Extraction and Purification of Acanthoside D and Chiisanoside from Acanthopanax chiisanensis

2001 2
acanthoside D, chiisanoside, HPLC analysis, acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.

acanthoside D, chiisanoside, HET P.
Abstract

The purpose of this study is to find the most suitable analytical conditions of HPLC in the separation of acanthoside D and chiisanoside after solvent extraction from the trunk and leaves of *Acanthopanax chiisanensis*, a tree used in herbal medicine. The experiments were performed with various extraction solvents in order to isolate acanthoside D from the trunk, and chiisanoside from the leaves of *Acanthopanax chiisanensis*. Analytical conditions of compositions and flow rates of mobile phase were varied. Also, the experiments were further done by increasing injection volumes, considering the future preparative use of the product.

Acanthoside D was extracted from the trunk of *Acanthopanax chiisanensis* with methanol, ethanol and water, and the content of acanthoside D was the highest as 1.40% / 10g from dried trunk when methanol was used as an extraction solvent. The contents of acanthoside D in the trunk bark and core were 0.21% / 10g and 1.52% / 10g respectively. It means that there is a higher concentration of acanthoside D in the core than in the trunk bark of *Acanthopanax chiisanensis*.

For isolating acanthoside D from the trunk, a mobile phase composed of acetonitrile added to water was used; 12% ACN, 15% ACN, 18% ACN, and the best analytical result was with
15% ACN. The retention times and resolutions were decreased and HETP was decreased a little as the concentration of acetonitrile was increased in acanthoside D from the trunk. The retention time of acanthoside D was not changed remarkably as the injection volume of sample increased from 30μl to 100μl.

Chiisanoside was extracted from the leaves of *Acanthopanax chiisanensis* with methanol, ethanol and water, and the content of chiisanoside was the highest as 6.21μg/10g from dried leaves when methanol was used as an extraction solvent.

For isolating chiisanoside from the leaves, a mobile phase composed of methanol added to water was used: 88% MeOH, 90% MeOH, 92% MeOH, and the best analytical result was with 90% MeOH. The retention times and HETP were not varied little while resolutions was decreased a little as the concentration of methanol was increased in chiisanoside from the leaves. The retention time of chiisanoside was not changed remarkably, as the injection volume of sample increased from 30μl to 100μl.
List of Tables

Table 1. HPLC condition for the determination of acanthoside D and chiisanoside in A. chiisanensis (Waters) .................................. 13

Table 2. HPLC condition for the determination of acanthoside D and chiisanoside in A. chiisanensis (ThermoQuest) .................. 14

Table 3. Peak area of the standard chromatogram of acanthoside D and chiisanoside according to wavelength variation in HPLC .................................................. 22

Table 4. Reproducibility of acanthoside D and chiisanoside standard (n=5) ........................................................................ 26

Table 5. Retention time, resolution and content of acanthoside D and chiisanoside by the variation of mobile phase composition and extract solvent in A. chiisanensis .................................. 39

Table 6. Comparison of retention time and peak area in trunk and leaves extracts by variation of flow rate. .................. 46
List of Figures

Fig. 1. Structure of acanthoside D(a) and chiisanoside(b). .......... 12

Fig. 2. Flow diagram of the sample preparation for separation of acanthoside D and chiisanoside in A. chiisanensis. .......... 17

Fig. 3. UV/VIS Spectrophotometer spectrum of acanthoside D and chiisanoside standard by concentration of acanthoside D (4, 5, 10) and chiisanoside (40, 70, 100). .......... 23

Fig. 4. Comparison of chromatogram peak area of acanthoside D and chiisanoside standard by wavelength variation of wavelength (200, 210, 214, 220, 254, 270). .......... 24

Fig. 5. Separation of acanthoside D from trunk extract of A. chiisanensis. Column: µ-Bondapak C18 (3.9 × 300 mm, 10 µ), acetonitrile/water: 15/85 vol.%, wavelength: 210 nm, inj. vol.: 20 µl, flow rate: 1.0 ml/min. .......... 28

Fig. 6. Comparison of the acanthoside D content in the trunk core and trunk bark of A. chiisanensis. .......... 30

Fig. 7. Separation of the chiisanoside from leaves extract of A. chiisanensis. Column: µ-Bondapak C18 (3.9 × 300 mm, 10 µ), methanol/water: 90/10 vol.%, wavelength: 210 nm, inj. vol.: 20 µl, flow rate: 1.0 ml/min. .......... 32

