

HPLC and GC-MS Analysis of Phenolic Substances in *Acer tegmentosum*

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Abstract – The stem barks, heartwoods, and leaves of *Acer tegmentosum* (Aceraceae) are widely used in Korea to treat hepatic or cerebral disorders mainly due to alcohol poisoning. This study was aimed to analyze phenolic substances in *A. tegmentosum*. Quantitative analysis of the three phenolic substances (salidroside, (+)-catechin and scopoletin) was performed by HPLC and the identification of volatile phenolic substances were done by GC-MS. The contents of the three compounds in the three MeOH extracts were higher in the stem bark (salidroside: 80.22 mg/g, (+)-catechin: 23.31 mg/g, and scopoletin: 9.45 mg/g) compared to the heartwoods and leaves. And GC-MS analysis of the stem bark extract demonstrated that *p*-tyrosol is a main substance of twenty-one compounds identified.

Keywords – *Acer tegmentosum*, Aceraceae, Salidroside, HPLC, GC-MS

Introduction

Acer tegmentosum, a deciduous tree belonging to the Aceraceae family, is widely used in Korea to prevent alcohol poisoning due to drunkenness, insomnia and stress.^{1,2} Several antioxidative constituents including salidroside, (+)-catechin, and quercitrin have been isolated from this plant's stem.³

It has been reported that salidroside attenuates cognitive dysfunction through the inhibition of oxidative stress and inflammatory mediators induced by NF-κB activation.⁴ Salidroside also prevents a glutamate-induced apoptotic cell death in the hippocampal neurons of rats⁵ as well as a tacrine-induced hepatotoxicity in hepatocytes.⁶ In addition, the extract of *Rhodiola rosea* containing salidroside has anxiolytic, cognitive, and antidepressive-like effects.⁷ *R. rosea* containing salidroside exhibits its antidepressant effect through a pathway other than the benzodiazepine site of the GABA_A receptor.⁸ (+)-Catechin⁹ and scopoletin¹⁰ have been reported to have hepatoprotective activities, although the actions on the central nervous system (CNS) are still unclear.

In the present study, we comparatively examined the contents of salidroside, (+)-catechin, and scopoletin among

the stem bark, heartwood, and leaf of *A. tegmentosum* by HPLC. This study will help us decide which compound is responsible for the folkloric medicinal use, and which part of the plant is the crude drug. GC-MS study was also undertaken to identify volatile phenolic substances in the extract.

Experimental

Instruments and reagents – The HPLC system consists of a ProStar 210 pump, a ProStar 325 UV-Vis detector, and a Shiseido Capcell PAK C18 column (5 μm, 4.6 mm × 250 mm, Japan) for analysis. The HPLC solvents of H₂O and MeOH were purchased from J.T.Baker Co. (Phillipsburg, NJ, USA). Data obtained from the HPLC analysis were processed by a Varian Star Workstation.

Plant materials – Stem barks, stems, and leaves of *A. tegmentosum* were purchased from the Korean traditional market in Jinbu, Pyongchang-gun, Gangwon-do, Korea. These plant materials were identified by Prof. Sang-Cheol Lim, Department of Horticulture and Landscape Architecture, Sangji University. The stems were peeled to obtain the heartwood parts. The stem barks, heartwoods, and leaves were sufficiently dried under shadow and then cut before extraction. The voucher specimens (#natchem-52 for stem barks, #natchem-53 for heartwoods, and #natchem-54 for leaves) were deposited at the Laboratory of Natural Products

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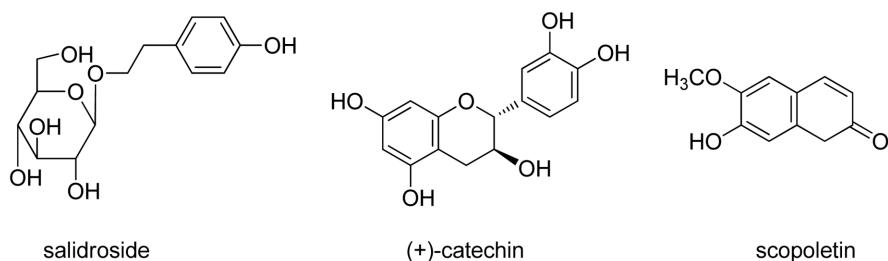


Fig. 1. Structure of salidroside, (+)-catechin, and scopoletin from *A. tegmentosum*.

