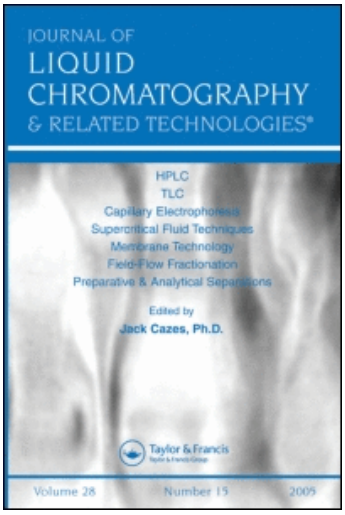

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Rapid Analysis of the Stem Bark of *Acanthopanax giraldii Harms* by HPLC/DAD/ESI/MS^2

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Abstract: A method has been developed for rapid analysis of the stem bark of *Acanthopanax giraldii Harms* (A.G) by HPLC/DAD/ESI/MS^2. The separation was performed on Zorbax SB-C_{18} (250 × 4.6 mm, 5 μm) column with gradient elution of acetonitrile-water as mobile phase. The mass spectral analysis was carried out on the Agilent 1100 series ion trap mass spectrometer with an electrospray ionization interface in negative ion mode. Uridine, Guanosine, Adenosine, Liriodendrin, Protocatechuic acid, Syringin, Chlorogenic acid, Caffeic acid were identified in the stem bark of A.G by the comparison of the HPLC retention time, UV spectra, and the mass spectra with corresponding authentic samples using the LC/DAD/ESI/MS^2 technique; the last 4 components identified in the A.G have not been reported to date.

Keywords: *Acanthopanax giraldii Harms*, LC/MS, Identification

INTRODUCTION

The herbal plant *Acanthopanax giraldii Harms* (A.G) is a kind of traditional Chinese medicine which has wide distribution in Sichuan, Gansu, and Ningxia province, China. The stem bark of this plant has long been used in the treatment of rheumatism as well as a tonic.[1] Extensive biological studies were carried out and reported on immunomodulations[2] anti-tumor,[3] anti-inflammation,[4] anti-virus,[5] and sedative[6] pharmacological

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actions. Chemical constituents studies on this herb revealed the following constituents: nucleotide, lignan, triterpane saponins, steroid glycosides, and phenolic compounds.[7–9]

The LC/MS technique offers a powerful, simple, and rapid method to detect known compounds in crude plant extracts. In the course of our study of biological active components in the A.G, uridine, guanosine, adenosine, liriodendrin, protocatechuic acid, syringin, chlorogenic acid, and caffeic acid were identified by the comparison of the HPLC retention time, UV spectra, and the mass spectra with corresponding authentic samples using the LC/ESI/MS^2 technique. Moreover the last four components identified in the A.G have not been reported to date. Two unknown compounds were also inferred by their UV spectra, mass spectra, and fragments of their MS^2 spectra. The molecular structures of these compounds are shown in Figure 1.

EXPERIMENTAL

Materials and Reagents

The stem bark of A.G was collected in Sichuan province, China. uridine, guanosine, adenosine, protocatechuic acid, syringin, chlorogenic acid and caffeic acid were purchased from the National Institute for the Control of Pharmaceutical and Biological Products P.R. China (Beijing, China). Liriodendrin was obtained by preparation in our lab. Acetonitrile was of HPLC grade from Dikma, USA. Water was doubly distilled pure water. The other reagents were of the analytical reagent grade.

Sample Preparation

The stem bark of A.G was crushed and 1.5 g of powder was extracted under reflux with 150 mL distilled water for 1.5 h. The extract was evaporated under reduced pressure to a small volume. Then 95% ethanol was added to the concentrated extract to make the concentration of ethanol to 75%. The solution was stored at 4°C for 24 h and then filtered. The filtrate was evaporated to a small volume under reduced pressure, then diluted to 10 mL with distilled water and filtered through 0.45 μm filter before sample injection.

Apparatus and Conditions

The LC separation was performed on an Agilent 1100 series HPLC system that included a quaternary pump, diode-array detector (DAD), vacuum