Fig. 8. Comparison of the chromatogram of trunk in A. chiisanensis by extract solvent variation. .......... 34

Fig. 9. Comparison of the chromatogram of leaves in A. chiisanensis by extract solvent variation. .......... 35
Fig. 10. Comparison of the chromatogram of trunk extract by mobile phase composition \( \text{acetonitrile/water (vol.%) : } 12/88, 15/85, 18/82 \) ................................................. 37

Fig. 11. Comparison of the chromatogram of leaves extract by mobile phase composition \( \text{methanol/water (vol.%) : } 88/12, 90/10, 92/8 \) ......................................................... 38

Fig. 12. Effect of mobile phase composition on HETP in analytical column \( \mu - \text{Bondapack C}_{18} (10\text{mm}, 300\times3.9\text{mm I.D.}), 12\%, 15\%, 18\% \text{ACN}, 1.0\text{l/min}, 20\text{C} \) ................................................. 43

Fig. 13. Effect of mobile phase composition on HETP in analytical column \( \mu - \text{Bondapack C}_{18} (10\text{mm}, 300\times3.9\text{mm I.D.}), 88\%, 90\%, 92\% \text{MeOH}, 1.0\text{l/min}, 20\text{C} \) ................................................. 44

Fig. 14. Comparison of the chromatogram of acanthoside D in trunk extract by flow rate of mobile phase \( \text{flow rate (l/min) : } 0.8, 1.0, 1.2 \) ................................................................. 47

Fig. 15. Comparison of the chromatogram of chiisanoside in leaves extract by flow rate of mobile phase \( \text{flow rate (l/min) : } 0.8, 1.0, 1.2 \) ................................................................. 48

Fig. 16. Comparison of the chromatogram of acanthoside D in trunk extract by injection volume \( \text{injection vol. (l) : } 30, 50, 80, 100 \) ................................................................. 50

Fig. 17. Comparison of the chromatogram of chiisanoside in leaves extract by injection volume \( \text{injection vol. (l) : } 30, 50, 80, 100 \) ................................................................. 51
1. 

- 前処理方法

chromatographic method

- 1.
Ovodov \( ^{2,3} \) 1965, Brekhmann \( ^{4} \) Elyakov \( ^{9} \) Eleuthrococcus

1.1 \( \textit{Acanthopanax Cortex} \)

- 2 -
A CANTHOPANAX

1.1.1

(A canthopanax

1.1.2

acanthoside B, D

lignan
chiisanoside [10] was identified in Acanthopanax chiisanensis, along with other compounds such as acanthoside D [10].

Adaptogenic activities [16] have been associated with A. chiisanensis since 1967 [16].

Silverian ginseng (Acanthopanax chiisanensis) is a plant species with potential adaptogenic activities, as noted in [16].

Adaptogenic activities and chiisanoside [10] have been studied in this context.

- 4 -
1.2 (A canthopanax chiisanensis Nakai)

...
1.3 A. chinensis

2. 

2.1 

\[ K = \frac{C_S}{C_M} \]  (1)

\[ k' = \frac{C_S V_S}{C_M V_M} = K \frac{V_S}{V_M} \]  (2)

\[ t_R = \frac{L}{u(1 + k')} = \frac{L}{u} \left(1 + K'\right) \]  (3)

- 7 -
(adjusted retention time)\( t_R \), \( t_0 \), \( V_s \), \( V_M \) (4)

\[ t_R = t_0 (1 + k) = t_0 \left[ 1 + K \frac{V_s}{V_M} \right] \] (4)

2.2  

\[ R = \frac{t_{R2} - t_{R1}}{(W_2 + W_1)/2} = \frac{2\Delta t}{W_2 + W_1} \] (5)
2.3 HETP

HETP refers to the height equivalent to a theoretical plate. It is a measure of peak sharpness. HETP is typically associated with the efficiency of a chromatographic column, where a lower HETP value indicates a more efficient separation.

Column C18 methanol, acetonitrile as organic modifier, methanol, acetonitrile as organic modifier, methanol, acetonitrile as organic modifier. The HETP values are typically reported in units of plates per meter.
peak\[\]  \[\] \[\] HETP[\] \[\] ...
3.1 

3.1.1 

A canthopanax (A. canthopanax) and A. canthopanax chiisanensis were prepared by using methanol, acetonitrile, and water as solvents in a water bath at 46°C. Rotavaprot (BUCHI, Switzerland) was used for the evaporation of the solvents.

