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Antioxidative Activity of *Prunus sargentii* Outer Bark Extractives*1

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ABSTRACT

The outer bark of *Prunus sargentii* was collected, air-dried and extracted with 70% aqueous acetone. Then it was successively partitioned with *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and H_2O. From the EtOAc soluble fraction, four compounds were isolated by the repeated Sephadex LH-20 column chromatography. The isolated compounds were determined as (+)-catechin (**1**), (-)-epicatechin (**2**), taxifolin (**3**), and neosakuranin (**4**) by the spectroscopic analysis including ^{1}H, ^{13}C-NMR, and 2D-NMR spectrometers. The antioxidative activities on the isolated compounds and the separated fractions were evaluated by DPPH radical scavenging assay. The crude, EtOAc, and H_2O soluble fractions indicated good antioxidative potential compared to the CH_2Cl_2 and *n*-hexane soluble fractions.

Keywords : *Prunus sargentii*, bark, EtOAc fraction, neosakuranin, antioxidant

1. INTRODUCTION

Prunus sargentii Rehder (Rosaceae) is a native cherry tree to Korea, Japan and Sakhalin (Russia). This is a deciduous tree that grows 6 ~8 m in height. The crown spreads to a width of 6~8 m and can tolerate wind, but not air pollution[1]. The tree is suitable for use as a roadside tree. The bark has been used for the treatment of inflammatory or skin diseases. The main constituents of the species are taxifolin, naringenin, pinostobin including sakuranin[1] and the domestic previous works have reported antioxidant, immunosuppression and tyrosinase inhibition activities on the heartwood extractives of *Prunus sargentii*[1,2]. However, they have not studied on the chemical constituents and biological activities of the bark which can have rather the higher functional potential.

Thus, this work was to carried out to study the chemical constituents and antioxidative activity of the outer bark to evaluate the bioactive potential for the functional uses.

2. MATERIALS and METHODS

2.1. Plant Material

The bark of *P. sargentii* was collected in April 2011 on the campus forest, Kangwon

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National University and debarked. Bark were dried for four weeks at room temperature and then ground to a fine powder using a Wiley mill before extraction.

2.2. General Experimental

^{1}H, ^{13}C-NMR, including 2D-NMR HMBC (Heteronuclear Multiple Bond Correlation) and HMQC (Heteronuclear Multiple Quantum Correlation) were measured with a Bruker DPX 400 (Germany) spectrometer using tetramethylsilane (TMS) as an internal standard, and chemical shift were given in δ (ppm). Column chromatography was done on a lipophilic Sephadex LH-20 column. TLC was performed on DC-Plastikfolien Cellulose F (Merck, Germany) plates and developed with TBA [*t*-BuOH-HOAc-H_2O (3 : 1 : 1, v/v/v)] and 6% aqueous acetic acid (HOAc). Spots were detected under UV (254 and 365 nm) radiation and spraying with vanillin [vanillin : HCl : EtOH (4.8 : 12 : 480, w/v/v)] followed by heating.

2.3. Extraction and Isolation

The dried bark powder (780 g) was extracted with 70% aqueous acetone for 5 days at room temperature. The filtrates were combined and evaporated using a rotary evaporator under reduced pressure at 40°C. The crude extract was suspended in H_2O and then successively partitioned using *n*-hexane, CH_2Cl_2, and EtOAc.

A portion of the EtOAc fraction (9.46 g) was chromatographed on a Sephadex LH-20 column using aqueous MeOH-H_2O (1 : 1, v/v) to give 5 fractions (Fr. 1～5). Fr. 2 was reapplied on a Sephadex LH-20 column with MeOH-H_2O (3:7, v/v) to give compound **1** (148.9 mg). From Fr. 2-1 was rechromatographed using MeOH-H_2O (2 : 3, v/v) to get compound **2** (44.4 mg). Fr. 2-4 and 2-5 were further treated on a Sephadex LH-20 column with EtOH-hexane (3 : 1, v/v) to separate compound **3** and **4** (11.1 mg).

