Supporting Information

Isolation of Antithrombotic Phenolic Compounds from the Leaves of

_Crataegus pinnatifida_

Shao-Jiang Song¹,², Ling-Zhi Li¹,², Pin-Yi Gao³, Yan-Qiang Yuan⁵, Ru-Ping Wang¹,², Ke-Chun Liu⁴, Ying Peng⁴

Affiliation

¹ School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, PR China

² State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, PR China

³ College of Chemical Engineering, Shenyang University of Chemical Technology, Shenyang, PR China

⁴ School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, PR China

⁵ Biology Institute of Shandong Academy of Sciences, Jinan, PR China

Correspondence

_Prof Dr Ying Peng_

School of Pharmacy

Shenyang Pharmaceutical University
103 Wenhua Road

Shenyang Liaoning 110016

PR China

Phone: +86/24/23986361

Fax: +86/24/23986361

yingpeng99@yahoo.com.cn
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**General experimental procedures**

Mass spectra were determined on a HRESI-MS: MicroTOF spectrometer (Bruker Daltonics). NMR spectra were recorded on a Bruker ARX-300 (1D) and ARX-600 (2D) spectrometer with TMS as internal standard in chloroform-\textit{d}$_1$ or dimethyl sulfoxide-\textit{d}$_6$ (DMSO-\textit{d}$_6$). Silica gel (200-300 mesh; Qingdao Marine Chemical Co.); Sephadex LH-20 (25-100 µm; Greenherbs Science and Technology Development Co., Ltd.); MCI gel (CHP20P, 75–150 µm; Mitsubishi Chemical Corporation), and reversed-phase C$_{18}$ silica gel (60–80 µm; Merck) were used for column chromatography and silica gel GF254 (Qingdao Marine Chemical Co.) for TLC. Solvents were of industrial purity and distilled prior to use. Heparin sodium (2mL: 12500 U, CAS Number: 9041-08-1, purity: >95%, Adhoc-Tech. Co., Ltd).
2S Extraction schemes

Dry leaves of *Crataegus pinnatifida* (5 kg)

EtOH 70%, 95 °C, 2h/time, 3times

EtOH extract (492 g)

macroporous resin (D 101)

EtOH:H₂O (0:100-95:5)

Fraction B (120 g)

Diaion HP-20 eluted with MeOH/H₂O (60:40-100:0)

Fraction B1 (15 g)

CH₂Cl₂/MeOH
30:1, SiO₂

Sephadex LH-20, MeOH

comp 1 (12 mg)

comp 7 (500 mg)

Fraction B2 (20 g)

MeOH/H₂O 0:100-100:0 and HPLC

comp 2 (11 mg)

comp 3 (28 mg)

comp 5 (58 mg)

Fraction B3 (10 g)

C₁₈ silica gel
MeOH/H₂O 30:70-90:10 and HPLC

comp 4 (10 mg)

comp 6 (12 mg)

3S Antithrombotic assay

Four days post fertilization zebrafish (CD41:GFP) larvae were placed in a 24-well plate with 6 fish per well and one compound concentration (100µg/mL) per well. Zebrafish larvae were exposed to one concentration (100µg/mL) of the compound for 24 h. Meanwhile, we have added a little dimethyl sulphoxide (DMSO) (=500 µg L⁻¹) as cosolvent for improving the solubility of compounds [S1]. Then they were exposed to 1ppm FeCl₃, and we began to record the time until thrombosis. Control groups consisting of the vehicle or 5 U/mL heparin sodium (positive) were included in this assay. The thrombosis time of the control group was normalised to 100%, and results
from compound-exposed larvae are expressed as a percentage of control.

References:


Observation of experimental thrombus formation by means of stereofluoroscope
Antithrombotic activity of compounds 1-7 on transgenic zebra fish system

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control</th>
<th>Thrombosis (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.12±0.07</td>
<td>11.68±0.13</td>
</tr>
<tr>
<td>2</td>
<td>11.39±0.12</td>
<td>10.71±0.12</td>
</tr>
<tr>
<td>3</td>
<td>11.39±0.12</td>
<td>10.57±0.12</td>
</tr>
<tr>
<td>4</td>
<td>12.74±0.07</td>
<td>7.63±0.23</td>
</tr>
<tr>
<td>5</td>
<td>11.39±0.12</td>
<td>9.71±0.07</td>
</tr>
<tr>
<td>6</td>
<td>11.39±0.12</td>
<td><strong>19.04±0.10</strong></td>
</tr>
<tr>
<td>7</td>
<td>11.39±0.12</td>
<td>9.82±0.15</td>
</tr>
</tbody>
</table>

4S Spectroscopic data of compounds 5-7

Shanyenoside A (5) was obtained as colourless needles (MeOH). The ESI-MS showed a pair of sodium adduct ion peaks at m/z 431.0 [M+Na]+ and 838.7 [2M+Na]+. ¹H-NMR (300MHz, pyridine-d₅), δ 7.02 (2H, d, J=8.6 Hz, H-9, 11), 7.66 (2H, d, J=8.6 Hz, H-8, 12), 7.55 (1H, d, J=1.75 Hz, H-2), 7.13 (1H, d, J=1.75 Hz, H-6), 3.67 (3H, s, 10-OCH₃), 3.85 (3H, s, 5-OCH₃), 5.61 (1H, d, J=6.0 Hz, H-1’).