3.1.2 

Acanthoside D, chiisanoside, and ethanol, hexane, ethyl ether were analyzed using HPLC (MERCK, Germany) and UV/VIS Spectrophotometer (Hewlett-Packard, USA) Model 8453.
Fig. 1. Structure of acanthoside D(a) and chiisanoside(b).
Table 1. HPLC condition for the determination of acanthoside D and chiisanoside in *A. chiisanensis*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Waters Model 590 (USA)</td>
</tr>
<tr>
<td>Detector</td>
<td>HITACHI L-4000</td>
</tr>
<tr>
<td>Integrator</td>
<td>HITACHI D-2500</td>
</tr>
<tr>
<td>Column</td>
<td>μ-Bondapak C₁₈ (Waters : 10µ, 3.9×300µ)</td>
</tr>
<tr>
<td>Wavelength</td>
<td>210µ</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile/H₂O(15/85), MeOH/H₂O(90/10)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0µ/min</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.1AUFS</td>
</tr>
<tr>
<td>Chart speed</td>
<td>0.5µ/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20µ</td>
</tr>
</tbody>
</table>
Table 2. HPLC condition for the determination of acanthoside D and chiisanoside in *A. chiisanensis*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>ThermoQueat (USA)</td>
</tr>
<tr>
<td>Detector</td>
<td>SPECTRA System UV 3000</td>
</tr>
<tr>
<td>Auto sampler</td>
<td>SPECTRA System AS 3000</td>
</tr>
<tr>
<td>Pump</td>
<td>SPECTRA System P4000</td>
</tr>
<tr>
<td>Column</td>
<td>µ-Bondapak C18 (Waters : 10% , 3.9×300(\text{mm}))</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile/H_2O(15/85), MeOH/H_2O(90/10)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0(\text{ml}/\text{min})</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20(\text{μl})</td>
</tr>
</tbody>
</table>
3.2 藻酸酯

3.2.1 acanthoside D 2.0 / 200 50% 50% 50% acanthoside D (10.0 / )

3.2.2 . chiisanoside 5.0 / 50% 50% chiisanoside (100.0 / )
3.2.3 

Fig. 2. 

- 16 -
Fig. 2. Flow diagram of the sample preparation for separation of acanthoside D and chiisanoside in *A. chiisanensis*.
3.3  

3.3.1  

HPLC  Analyzing acanthoside D and chiisanoside\[\] in the extracts of the samples.  

A  HPLC  instrument equipped with a UV/Vis Spectrophotometer  was used.  

The  system  consisted of a  pump,  a  column  (µ-Bondapak C18,  30 cm × 3.9 mm),  a  detector,  and  a  computer  for  data  acquisition.  

The  mobile  phase consisted  of  acetonitrile  and  water,  with  the  following  composition:  40\%  acetonitrile  for  acanthoside  D  and  70\%  acetonitrile  for  chiisanoside.  

The chromatograms were  recorded  at  wavelengths  of  200,  210,  214,  220,  254,  and  270 nm.  

The  results  are  summarized  in  Table 1.  

Table 1:  HPLC  chromatograms of acanthoside D and chiisanoside.  

Table 2:  Peak areas of acanthoside D and chiisanoside.  

A  stock solution  of  50\%  acanthoside D  and  100\%  chiisanoside  was prepared.  

The  solutions  of  2.5,  5,  10,  20,  30,  and  40\%  were  prepared.  

- 18 -
Table 1: HPLC chromatogram peak area.

3.3.2 Acanthoside D

UV/VIS Spectrophotometer, HPLC chromatogram peak area.

Table 1: HPLC chromatogram peak area.

- 19 -
3.3.3 Chiisanoside

Chii soside

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>88/12 vol.%</td>
<td>90/10 vol.%</td>
</tr>
</tbody>
</table>

Table 1

Chromatogram

HPLC

4. - 20 -
4.1  

4.1.1  

Acanthoside D, chiisanoside.  

UV Detector, UV/VIS Spectrophotometer.  

190~300 nm, scanning.  

Fig. 3.  

4~10 nm, acanthoside D.  

210 nm, scanning.  

Chiisanoside.  

40~100 nm, 100 nm, 210 nm.  

210 nm, 40~100 nm, 100 nm.  