Chemistry, Department of Pharmaceutical Engineering, Sangji University.

Isolation of salidroside, (+)-catechin, and scopoletin – The stem barks of *A. tegmentosum* (1.00 kg) were extracted with MeOH (each, 6.0 L) under reflux for 5 h three times. The extracted solution was filtered and dried under reduced pressure to give a viscous MeOH extract, which were further dried using a freeze-drier. The MeOH extract was divided into CHCl₃- and BuOH fractions by a successive solvent fractionation. The BuOH fraction (5.0 g) was subjected to silica gel column (SiO₂, Art No. 7734, Merck, Germany, 320 g, 5 × 30 cm) chromatography. The eluate was collected to give seven fractions (I - VII) and monitored by checking a TLC. Fractions III and VI were crystallized in MeOH to yield (+)-catechin (64 mg) and salidroside (0.502 g) which were identified by comparisons of mp and ¹H- and ¹³C-NMR data with the literature data.^{11,12} The CHCl₃ fraction (4 g) was chromatographed on silica gel column (320 g, 5 × 30 cm) to yield scopoletin (48 mg) which was identified by comparisons of mp and ¹H- and ¹³C-NMR data with the literature data.¹³ The identifications were also confirmed by co-TLC and co-HPLC with authentic compounds. Chemical structures of the isolated compounds are shown in Fig. 1.

Extraction for quantification – For HPLC analysis, three parts (stem bark, heartwood, and leaf) of *Acer tegmentosum*, were used in this study. Twenty grams of dry material from each part were soaked in 250 mL of MeOH and extracted under reflux on a heating plate for 5 h at 80 °C. After cooling, the extract solution was filtered through a filter paper (Advantec, Toyo Ltd., Japan) and evaporated on a vacuum rotary evaporator. The extract was further dried by freeze-drying and then weighed.

HPLC analytical method – The mobile phase was 20% (v/v) aqueous MeOH added with 0.10% (v/v) of trifluoroacetic acid (TFA). The elution program was set at a flow rate of 0.8 mL/min under 25 °C column temperature, and recorded for 50 min. The injection volume for all samples was 20 µL and simultaneous monitoring was performed at 225 nm. The UV wavelength of 225 nm

was used because it was sensitive for simultaneous detection of salidroside, (+)-catechin, and scopoletin. The sample solutions were prepared by dissolving the three extracts in 60% (v/v) aqueous MeOH and filtered through a disposable syringe filter (0.50 µm, Dismic-25JP Advantec, Japan) prior to the injection onto the HPLC system.

Linearity of regression equation and determination of LOD (limit-of-detection) and LOQ (limit-of-quantification) – To assess the linearity of the regression equations, the standard stock solution (1000 µg/mL) was prepared by dissolving each standard compound in 60% (v/v) aqueous MeOH. The stock solutions were serially diluted in order to prepare working solutions of 31.25 - 1000.0 µg/mL for salidroside and 7.81 - 250.0 µg/mL for (+)-catechin and scopoletin. Calibration curve equations were determined by plotting the concentration (x axis) versus the peak area (y axis), and the linearity of equations was assessed by *R*² values. The values of LOD (limit-of-detection) and LOQ (limit-of-quantification) were determined by signal-to-noise (S/N) ratios of 3 and 10, respectively.

Gas chromatography - mass spectrometry – Volatile substances were analyzed by GC-MS under the following method. A capillary HP-5MS column (30 m × 0.25 mm; 0.25 µm 5% diphenyl-95% dimethylpolysiloxane) was equipped on a chromatograph (Agilent 6890N GC) coupled to a mass spectrometer (Agilent 5973N mass selective detector).

