture indicates higher ABTS•+ scavenging activity. The capability to scavenge the ABTS•+ was calculated using the formula given below: ABTS•+ scavenging activity (%) = [1-(S-SB)/(C-CB)]×100% where S, SB, C, and CB are the absorbencies of the sample, the blank sample, the control, and the blank control, respectively [1].

2. Assay of inhibitory activity to tyrosinase

In this assay, L-tyrosine was used as a substrate. 40 μL of mushroom tyrosinase solution (100 units/mL), 40 μL of 0.1 mg/mL L-tyrosine solution in phosphate-buffered saline (PBS) solution (25 mM, pH 6.8), 80 μL of PBS solution (25 mM, pH 6.8), and 40 μL of sample (compound 1-6 and arbutin) in 20% MeOH solution (at 500 μg/mL) were added to a 96-well microplate. The assay mixture was incubated at 37°C for 30 min. A 20% MeOH solution was added to a blank solution. Before and after incubation, the amount of dopachrome produced in the reaction mixture was measured at 492 nm in the microplate reader. The percentage of the inhibition of tyrosinase activity was calculated by the following equation: inhibition (%)=[(A-B)-(C-D)]/(A-B)×100, where A is absorbance of blank solution after incubation, B is absorbance of blank solution before incubation, C is absorbance of sample solution after incubation, and D is absorbance of sample solution before incubation [2].

References

1 Wu SB, Wu J, Yin ZW, Zhang JZ, Long CL, Kennelly EJ, Zheng SP. Bioactive and


3. NMR, UA, IR, CD spectra and HRESIMS of compounds 1-4, 1a and 2a

**Figures 1S-11S** Spectra of pinnatifidaninside A (1)

**Figures 12S-20S** Spectra of pinnatifidaninside B (2)

**Figures 21S-30S** Spectra of pinnatifidaninside C (3)

**Figures 31S-38S** Spectra of pinnatifidaninside D (4)

**Figure 39S** $^1$H NMR spectrum of the aglycone of pinnatifidaninside A (1a)

**Figure 40S** $^1$H NMR spectrum of the aglycone of pinnatifidaninside B (2a)
Fig. 1S $^1$H NMR spectrum of compound 1.

Fig. 2S $^{13}$C NMR spectrum of compound 1.
Fig. 3S DEPT spectrum of compound 1.

Fig. 4S HSQC spectrum of compound 1.
**Fig. 5S** HMBC spectrum of compound 1.

**Fig. 6S** $^1$H-$^1$H COSY spectrum of compound 1.
**Fig. 7S** NOESY spectrum of compound 1.

**Fig. 8S** UV spectrum of compound 1.
Fig. 9S CD spectrum of compound 1.

Fig. 10S IR spectrum of compound 1.
**Fig. 11S** HRESIMS spectrum of compound 1.

**Fig. 12S** $^1$H NMR spectrum of compound 2.
Fig. 13S $^{13}$C NMR spectrum of compound 2.

Fig. 14S HMBC spectrum of compound 2.
Fig. 15S $^1\text{H}-^1\text{H}$ COSY spectrum of compound 2.

Fig. 16S NOESY spectrum of compound 2.
Fig. 17S UV spectrum of compound 2.

Fig. 18S CD spectrum of compound 2.
Fig. 19S IR spectrum of compound 2.

Fig. 20S HRESIMS spectrum of compound 2.
Fig. 21S $^1$H NMR spectrum of compound 3.

Fig. 22S $^{13}$C NMR spectrum of compound 3.
**Fig. 23S** DEPT spectrum of compound 3.

**Fig. 24S** HSQC spectrum of compound 3.
Fig. 25S HMBC spectrum of compound 3.

Fig. 26S NOESY spectrum of compound 3.
Fig. 27S UV spectrum of compound 3.

Fig. 28S CD spectrum of compound 3.
Fig. 29S IR spectrum of compound 3.

Fig. 30S HRESIMS spectrum of compound 3.
Fig. 31S $^1$H NMR spectrum of compound 4.

Fig. 32S $^{13}$C NMR spectrum of compound 4.
Fig. 33S HMBC spectrum of compound 4.

Fig. 34S NOESY spectrum of compound 4.
Fig. 35S UV spectrum of compound 4.

Fig. 36S CD spectrum of compound 4.
Fig. 37S IR spectrum of compound 4.

Fig. 38S HRESIMS spectrum of compound 4.
Fig. 39S $^1$H NMR spectrum of compound 1a.

Fig. 40S $^1$H NMR spectrum of compound 2a.