205 nm.  

HPLC.  

UV detector.  

200, 210, 214, 220, 254, 270 nm. 

Table 1.  

Acanthoside D, chiisanoside.  

Chromatogram, peak area.  

Table 3.  

UV Cutoff.  

Fig. 4.  

Acanthoside D, chiisanoside, 210 nm.  

Peak.  

UV Cutoff.  

190 nm.
Table 3. Peak area of the standard chromatogram of acanthoside D and chiisanoside according to wavelength variation in HPLC

<table>
<thead>
<tr>
<th>Component</th>
<th>Acanthoside D (100% /%)</th>
<th>Chiisanoside (100% /%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>ACN/H$_2$O(15/85 vol.% )</td>
<td>MeOH/H$_2$O(90/10 vol.% )</td>
</tr>
<tr>
<td>Wavelength(Å )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>242664</td>
<td>21645</td>
</tr>
<tr>
<td>210</td>
<td><strong>573683</strong></td>
<td><strong>784544</strong></td>
</tr>
<tr>
<td>214</td>
<td>456121</td>
<td>458431</td>
</tr>
<tr>
<td>220</td>
<td>245642</td>
<td>257172</td>
</tr>
<tr>
<td>254</td>
<td>121977</td>
<td>22690</td>
</tr>
<tr>
<td>270</td>
<td>5839</td>
<td>28324</td>
</tr>
</tbody>
</table>

UV-cutoff: 205 Å

- 22 -
Fig. 3. UV/VIS Spectrophotometer spectrum of acanthoside D and chiisanoside standard by concentration. [ ] acanthoside D ( / ): 4, 5, 10, chiisanoside ( / ): 40, 70, 100
Fig. 4. Comparison of chromatogram peak area of acanthoside D and chiisanoside standard by wavelength variation. 
[] wavelength( ) : 200, 210, 214, 220, 254, 270[]

4.1.2
UV detector 210 HPLC
organic modifier 2 acetonitrile methanol
acanthoside D chiisanoside
Table 1 Table 2
acanthoside D acetonitrile/water methanol/water
Fig. 5 acetonitrile/water 15/85 vol.%, methanol/water
Fig. 7 methanol/water 90/10 vol.%
chromatogram

4.1.3 acanthoside D chiisanoside stock solution
2.5, 5, 10, 20, 30, 40, 50 (/>1) y = 64.156x - 4957.6
acanthoside D chiisanoside
y = 64.156x - 4957.6, chiisanoside
\[ y = 16549x - 34634 \]

0.9993 \quad 0.9969 \quad 1 \quad 1 \quad 0.9969 \quad 0.9959 \quad 2.

\[ 5 \sim 50.0 / \mu \text{g} \quad \text{acanthoside D} \quad \text{chiisanoside} \]

\[ 1 \quad \text{acanthoside D} \quad \text{chiisanoside} \]

\[ \text{Table 1: HPLC chromatogram peak area} \]

\[ \text{Table 4: Reproducibility of acanthoside D and chiisanoside standard. (n=5)} \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Run</th>
<th>Mean</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthoside D</td>
<td>571240</td>
<td>584173</td>
<td>585249</td>
</tr>
<tr>
<td>Chiisanoside</td>
<td>301674</td>
<td>296041</td>
<td>292527</td>
</tr>
</tbody>
</table>
4.2 Acanthoside D

Acanthoside D

Table 1

HPLC analysis: µ-Bondapak C18 (10 μ, 3.9×300 mm), UV detector, 210 nm. The elution was performed at a flow rate of 15/85 vol.% water/ethanol.

Table 2

Acanthoside D chromatogram: Fig. 5. Table 1 details the results of the HPLC analysis.

10 g of Acanthoside D was extracted and analyzed.

Chromatogram: Acanthoside D content was determined to be 1.40%.