A 1.0 µL sample was injected with a pulsed splitless mode (injector temperature 260 °C, injector pulse pressure 10.00 psi, purge flow to split vent 60.0 ml/min) on a capillary column. The carrier gas (He) was flowed at the rate of 1.0 ml/min. The oven temperature was increased from 80 to 290 °C for 30 min. Mass spectrometry condition was as follows: solvent delay (3.0 min), full scan mode, mass range (40 - 550 amu), and scan velocity (2.89 scans/sec). Electron impact ionization voltage was 70 eV. Each compound was identified by comparisons of the *m/z* value and relative abundance of molecular and fragment ion peaks, which were compared with the spectra of standard substances (CAS numbered compound) in the database.

Results and Discussion

HPLC quantitative analysis – To optimize HPLC conditions, four parameters including mobile phase, flow rate, column temperature, and wavelength of the UV detector were considered. After optimization experiments, the following condition was chosen: isocratic elution with

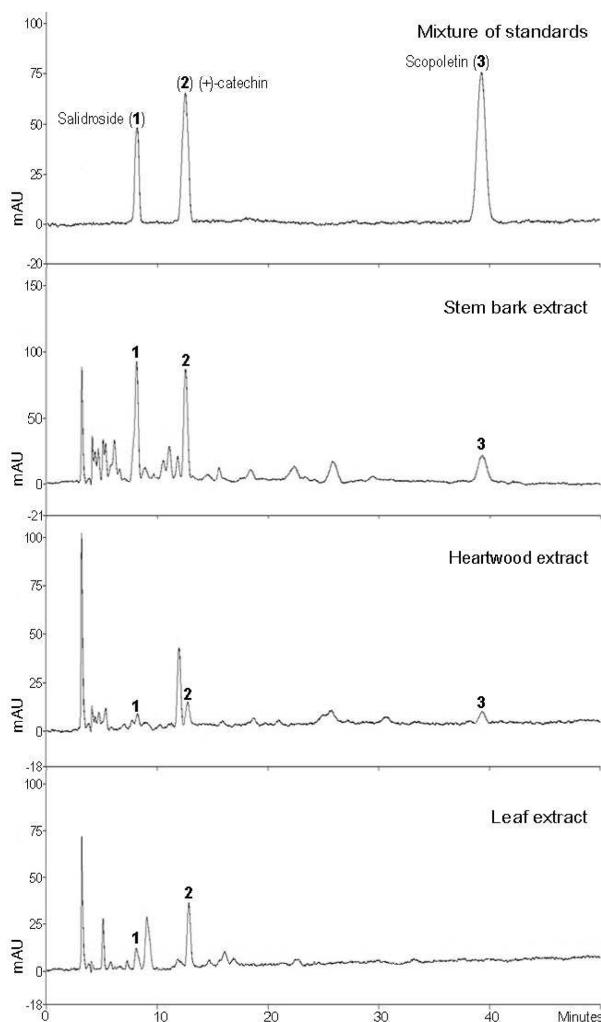


Fig. 2. HPLC chromatograms of mixed standard compounds and MeOH extracts of three parts of *A. tegmentosum*.

20% aqueous MeOH with 0.10% TFA as the mobile phase, at a flow rate of 0.80 mL/min, column temperature of 25 °C and 225 nm as the detection wavelength. This condition was ideal for separation and sensitivity on HPLC chromatogram.

As shown in Fig. 2, salidroside, (+)-catechin, and scopoletin were shown at the retention times of 8.15, 12.28, and 38.73 min, respectively. As shown in Table 1, the regression equations with $R^2 > 0.999$ were determined to verify their linearity. The LOD (0.95 - 5.86 µg/mL) and LOQ (3.19 - 19.54 µg/mL) values were low enough for HPLC detection and quantification, respectively.