2.3.1. Compound 1

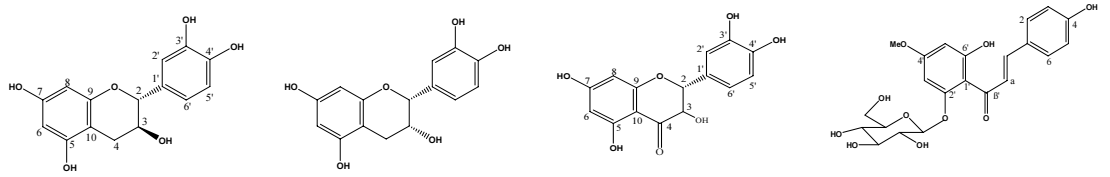
^{1}H-NMR (400 MHz, δ, CD_3OD) : 2.41 (1H, *dd*, *J* = 8.1 and 16.1 Hz, H-4ax), 2.75 (1H, *dd*, *J* = 5.4 and 16.1 Hz, H-4eq), 3.89 (1H, *m*, H-3), 4.47 (1H, *d*, *J* = 7.5 Hz, H-2), 5.77 (1H, *d*, *J* = 2.2 Hz, H-6), 5.84 (1H, *d*, *J* = 2.2 Hz, H-8), 6.64 (1H, *dd*, *J* = 1.8 and 8.1 Hz, H-6′), 6.68 (1H, *d*, *J* = 8.1 Hz, H-5′), 6.75 (1H, *d*, *J* = 1.8 Hz, H-2′). ^{13}C-NMR (100 MHz, δ, CD_3OD) : 28.55 (C-4), 68.84 (C-3), 82.88 (C-2), 95.53 (C-8), 96.32 (C-6), 100.85 (C-10), 115.28 (C-2′), 116.12 (C-5′), 120.08 (C-6′), 132.24 (C-1′), 146.26 (C-3′), 146.28 (C-4′), 156.95 (C-9), 157.61 (C-5), 157.86 (C-7).

2.3.2. Compound 2

^{1}H-NMR (400 MHz, δ, CD_3OD) : 2.63 (1H, *dd*, *J* = 2.9 and 16.8 Hz, H-4ax), 2.83 (1H, *dd*, *J* = 4.5 and 16.7 Hz, H-4eq), 4.08 (1H, *m*, H-3), 4.75 (1H, *s*, H-2), 5.81 (1H, *d*, *J* = 2.4 Hz, H-6), 5.84 (1H, *d*, *J* = 2.4 Hz, H-8), 6.75 (1H, *d*, *J* = 7.5 Hz, H-5′), 6.80 (1H, *dd*, *J* = 1.8 and 8.3 Hz, H-6′), 6.8 (1H, *d*, *J* = 1.8 Hz, H-2'). ^{13}C-NMR (100 MHz, δ, CD_3OD) : 28.28 (C-4), 66.49 (C-3), 78.87 (C-2), 94.94 (C-8), 95.44 (C-6), 99.12 (C-10), 114.34 (C-2′), 114.94 (C-5′), 118.45 (C-6′), 131.30 (C-1′), 144.77 (C-3′), 144.94 (C-4′), 156.38 (C-9), 156.65 (C-5), 157.00 (C-7)

2.3.3. Compound 3

^{1}H-NMR (400 MHz, δ, CD_3OD) : 4.50 (1H, *d*, *J* = 11.5 Hz, H-3), 4.96 (1H, *d*, *J* = 11.6 Hz, H-2), 5.89 (1H, *d*, *J* = 2.0 Hz, H-6), 5.92 (1H, d, *J* = 1.6 Hz, H-8), 6.80 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.84 (1H, *dd*, *J* = 1.8 and 9.2 Hz, H-6′), 6.96 (1H, *d*, *J* = 1.8 Hz, H-2′). ^{13}C-



(+)-catechin (1) (-)-epicatechin (**2**) Taxifolin (**3**) Neosakuranin (**4**)

Fig. 1. Chemical structures of the isolated compounds from the P. sargentii outer bark.

NMR(100 MHz, CD_3OD) : 73.689 (C-3), 85.14 (C-2), 96.58 (C-8), 97.34 (C-6), 102.00 (C-10), 116.17 (C-2′), 116.36 (C-5′), 120.93 (C-6′), 130.3 (C-1′), 146.33 (C-3) 147.16 (C-4′), 164.57 (C-9), 165.32 (C-5), 168.72 (C-7), 198.54 (C-4).

2.3.4. Compound 4

^{1}H-NMR (400 MHz, δ, CD_3OD) : 3.24～3.55 (4H, m, H-2″,3″,4″,5″), 3.72 (1H, *dd*, J = 5.8 and 16.1 Hz, Ha-6″), 3.81 (3H, s, OCH_3), 3.91 (1H, *dd*, J = 2.1 and 12.2 Hz, Hb-6″), 5.17 (1H, *d*, J = 7.5 Hz, H-1″), 6.15 (1H, *d*, J = 2.2 Hz, H-5′), 6.34 (1H, *d*, J = 2.3 Hz H-3′), 6.83 (2H, *d*, J = 8.6 Hz, H-3,5), 7.61 (2H, *d*, J = 8.7 Hz, H-2,6), 7.70 (1H, *d*, J = 15.5 Hz, H-β), 8.01 (1H, *d*, J = 15.3 Hz, H-α). ^{13}C-NMR (100 MHz, δ, CD_3OD) : 54.75 (4′-OMe), 61.04 (C-6″), 69.88 (C-4″), 73.67(C-2″), 77.10 (C-3″), 77.20 (C-5″), 93.63 (C-3′), 95.15 (C-5′), 100.58 (C-1″), 106.95 (C-1′), 115.51 (C-3,5), 124.38 (C-α), 127.03 (C-1), 130.48 (C-2,6), 143.20 (C-β), 159.81 (C-22), 159.99 (C-4), 165.79 (C-6′), 166.19 (C-4′), 193.35 (C=O).