Concentrations: 0.315%, 0.397%, 0.388%

Acanthoside D was analyzed at 27°.
Fig. 5. Separation of acanthoside D from trunk extract of *A. chiisanensis* column: μ-Bondapak C_{18}(3.9×300 mm, 10 μ), acetonitrile/water: 15/85 vol.%, wavelength: 210 nm, injection volume: 20 μL, flow rate: 1.0 mL/min.
4.3 Acanthoside D

acanthoside D

10g acanthoside D 0.21

1.52 acanthoside D

Fig. 6 chromatogram

Table 1 Table 2 HPLC chromatogram

Table 2 chromatogram
Fig. 6. Comparison of the acanthoside D content in the trunk core and trunk bark of *A. chiisanensis*.
4.4 Chiisanoside

Table 1 Table 2

2. HPLC μ-Bondapak C18 (10 μ, 3.9×300 μ) 210
1.0/min 10 vol.%, 20 10 g

Table 2 chromatogram Fig. 7

Table 1 chromatogram

10 g 6.21 acanthoside D

- 31 -
Fig. 7. Separation of the chiisanoside from leaves extract of A. chiisanensis. Column: µ-Bondpak C18 (3.9x 300 mm, 10µ), methanol/water: 90/10 vol.%, wavelength: 210nm, injection volume: 20µl, flow rate: 1.0ml/min.
4.5 Table 2

<table>
<thead>
<tr>
<th>compoud</th>
<th>peak 1</th>
<th>peak 2</th>
<th>peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>acanthoside D</td>
<td>1.40</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>chiisanoside</td>
<td>6.21</td>
<td>6.09</td>
<td>6.05</td>
</tr>
</tbody>
</table>

Fig. 8, Fig. 9, Table 2
Fig. 8. Comparison of the chromatogram of trunk in *A. chiisanensis* by extract solvent variation.
Fig. 9. Comparison of the chromatogram of leaves in *A. chiisanensis* by extract solvent variation.
4.6 ของ รายละเอียด

acanthoside D  ชิวานโซไซด์ D
chiisanoside  ชิวานโซไซด์ D

Table 5  รายละเอียดของ chromatogram Fig. 10
Table 2  รายละเอียดของ chromatogram Fig. 11
Fig. 10. Comparison of the chromatogram of trunk extract by mobile phase composition: acetonitrile/water (vol.%): 12/88, 15/85, 18/82.
Fig. 11. Comparison of the chromatogram of leaves extract by mobile phase composition. [ methanol/water (vol.%) ]
: 88/12, 90/10, 92/8
Table 5. Retention time, resolution and content of acanthoside D and chiisanoside by the variation of mobile phase composition and extract solvent in *A. chiisanensis*.

<table>
<thead>
<tr>
<th>Mobile phase compositions (vol.%</th>
<th>Extract solvent</th>
<th>Retention time (min)</th>
<th>Resolution</th>
<th>Content ([□] / 10g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acanthoside D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12%ACN</td>
<td>MeOH</td>
<td>32.94</td>
<td>1.78</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>EtOH</td>
<td>32.24</td>
<td>1.51</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>H2O</td>
<td>30.43</td>
<td>1.39</td>
<td>0.68</td>
</tr>
<tr>
<td>15%ACN</td>
<td>MeOH</td>
<td>13.63</td>
<td>1.00</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>EtOH</td>
<td>13.32</td>
<td>0.99</td>
<td>0.78</td>
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<tr>
<td></td>
<td>H2O</td>
<td>13.70</td>
<td>0.99</td>
<td>0.71</td>
</tr>
<tr>
<td>18%ACN</td>
<td>MeOH</td>
<td>6.97</td>
<td>0.74</td>
<td>1.80</td>
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<tr>
<td></td>
<td>EtOH</td>
<td>7.04</td>
<td>0.72</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>H2O</td>
<td>7.05</td>
<td>0.68</td>
<td>1.10</td>
</tr>
<tr>
<td><strong>Chiisanoside</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>88%MeOH</td>
<td>MeOH</td>
<td>2.75</td>
<td>0.96</td>
<td>5.88</td>
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<tr>
<td></td>
<td>EtOH</td>
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<td>0.88</td>
<td>5.63</td>
</tr>
<tr>
<td></td>
<td>H2O</td>
<td>2.73</td>
<td>0.65</td>
<td>5.67</td>
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<tr>
<td>90%MeOH</td>
<td>MeOH</td>
<td>2.76</td>
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<td>6.21</td>
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<td>H2O</td>
<td>2.79</td>
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<td>6.05</td>
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<tr>
<td>92%MeOH</td>
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<td>2.75</td>
<td>0.71</td>
<td>5.97</td>
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<tr>
<td></td>
<td>EtOH</td>
<td>2.75</td>
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<tr>
<td></td>
<td>H2O</td>
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<td>0.58</td>
<td>5.59</td>
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</table>
4.6.1  

