Supporting Information

Novel flavonoids from the leaves of *Actinidia valvata* Dunn: structural elucidation and antioxidant activity

Hailiang Xin¹,², Yingchun Wu³#, Yonghua Su¹, Jiayu Sheng¹, Changquan Ling¹

# co –first author

Affiliation

¹ Department of Traditional Chinese Medicine, Changhai Hospital, the Second Military Medical University, Shanghai, P. R. China

² School of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai, P. R. China

Correspondence

Chang-Quan Ling

Department of Traditional Chinese Medicine

Changhai Hospital

The Second Military Medical University
Bioassays

1 Scavenging activity of DPPH free radicals
DPPH radical scavenging activity was measured according to the method described previously (Cho et al., 2004). The reaction mixture containing various concentrations of the test sample and DPPH methanolic solution (150 μM) was incubated at 37 °C for 30 min, and the absorbance was measured at 517 nm. DPPH radical-scavenging activity was calculated by using the following formula:

\[
\text{Scavenging effect (\%) = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100}
\]

2 Scavenging activity of superoxide anion radicals
Various concentrations of the tested sample dissolved in methanol were added to a 1.0 mL mixture of 0.4 mM xanthine and 0.24 mM nitro blue tetrazolium chloride (NBT) in 0.1 M phosphate buffer (pH 7.8) containing 0.1 mM EDTA. A 1.0 mL sample of xanthine oxidase (0.05 U/ml), diluted in the same phosphate buffer, was added to the above solution, and the resulting mixture was incubated in a water bath at 37°C for 40 min. The reaction was terminated by adding 1.0 mL of 69 mM sodium dodecylsulphate solution (SDS), and the absorbance of reduced NBT was measured at 560 nm. The lower absorbance indicated higher scavenging activity. Superoxide scavenging activity was calculated using the following formula:

\[
\text{Scavenging effect (\%) = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100}
\]

3 Scavenging activity of hydroxyl radicals
Effects of compounds 1-3 on hydroxyl radicals were determined by the fenton system described by Smimoff with some modifications. The reaction mixture (3.0 ml), containing FeSO4 (0.15 mmol/L), H2O2 (6 mmol/L), and natrium salicylicum (2 mmol/L), as well as various concentrations of the tested sample, was incubated for 1 hour at 37°C, and the absorbance was measured at 510 nm (Valentova et al., 2003). Hydroxyl radical-scavenging activity was calculated using the following formula:

\[
\text{Scavenging effect (\%) = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100}
\]

4 Inhibitory activity of lipid peroxidation
Lipid peroxidation was initiated by Fe2+ (10 μM) and ascorbic acid (100 μM) in mouse liver homogenates and assayed according to previous report (Smirnoff et al., 1989). In brief, the
The reaction mixture was incubated at 37°C for 1 h in the absence or presence of the compounds. The reaction was stopped by the addition of trichloroacetic acid (TCA; 28% w/v) and thiobarbituric acid (TBA; 1% w/v) in succession, and the mixture was then heated at 100°C for 15 min. After centrifugation to remove precipitates, the absorbance was measured at 532 nm (Cho et al., 2004; Sun et al., 2003). The inhibitory activity was calculated using the following formula:

\[
\text{Inhibitory effect (\%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100}
\]

**Fig. 1S** IR spectrum of compound 1.
**Fig. 2S** ESI-MS spectrum of compound 1.
**Fig. 3S** HR-ESI-MS spectrum of compound 1.
**Fig. 4S** $^1$H-NMR spectrum of compound 1.
**Fig. 5S** $^{13}$C-NMR spectrum of compound 1.
**Fig. 6S** DEPT spectrum of compound 1.
**Fig. 7S** H,H-COSY spectrum of compound 1.
**Fig. 8S** H,H-COSY spectrum of compound 1.
**Fig. 9S** HMBC spectrum of compound 1.
**Fig. 10S** NOSEY spectrum of compound 1.
**Fig. 11S** TOCSY spectrum of compound 1.
**Fig. 12S** IR spectrum of compound 2.
**Fig. 13S** ESI-MS spectrum of compound 2.
**Fig. 14S** HR-ESI-MS spectrum of compound 2.
**Fig. 15S** $^1$H-NMR spectrum of compound 2.
**Fig. 16S** $^{13}$C-NMR spectrum of compound 2.
**Fig. 17S** DEPT spectrum of compound 2.
**Fig. 18S** H,H-COSY spectrum of compound 2.
**Fig. 19S** HMQC spectrum of compound 2.
**Fig. 20S** HMBC spectrum of compound 2.
**Fig. 21S** NOSEY spectrum of compound 2.
**Fig. 22S** TOCSY spectrum of compound 2.
**Fig. 23S** HPLC chromatogram for identification of sugar linkage of compound 1.
**Fig. 24S** HPLC chromatogram for identification of sugar linkage of compound 2.