2.4. Antioxidative activity (DPPH Radical Scavenging Assay)

The antioxidative activity was performed on the basis of their radical scavenging activity in the presence of DPPH (2,2-Diphenyl-1-picryl-hydrazyl) free radical using the method introduced by Blois[3] with slight modification. Samples of different concentration (50, 100, 250, 500, and 1000 ppm) were added to a solution of DPPH (0.15 mM) in MeOH. After mixing gently and standing at room temperature for 30 min in the dark, the absorbance was measured at 540 nm with a UV-visible spectrophotometer (Libra S32, Biochrom LTD). Ascorbic acid was used as a control for the comparison.

The values were calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1 - \frac{Ab(Control) - Ab(Sample)}{Ab(Control)} \times 100]$$

2.5. Total Polyphenol Content

Total polyphenolic content was evaluated using the Folin-Ciocalteu method[4] with a slight modificattion. The crude extract solution was mixed with Folin-Ciocalteu reagent for 3 min and sodium carbonate (Na_2CO_3) was then added. After incubation at room temperature for 1 hr, the absorbance of the reaction mixture was measured at 725 nm against a MeOH blank. Caffeic acid was used as a standard to calibrate the curve (y = 1.0027x + 0.0119 (R^2 = 0.9997)).

3. RESULTS and DISCUSSION

As shown in Fig. 1, four compounds were isolated by repeated Sephadex LH-20 column

Table 1. DPPH radical scavenging activity (%) of the separated fractions from the *P. sargentii* outer bark

Sample	DPPH radical scavenging activity (%)				
	50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm
Ascorbic acid	98.07	98.25	98.29	98.35	98.29
Crude	37.72	65.62	96.97	96.73	96.31
n-hexane	3.51	2.74	3.33	9.04	28.26
CH_2Cl_2	2.68	7.91	21.71	43.07	70.43
EtOAc	44.91	69.78	95.95	96.43	96.61
H_2O	27.07	45.21	89.73	87.45	82.81

Table 2. Total polyphenol content of the extractives of the *P. sargentii* outer bark

	Sample	Polyphenol content (mg/100 g)
Fractions	Crude	475.22
	n-hexane	35.47
	CH_2Cl_2	189.42
	EtOAc	610.28
	H_2O	387.32

chromatography of EtOAc soluble fraction.

Compound **1** and **2** are very common in many plant sources and characterized as (+)-catechin and (-)-epicatechin using authentic literature data[5].

Compound **3** was isolated from the EtOAc soluble fraction of the outer bark as a yellowish amorphous powder. On a cellulose TLC plate, it exhibited positive reactions with vanillin-HCl-EtOH. Compound **3** was also elucidated taxifolin (3′,4′,5,7-tetrahydroxyflavanonol), which was in good agreement with the previous literature[6-8].

Compound **4** was obtained as a yellowish amorphous powder. In the ^{1}H-,^{13}C-NMR and 2D-NMR spectrum, the compound **4** showed the presence of a pair of *meta*-coupled aromatic protons at δ 6.34, 6.15 (1H, *d*, *J* = 2.3, 2.2 Hz, H-3′,5′), *ortho*-coupled aromatic protons at δ 6.83, 7.61 (2H, *d, J*= 8.6, 8.7 Hz, H-3,5 and H-2,6) and a pair of *trans*-configuration protons at δ 8.01, 7.70 (1H, *d, J* = 15.3, 15.5 Hz, H-α, β). Also, the anomeric proton gave rise to a doublet signal at δ 5.17 and its coupling constant 7.5 Hz indicating the presence of the β-glucose moiety. The other proton signals appeared at 3.24～3.55, which is characteristic of typical glucose protons. The aromatic methoxyl group was observed at δ 3.81 (3H, s, -OCH3) [9]. The characteristic *trans*-configuration protons at δ 8.01, 7.70 and the ^{13}C-NMR signal at δ 193.35 (C=O) suggested that compound **4** was a chalcone. The connectivity of glucose moiety methoxyl group was determined based on HMBC. In the HMBC spectrum of **4**, correlation of methoxyl proton (δ 3.81, s), H-3′ (δ 6.34, s), and H-5′ (δ 6.15, s) with C-4′ (δ 166.19) was observed. HMBC correlation of the anomeric proton (δ 5.17, *d, J* =7.5 Hz, H-1″) with the carbon at δ 159.81 (C-2′) indicated the presence of sugar moiety at C-2′ position[10,11]. According to the above data comparison with the literature[11,12], the compound **4** was determined as neosakuranin, a chalcone glucoside, and reported for the first time in domestic from the outer bark of *P. sargentii*.