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>12% ACN</th>
<th>15% ACN</th>
<th>18% ACN</th>
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<tbody>
<tr>
<td>Acanthoside D</td>
<td></td>
<td>32.9</td>
<td>27</td>
<td>21.5</td>
</tr>
<tr>
<td>Chiisanoside</td>
<td></td>
<td>13.3</td>
<td>11.9</td>
<td>11.4</td>
</tr>
<tr>
<td>Peak 1.5</td>
<td></td>
<td>7</td>
<td>6.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Peak 15% ACN</td>
<td></td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12% ACN</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>18% ACN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 40 -
4.6.2

Table 5

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Elution time (min)</th>
<th>Acanthoside D</th>
<th>Chiisanoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>15% ACN</td>
<td>1.40</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>12% ACN</td>
<td>1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18% ACN</td>
<td>1.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10g of each compound was injected and eluted at different mobile phase.

Table 6

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Elution time (min)</th>
<th>Acanthoside D</th>
<th>Chiisanoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>90% MeOH</td>
<td>6.21</td>
<td>6.09</td>
<td>6.05</td>
</tr>
<tr>
<td>90% MeOH</td>
<td>5.88</td>
<td>5.97</td>
<td></td>
</tr>
<tr>
<td>88% MeOH</td>
<td>6.21</td>
<td>5.88</td>
<td>5.97</td>
</tr>
<tr>
<td>92% MeOH</td>
<td>6.21</td>
<td>5.88</td>
<td>5.97</td>
</tr>
</tbody>
</table>
4.6.3 HETP

HETP peak 1/2 peak 1/2
HETP LC peak 1/2 peak 1/2
12% ACN, 15% ACN, 18% ACN
HETP Fig. 12
HETP peak 1/2 peak 1/2
88% MeOH, 90% MeOH, 92% MeOH
chromatogram HETP Fig. 13
HETP peak 1/2 peak 1/2
88% MeOH, 92% MeOH HETP Fig. 14
90% MeOH
88% MeOH, 92% MeOH
chiisanoside peak 1/2 peak 1/2
90% MeOH peak 1/2 peak 1/2.
Fig. 12. Effect of mobile phase composition on HETP in analytical column $\mu$-Bondapack C$_{18}$ (100, 300×3.9mm I.D.), 12%, 15%, 18% ACN, 1.0 mL/min, 20°C.
Fig. 13. Effect of mobile phase composition on HETP in analytical column. µ-Bondapack C<sub>18</sub> (100Å, 300×3.9 mm I.D.), 88%, 90%, 92% MeOH, 1.0 l/min, 20°C.
4.7 The chromatograms of HPLC analysis shown in Table 2. HPLC analysis results are shown in Table 6. Figure 14 shows the chromatogram of acanthoside D peak at 0.8 /min, 1.0 /min, 1.2 /min respectively. Figure 15 shows the chromatogram of chiisanoside peak at 0.8 /min, 1.2 /min respectively. The retention time of acanthoside D peak is 15.9 min, and the retention time of chiisanoside peak is 2.29 min. The retention time of acanthoside D peak is 10.8 min, and the retention time of chiisanoside peak is 3.43 min. The retention time of acanthoside D peak is 12.5 min, and the retention time of chiisanoside peak is 10.0 min.
Table 6. Comparison of retention time and peak area in trunk and leaves extracts by variation of flow rate.

<table>
<thead>
<tr>
<th>Flow rate (l/min)</th>
<th>Trunk extract</th>
<th>Leaves extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>peak area</td>
</tr>
<tr>
<td>0.8</td>
<td>15.87</td>
<td>62999</td>
</tr>
<tr>
<td>1.0</td>
<td>12.56</td>
<td>48351</td>
</tr>
<tr>
<td>1.2</td>
<td>10.77</td>
<td>27149</td>
</tr>
</tbody>
</table>
Fig. 14. Comparison of the chromatogram of acanthoside D in trunk extract by flow rate of mobile phase: 0.8 ml/min, 1.0 ml/min, 1.2 ml/min.
Fig. 15. Comparison of the chromatogram of chiisanoside in leaves extract by flow rate of mobile phase. - 0.8 ml/min, 1.0 ml/min, 1.2 ml/min -
4.8 µ-Bondapack C18 (100Å, 300×3.9 mm I.D.)