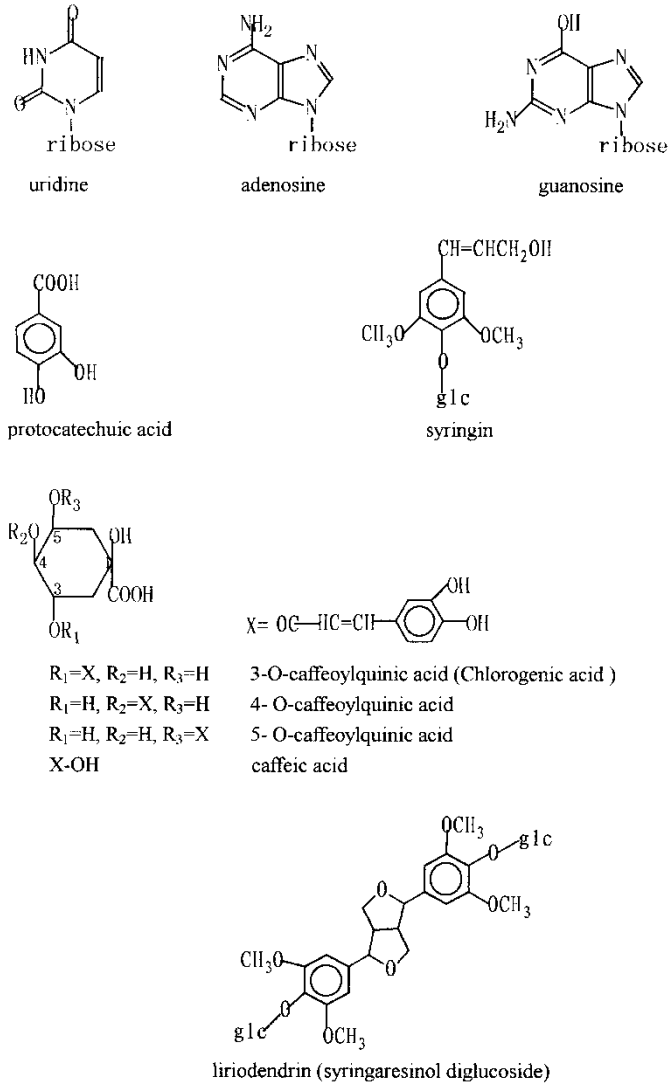

Figure 1. Structure of components in the A.G.

degasser, auto-sampler, and thermostatted column compartment. The separation was carried out on Zorbax SB-C_{18} (250 × 4.6 mm, 5 μm) column. The mobile phase consisted of solvent A (acetonitrile) and solvent B (0.2% formic acid water) with gradient elution as was shown in Table 1. The flow rate was 1.0 mL · min^{-1}. The column oven temperature was at 30°C. The injection was by an auto-sampler with the injection volume of 10 μL.

Table 1. Mobile phase gradient program

Time (min)	Solvent A (%)	Solvent B (%)
0	10	90
15	15	85
25	20	80
32	20	80
45	35	65

The mass spectral analysis was performed on an Agilent 1100 Series SL ion trap mass spectrometer with an eletrospray ionization interface in negative ion detection mode. The nebulizer nitrogen gas pressure was set at 0.24 Mpa and the drying nitrogen gas rate was set at $9\,L \cdot min^{-1}$. Drying gas temperature was 350°C. The scan range was 50–800 m/z.

After UV detection, 10% of the eluate was split off and introduced into the ESI/MS system.

Total Ion Chromatogram (TIC) and Extracted Ion Chromatogram (EIC)

Components of the sample were firstly separated by HPLC, and then were analyzed by ESI/MS. There was a delay between the UV chromatogram and the total ion chromatogram (TIC). The UV chromatogram and TIC of the stem bark of A.G were shown in Figure 2. EIC of components in the A.G were shown in Figure 3.

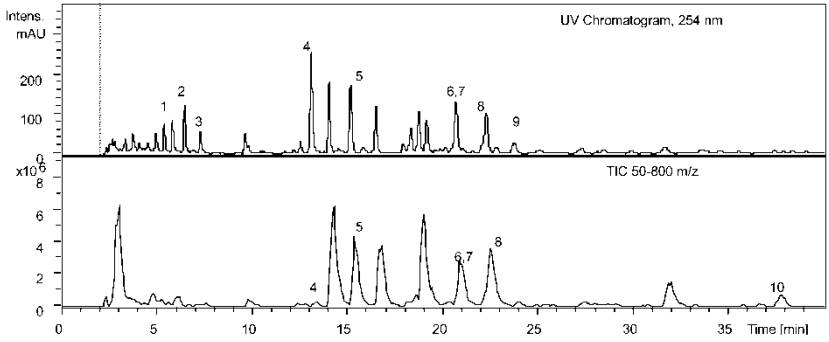

Figure 2. UV chromatogram and negative TIC of the extract of A.G. 1, uridine; 2, adenosine; 3, guanosine; 4, protocatechuic acid; 5, unknown; 6, syringin; 7, chlorogenic acid (3-O-caffeoylquinic acid); 8, unknown; 9, caffeic acid; 10, liriodendrin.