The contents of the test compounds in the MeOH extracts were shown in Table 2 together with the extraction rate (%). The extraction rate was highest in the stem bark (12.7%), and it was lowest in the heartwood (2.65%). The extraction rate of the leaves was 10.8%. The content of salidroside was much higher in the stem bark extract (80.22 mg/g) compared to the extracts of heartwood (5.56 mg/g) and leaf (9.81 mg/g). (+)-Catechin was quantitatively higher in the stem bark (23.31 mg/g) than in the heartwood and leaf. The content of scopoletin in the stem bark (9.45 mg/g) was much lower than that of salidroside. Therefore, the stem bark is recommended as the active part of *A. tegmentosum*, since biological active substance is considered salidroside in this crude drug. Perfumi and Mattioli⁷ suggested that the extract of *Rhodiola rosea* containing salidroside has anti-depressant-like, adaptogenic, anxiolytic, and stimulating effects. However, the content of salidroside in *A. tegmentosum* (10.19 mg/g dried weight) was much higher than *R. rosea* (1.13 mg/g),⁸ when calculated as the unit of mg/g dried weight.

The pharmacological action of salidroside on the CNS includes anxiolytic and memory-enhancing activities. The mechanism of action is attributed to the reduction of oxidative stress and modulation of inflammatory mediators.⁴ Medicinal drugs acting on the CNS should pass through the blood-brain-barrier. Therefore, they usually have a low molecular weight and less polar properties. The natural products of carvacrol,¹⁴ α-asarone,¹⁵ and gastrodin¹⁶ with cognition-enhancing activities belong to such examples.

Table 1. Linearities and limits of detection and quantification (LOD - LOQ) of the analytes

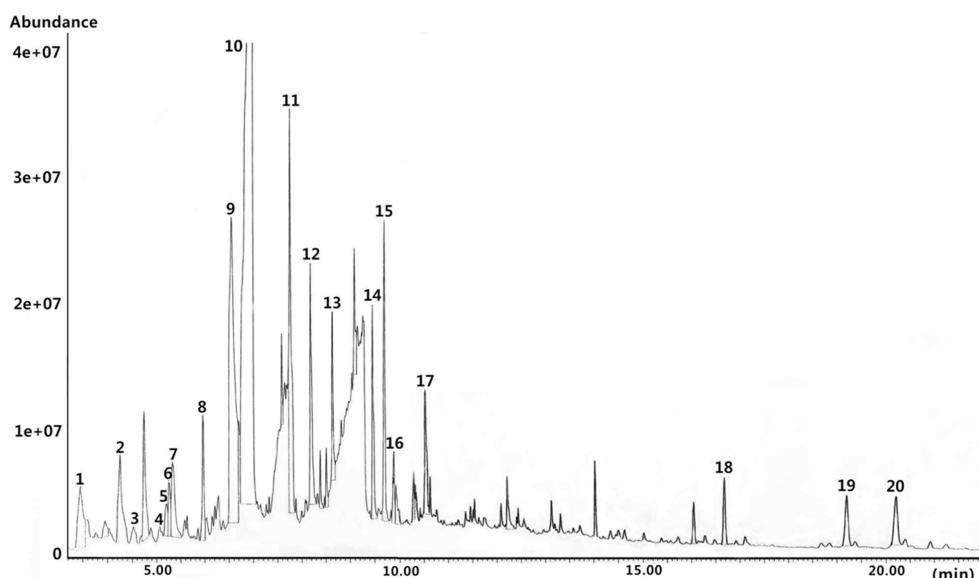
Analyte	t_R (min)	Equation of the linear regression ^a	Linear range (µg/mL)	R^2 ^b	LOD ^c (µg/mL)	LOQ ^d (µg/mL)
Salidroside	8.15	$y = 37.05x + 52.86$	31.25 - 1000.0	0.9995	5.86	19.54
(+)-Catechin	12.28	$y = 151.77x + 59.13$	7.81 - 250.0	0.9995	1.39	4.63
Scopoletin	38.73	$y = 212.75x + 66.17$	7.81 - 250.0	0.9996	0.95	3.19

^a y , peak area at 225 nm; x , concentration of the standard (µg/mL); ^b R^2 , correlation coefficient for 6 data points in the calibration curves ($n = 4$); ^c LOD, limit of detection ($S/N = 3$); ^d LOQ, limit of quantification ($S/N = 10$).