Eriodictyol (6) was obtained as amorphous yellow powder. ¹H-NMR(300MHz, DMSO-d₆) δ 12.14 (1H, s, 5-OH), 6.87 (1H, br.s, H-2’), 6.74 (2H, m, H-5’, 6’), 5.87 (2H, d, J=2.4 Hz, H-6, H-8), 5.37 (1H, dd, J=3.0, 12.6 Hz, H-2), 3.18 (1H, dd, J=12.6, 17.1 Hz, Hₐ-3), 2.66 (1H, dd, J=3.0, 17.1 Hz, Hₐ-3).

2"-O-Rhamnosyl vitexin (7) was obtained as amorphous yellow powder. ¹H-NMR (300MHz, DMSO-d₆) δ 13.1 (1H, s, 5-OH), 8.04 (2H, d, J=8.5 Hz, H-2’, 6’), 6.92 (2H, d, J=8.5 Hz, H-3’, 5’), 6.79 (1H, s, H-3), 6.28 (1H, s, H-6), 0.47 (3H, d, J=6.0
Hz, Rha-CH<sub>3</sub>). <sup>13</sup>C-NMR (75 MHz, DMSO-<i>d</i><sub>6</sub>) δ 164.1 (C-2), 102.5 (C-3), 182.2 (C-4), 161.4 (C-5), 98.5 (C-6), 162.6 (C-7), 104.6 (C-8), 155.9 (C-9), 104.2 (C-10), 121.7 (C-1'), 129.1 (C-2'), 116.0 (C-3'), 160.7 (C-4'), 116.0 (C-5'), 129.1 (C-6'), 71.6 (C-1''), 75.2 (C-2''), 80.0 (C-3''), 70.6 (C-4''), 81.9 (C-5''), 61.2 (C-6''), 100.4 (C-1'''), 70.4 (C-2''''), 71.6 (C-3'''), 70.4 (C-4''''), 68.3 (C-5'''), 17.8 (C-6''').
**Fig. 1** $^1$H NMR spectrum (300MHz, DMSO-$d_6$) of compound 1
Fig. 2S $^{13}$C NMR spectrum (300MHz, DMSO-d$_6$) of compound 1

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

14.641 16.680 26.780 28.355 52.641 61.301 61.796 70.394 70.827 74.043 74.477 77.384 77.817 100.952 102.312 114.746 122.468 123.354 127.873 129.468 130.886 132.570 136.912 159.686 166.864
Fig. 3S HMBC spectrum (150MHz, DMSO-\textit{d6}) of compound 1
Mass Spectrum Molecular Formula Report

Analysis Info
Analysis Name: D:/Data/20110113/YE-55.d
Method: tune low 100-500 m/z
Sample Name: YE-55

Acquisition Parameter
Source Type: ESI
Focus: Active
Scan Begin: 50 m/z
Scan End: 2000 m/z
Scan Spacing: 0.05 V
Set Collision Cell RF: 200.0 Vpp
Set Nebulizer: 0.4 Bar
Set Dry Heater: 190 °C
Set Dry Gas: 4.0 L/min
Set Divert Valve: Source

Generated Molecular Formula Parameter
Formula, m/z: C30H44Na14Np
Measured m/z: 651.262
Tolerance: 3 ppm
Charge: 1

NH-Rule: no
Electron Configuration: both
Minimum: 0
Maximum: 3

Formula, max: C30H44Na14Np

Error Parameter
Sigma: 0.173
Err (ppm): 0.14
Mean Err (ppm): 0.14
Err (Intensity): -0.09

NH-Rule: a even

Fig. 4S: HREIMS spectrum of compound 1
Fig. 5S $^1$H NMR spectrum (300MHz, DMSO-$d_6$) of compound 2
Fig. 6S $^{13}$C NMR spectrum (300MHz, DMSO-$d_6$) of compound 2
Fig. 7S HMBC spectrum (150MHz, DMSO-$d_6$) of compound 2
Fig. 9S $^1$H NMR spectrum (300MHz, DMSO-$d_6$) of compound 3
Fig. 10S $^{13}$C NMR spectrum (300MHz, DMSO-$d_6$) of compound 3
Fig. 11S HMBC spectrum (150MHz, DMSO-$d_6$) of compound 3
Mass Spectrum Molecular Formula Report

Analysis Info
Analysis Name D1Data20110107YE-41.d
Method Tune low 100-500 m/z
Sample Name YE-41
Comment
Acquisition Date 1/7/2011 5:04:53 PM
Operator Bruker Custumer Inst.
Instrument / Ser# microTOF-Q 125

Acquisition Parameter:
Source Type EI
Ion Polarity Positive
Set Nebulizer 0.4 Bar
Set Capillary 4500 V
Set Dry Heater 180 °C
Set End Plate Offset 200 V
Set Dry Gas 4.0 l/m
Set Collision Cell RF 50.0 Vpp
Set Divert Valve Source

Generate Molecular Formula Parameter
Formula, max. C20H24O6P5a
Measured m/z 431.131
Tolerance 3 ppm
Charge 1
Check Valence no
Minimum 0
Maximum 0
Molten Rule no
Electron Configuration both
Filter HC Ratio no
Minimum 0
Maximum 3
Estimate Carbon yes

![Mass Spectrum Molecule Formula Report](image)

Fig. 12S: HREIMS spectrum of compound 3
Fig. 13S $^1$H NMR spectrum (300MHz, DMSO-$d_6$) of compound 4
Fig. 14S $^{13}$C NMR spectrum (300MHz, DMSO-$d_6$) of compound 4
Fig. 15S HSQC spectrum (150MHz, DMSO-$d_6$) of compound 4
Fig. 16S HMBC spectrum (150MHz, DMSO-$d_6$) of compound 4
Fig. 17S HREIMS spectrum of compound 4.