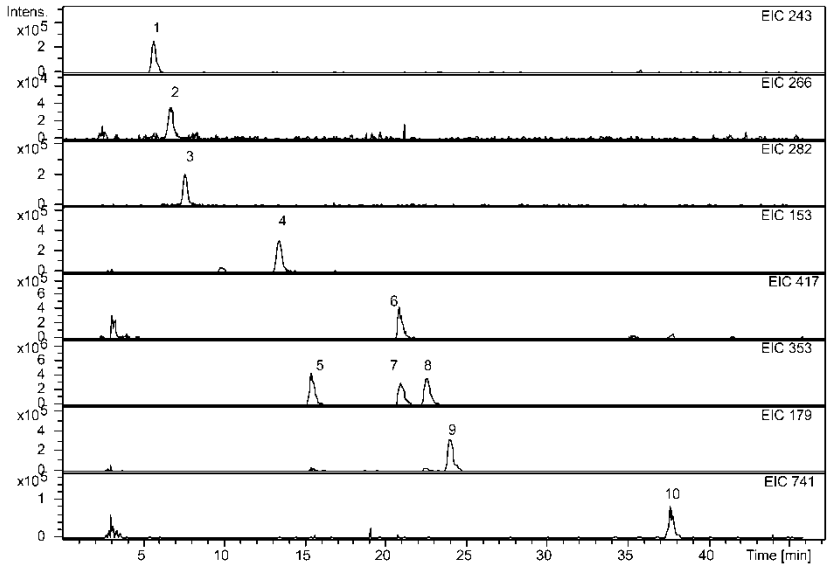

Figure 3. EICs of components in the A.G,1–10 are as in Figure 2.

In the negative ESI mode, the deprotonated molecule of $[M\text{-}H]^-$ was mainly generated, and adductive ions of $[[M\text{-}H + HCOOH]^-$ and $[M\text{-}2H + Na]^-$ were also observed in the mass spectra. The mass spectra of components were as in Figure 4.

It also should be noted that, in Figure 2, compound 6 (syringin) and 7 (chlorogenic acid) were co-eluting, which would not be distinguished without the detection of mass spectrometry.

In Figure 3, the EIC of m/z 353 has 3 peaks, and the middle peak has been identified as that of chlorogenic acid (3-O-caffeoylquinic acid) by comparison with the authentic sample. The 3 peaks have almost the same UV spectra, mass spectra, and fragments of their MS/MS spectra. The overlaid UV spectrum of the 3 peaks was shown in Figure 6. As we have known, the caffeoylquinic acid has 3-, 4-, 5-O-substitutes, so the other 2 components were probably the 4-, or 5-O-caffoylquinic acid. Further structure confirmation would be made by NMR and IR after preparation of these compounds.

Attributes of MS and MS/MS Spectra of Components in the A.G

Compound 1: Uridine (MW 244) m/z: $311[M\text{-}2H + Na + HCOOH]^-$, $243[M\text{-}H]^-$, $200[M\text{-}H\text{-}NHCO]^-$, $111[M\text{-}H\text{-}rib]^-$. Compound 2: Adenosine