Table 2. Content of analytes in the MeOH extracts and dried materials of three parts of *Acer tegmentosum* (unit, mg/g)

Plant part	Yield of extract (%)	Salidroside			(+)-Catechin			Scopoletin			Sum	
		extract	DW	RSD	extract	DW	RSD	extract	DW	RSD	extract	DW
Stem bark	12.7	80.22	10.19	0.85	23.31	2.96	0.75	9.45	1.20	0.81	112.98	14.35
Heartwood	2.65	5.56	0.15	2.98	4.89	0.13	1.37	0.84	0.02	2.39	11.29	0.30
Leaf	10.8	9.81	1.06	1.45	7.79	0.84	0.33	< LOQ	—	—	17.60	1.90

DW: dry weight of plant material. RSD: relative standard deviation (%) for $n = 3$.

**Fig. 3.** GC chromatogram of the MeOH extract of *A. tegmentosum*.

Tyrosol, which is the aglycone of salidroside, may be the active moiety, since the former compound could be formed through the hydrolysis of the latter in the body. The pharmacological actions of salidroside other than CNS have been also reported: antioxidative,³ glucose-uptake¹⁷ and hepatoprotective effects.⁶ Therefore, *A. tegmentosum* with high content of salidroside could show pharmacological activities on both CNS and other tissues. (+)-Catechin and scopoletin were not main substance in *A. tegmentosum*.

GC-MS analysis – Gas chromatogram of the MeOH extract of the stem bark is shown in Fig. 3. The mass spectra of identified compounds were in good agreement with the mass spectra of CAS numbered compounds in database, and the quality ratio (%) was also noted (Table 3). Twenty-one compounds were identified: fatty acids (caprylic acid, palmitic acid, linoleic acid, 2-palmitoylglycerol), maltols (maltol, 3-hydroxy-2,3-dihydromaltol, and hydroxymaltol), a furfural (5-hydroxymethylfurfural), simple phenols (catechol, 4-vinylphenol, 4-vinylguaiacol, pyrogallol, and 3,5-dimethoxy-4-hydroxybenzyl alcohol),

phenylethanoids (phenethyl alcohol, *p*-tyrosol, homovanillic acid, and methyl homovanillate), phenylpropanoids (dihydrostyrinyl alcohol, scopoletin), and steroids (stigmasterol, β -sitosterol). In Table 1, mass spectra of the standard compounds that have been compared with each compound of the peak were noted as the chemical abstract number (CAS#). Of these, *p*-tyrosol, which is the aglycone of salidroside, occupied 40.64% of the peak areas on GC chromatogram, suggesting that it is the predominant substance of the observed peaks.

In plants, it is known that simple phenols like phenol, catechol and pyrogallol could be produced from the lignin structure of the tree by the mechanism of free radical-chain reaction during the process of pyrolysis or toasting.¹⁸ Furthermore, volatile substances like maltol derivatives can be generated from sugars by a heating process.¹⁹ Therefore, it cannot be excluded that these substances could be generated during a drying process period or during the growth of this tree. However, the most abundant compound, *p*-tyrosol, is the aglycone of salidroside rather than artifact, suggesting that it could be considered as one

Table 3. GC-MS data and identification of the constituents from *Acer tegmetosum*