These compounds were separated by HPLC using a µ-Bondapack C18 (100Å, 300×3.9 mm I.D.) column. The mobile phase consisted of water and acetonitrile, with a gradient from 30% to 100% acetonitrile over 30 min. The flow rate was 1 mL/min and the detection wavelength was 210 nm. The retention times of the compounds were: Acanthoside D 30 min, Chiisanoside 30 min, and peak 30 min.

Fig. 16: Acanthoside D chromatogram

Fig. 17: Chiisanoside chromatogram

The chromatograms showed good separation of the compounds, allowing for accurate quantification and identification.
Fig. 16. Comparison of the chromatogram of acanthoside D in trunk extract by injection volume. Injection vol: 30, 50, 80, 100 μl.
Fig. 17. Comparison of the chromatogram of chiisanoside in leaves extract by injection volume injection vol.: 30, 50, 80, 100
5.  

A canthopanax chiisanensis (A canthopanax chiisanensis)  173  
acanthoside D  chiisanoside  HPLC  
UV/VIS Spectrophotometer  HPLC  
UV  190~300  scanning  HPLC  
acanthoside D  chiisanoside  210  
acanthoside D  chiisanoside  HETP  
UV  1.0,  1.40 /10g.
ヒサノサイドを、90/10 vol.%の水/アルコール (/ ) (90/10 vol.% ) 2.7, 1.6, 6.21 10g 1 2.7, 1.6, 6.21 10g 1.2/min 1.0/min HETP 30 100


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12. ÏëÇѾàÀüÁ¦ 7°³Á¤, p.752 (1998)

13. ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, pp370 (1989)

14. Petkov V. Hulgarian Academy of Sciences, pp73~137 (1958)

15. Petkov V. Materials for studies on Ginseng and Schizandra cheinensis IV, pp191~202 (1960)


17. ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, pp140~141 (1993)

18. ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦ ÏëÇѾàÀüÁ¦, pp359 (1997)


22. ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦, ÏëÇѾàÀüÁ¦ : ÏëÇѾàÀüÁ¦ ÏëÇѾàÀüÁ¦ ÏëÇѾàÀüÁ¦ ÏëÇѾàÀüÁ¦ ÏëÇѾàÀüÁ¦. ÏëÇѾàÀüÁ¦, 40(3), pp251 (1996)


1. Calibration curve for acanthoside D and chiisanoside standard.

![Calibration curve graph]

\[ y = 641.56x - 4057.8, \quad R^2 = 0.9993 \]
\[ y = 185.49x - 3463.4, \quad R^2 = 0.9999 \]

2. Column Efficiency

Column\[ \text{HPLC column} \]

1~6 i.d. column\[ \text{multiple path effect} \]

Column efficiency\[ \text{column efficiency} \]
packing \[ 31 \]

pulse\[ \] peak\[ \] peak broadening\[ \]; Multiple paths, \[33\] (number of theoretical plates) \[29\]

\[
N = 16 \left[ \frac{f_R}{W} \right]^2 = 5.54 \left[ \frac{f_R}{W_{1/2}} \right]^2
\] (6)

\[ W \] \[ W_{1/2} \] \[ (half width) \] \[ N \] \[ (height equi- valent to a theoretical plate: HETP) \] \[ H \] \[ \frac{L}{N} \] (7)
3. Mobile phase

carrier, eluent

<table>
<thead>
<tr>
<th>Item</th>
<th>Normal-phase</th>
<th>Reversed-phase</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solvent</th>
<th>UV Cutoff($)</th>
<th>Solvent</th>
<th>UV Cutoff($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>180</td>
<td>n-Heptane</td>
<td>197</td>
</tr>
<tr>
<td>Methanol</td>
<td>205</td>
<td>Cyclohexane</td>
<td>200</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>205</td>
<td>Carbon tetrachloride</td>
<td>265</td>
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<tr>
<td>Acetonitrile</td>
<td>190</td>
<td>Chloroform</td>
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<tr>
<td>THF</td>
<td>230</td>
<td>Benzene</td>
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<td>Acetone</td>
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<tr>
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<td>Methylene chloride</td>
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<tr>
<td>Ethyl acetate</td>
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<td>Tetrachloroethylene</td>
<td>280</td>
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<tr>
<td>Nitromethane</td>
<td>380</td>
<td>1,2-Dichloroethane</td>
<td>225</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>205</td>
<td>Ethanol</td>
<td>210</td>
</tr>
</tbody>
</table>