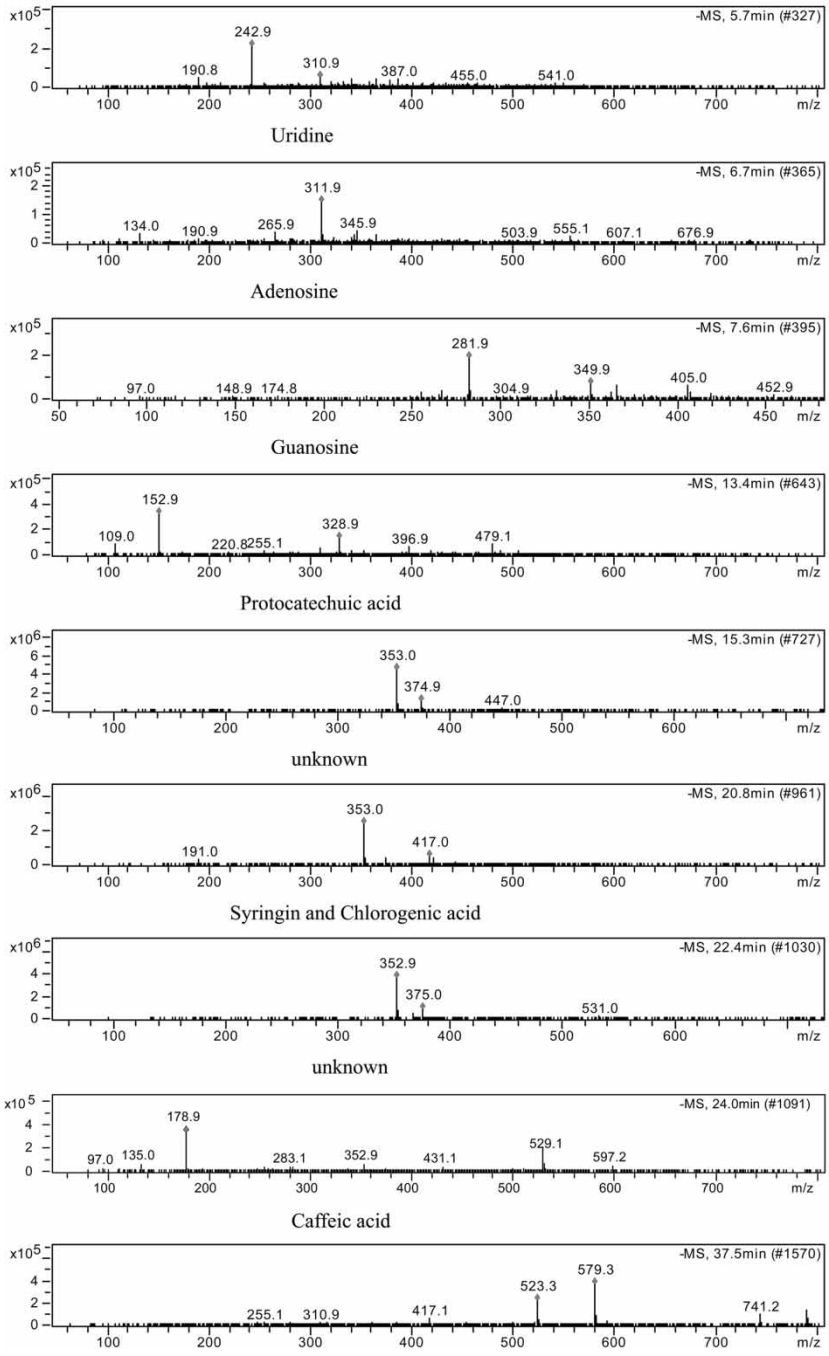

Figure 4. MS spectra of components in the A.G.

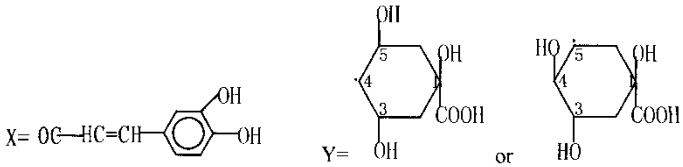

Figure 5. Structure of X, Y.

(MW 267) m/z: 312[M-H + HCOOH]$^-$, 266[M-H]$^-$, 134[M-H-rib]$^-$. Compound 3: Guanosine (MW 283) m/z: 350[M-2H + Na + HCOOH]$^-$, 282[M-H]$^-$, 150[M-H-rib]$^-$. Compound 4: Protocatechuic acid (MW 154) m/z: 329[2M-2H + Na]$^-$, 153[M-H]$^-$, 109 [M-H-CO_2]$^-$. Compound 5 and 8: Unknown (MW 354) m/z: 375[M-2H + Na]$^-$, 353[M-H]$^-$, 201[M-2H + Na-Y]$^-$, 191[M-H-X]$^-$, 179[M-H-Y]$^-$, 135[M-H-Y-CO_2]$^-$(structure of X, Y was shown in Fig. 5). Compound 6: Syringin (MW 372) m/z: 417[M-H + HCOOH]$^-$, 371[M-H]$^-$, 209 [M-H-glc]$^-$. Compound 7: Chlorogenic acid [3-O-caffeoylquinic acid] (MW 354) m/z: 353[M-H]$^-$, 191[M-H-X]$^-$, 135[M-H-Y-CO_2]$^-$. Compound 9: Caffeic acid (MW 180) m/z: 179[M-H]$^-$, 135[M-H-CO_2]$^-$. Compound 10: Liriodendrin (MW 742) m/z: 741[M-H]$^-$, 579 [M-H-glc]$^-$, 417 [M-H-2glc]$^-$.

CONCLUSION

By the LC/DAD/ESI/MS^2 technique, 8 compounds in the stem bark of A.G have been identified, and 4 of them have not been reported in the A.G to date.

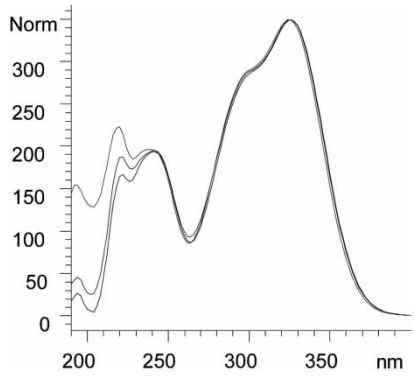

Figure 6. Overlaid UV spectra of peak 5, 7, and 8.

Another 2 quinic acid derivatives were also inferred by their UV spectra, mass spectra, and fragments of their MS/MS spectra. This paper provides a rapid, accurate, and powerful method for appraisal of the quality of A.G and other traditional Chinese medicines.

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