Peak	<i>t</i> _R (min)	Identity	<i>m/z</i> (%) M ⁺	Fragment ion (%)	Peak area (%)	Quality (%)	CAS #
1	3.46	Caprylic acid	144 (5.7) [C ₈ H ₁₆ O ₂] ⁺	87 (15.7), 73 (62.8), 60 (base)	2.55	80	000142-62-1
2	4.26	maltol	126(78.3) [C ₇ H ₈ O ₂] ⁺	95 (19.8), 56 (43.5)), 43 (base)	3.99	80	000118-71-8
3	4.50	Phenethyl alcohol	122 (25.7) [C ₈ H ₁₀ O] ⁺	91 (base), 65 (20), 51 (7.1)	1.10	85	000060-12-8
4	4.75	3-OH-2,3-diHmaltol	144 (44.2) [C ₆ H ₈ O ₄] ⁺	101 (39.1), 72 (29.4), 43 (base)	2.63	95	028564-83-2
5	5.06	Hydroxymalton	142 (base) [C ₆ H ₆ O ₄] ⁺	113 (17.1), 85 (27.1), 43 (72.8)	1.33	90	001073-96-7
6	5.21	Catechol	110 (base) [C ₆ H ₆ O ₂] ⁺	81 (20.0), 64 (24.0), 43 (18.6)	0.73	93	000120-80-9
7	5.26	4-Vinylphenol	120 (base) [C ₈ H ₈ O] ⁺	91 (52.9), 65 (21.9), 39 (17.1)	0.93	90	000027-44-3
8	5.33	5-hydroxymethylfurfural	126 (58.6) [C ₆ H ₆ O ₃] ⁺	97 (base), 69 (41.4), 41 (65.7)	2.19	99	000067-47-0
9	5.96	4-Vinylguaiacol	150 (base) [C ₉ H ₁₀ O ₂] ⁺	135 (91.4), 107 (41.4), 77 (54.2)	1.85	93	007786-61-0
10	6.57	Pyrogallol	126 (base) [C ₆ H ₆ O ₃] ⁺	108 (28.6), 80 (34.3), 52 (50.0)	13.28	98	000533-73-3
11	6.98	p-Tyrosol	138 (70.0) [C ₈ H ₁₀ O ₂] ⁺	107 (base), 77 (65.7), 51 (21.4)	40.64	94	000501-94-0
12	8.20	Homovanillic acid	182 (41.4) [C ₁₀ H ₁₄ O ₃] ⁺	137 (base), 122 (14.3), 60 (15.7)	3.52	99	000306-08-1
13	8.51	3,5-diOCH ₃ -4-OHbenzyl alcohol	184 (base) [C ₉ H ₁₂ O ₄] ⁺	167 (34.4), 141 (14.8), 123 (42.5)	0.63	98	000530-56-3
14	8.65	Methyl homovanillate	196 (32.9) [C ₁₀ H ₁₂ O ₄] ⁺	137 (base), 122 (11.4), 94 (18.6)	1.86	84	000306-08-1
15	9.47	Dihydrosinapyl alcohol	212 (67.1) [C ₁₁ H ₁₆ O ₄] ⁺	168 (base), 153 (20.0), 137 (18.6)	2.51	94	2000235-37-2
16	9.71	Palmitic acid	256 (28.6) [C ₁₆ H ₃₂ O ₂] ⁺	213 (22.9), 129 (37.1), 73 (base)	3.51	99	000057-10-3
17	9.93	Scopoletin	192 (base)	177 (62.6), 149 (47.5), 121 (19.3)	0.60	96	000092-61-5
18	10.55	Linoleic acid	358 (2.9) [C ₁₈ H ₃₄ O ₂] ⁺	264 (14.3), 107 (38.6), 55 (base)	1.99	99	000060-33-3
19	12.18	2-Palmitoylglycerol	330 (0.0) [C ₁₉ H ₃₈ O ₄] ⁺	299 (8.9), 239 (46.3), 98 (base)	0.83	94	023470-00-0
20	19.21	Stigmasterol	412 (51.4) [C ₂₉ H ₄₈ O] ⁺	394 (5.7), 255 (51.4), 55 (base)	1.27	99	000083-48-7
21	20.21	β-Sitosterol	414 (55.7) [C ₂₉ H ₅₀ O] ⁺	396 (28.6), 303 (35.7), 43 (base)	1.40	99	000083-46-5

Base (100% as relative intensity); ND (not detected)

of the active principles. The compounds identified from the present GC-MS have not been reported from *A. tegmentosum*.

In conclusion, the stem bark extract contained higher amounts of salidroside, (+)-catechin, and scopoletin than the extracts of the heartwood and leaf. GC-MS analysis of the extract demonstrated that *p*-tyrosol is a main substance of the detected ones. In particular, the stem bark extract of *A. tegmentosum* with a high content of salidroside could be used to treat patients suffering from CNS or hepatic disorders.

Acknowledgments

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