The antioxidative activity of the partitioned fractions including crude extractive was eval-

uated with the DPPH assay. As shown in Table **1**, the crude, EtOAc and H_2O fractions indicated higher antioxidative activity compared to CH_2Cl_2 and *n*-hexane fractions. The values were similar to the ascorbic acid as a control. Also, the polyphenol contents of the crude, EtOAc and H_2O fractions were higher than CH_2Cl_2 and *n*-hexane fraction, suggesting that the polyphenolic contents of the extractives could be one of important factors to improve the DPPH radical scavenging activity[13] and that the antioxidative activities of the each separated fractions were increased in proportion to the total phenolic contents (Table **2**). The isolated compounds, (+)-catechin (**1**), (-)-epicatechin (**2**), and taxifolin (**3**), were also indicated good antioxidative potentials using α-tocopherol (26 μg) and BHT (30 μg) (Butylated Hydroxy Toluene) as controls. The IC_{50}values were 11 (**1**), 12 (**2**), and 19 (**3**), separately[14]. Therefore, this fact means that the crude, EtOAc and H_2O fractions of the higher phenolic contents can have better antioxidative activities compared to the other fractions.

4. CONCLUSIONS

This study was carried out to investigate the chemical constituents and DPPH radical scavenging activity of the outer bark of *Prunus sargentii.* The dried sample was extracted with 70% aqueous acetone, concentrated under reduced pressure, and successively partitioned with *n*-hexane, CH_2Cl_2, EtOAc. A portion of the ethyl acetate fraction was chromatographed on a Sephadex LH-20 column using aqueous MeOH and EtOH-hexane mixture, and the isolated compounds were characterized as (+)-catechin (**1**), (-)-epicatechin (**2**), taxifolin (**3**), and neosakuranin (**4**) on the basis of spectroscopic analysis. The antioxidative values of compound 1,2, and 3 by IC_{50} were 11, 12, and 19, respectively.

The antioxidative activities of the crude and *n*-hexane, and EtOAc fractions were evaluated by measuring the total polyphenol content and DPPH radical scavenging activity. The results indicated that crude, EtOAc, and H_2O soluble fractions showed higher antioxidative potential compared to the CH_2Cl_2 and *n*-hexane fractions. The above results suggest that the antioxidative activity can be in proportion to the total polyphenol content of the extractives.

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REFERENCE

1. Lee, H. J., S. S. Lee, D. H. Choi, and A. Kato. 2001. Studied on Biological Activity of Wood Extractives -Flavanoids in heartwood of *Prunus sargentii*.-. Mokchae Konghak 29(2): 133～139
2. Lee, S. S., H. J. Lee, and D. H. Choi. 2001. Studied on Biological Activity of Wood ExtractivesⅧ: Antimicrobial and Antioxidation Activities of Extractives from the Heartwood of *Prunus sargentii.*-Mokchae Konghak 29(2): 140～145
3. Biois, M. S. 1985. Antioxidant determinations by the use of a stable free radical. Natural 26: 1199～1200.
4. Gutfinger, T. 1981. Polyphenols in olive oils. Journal of the American Oil Chemists' Society 58: 966～968.
5. Harborne, J. B. and T. J. Marby. 1982. The flavonoids: advances in research, Chapman and Hall LTd., London, UK, 6: 35～37.
6. Agrawal, P. K. 1989. Carbon-13 NMR of flavanoids, Elsevier, New York.
7. Ishimaru, K., T. Omoto, I. Asai, K. Ezaki, and K, Shimomura. 1995. Taxifolin 3-arabinoside from *Fragariax ananassa.* Phytochemistry 40(1): 345～347.

8. Jenkins, T., J. Bhattacharyya, G. Majetich, Q. Teng, A. M. Fatima, and R. Almeida. 1999. Flavonoids from the root-bark of *Dioclea grandiflra.* Phytochemistry 52(4): 723～730.
9. Bhatt, L. R, M. S. Bae, B. M. Kim, G. S. Oh, and K. Y. Chai. 2009. A Chalcone Glycoside from the Fruits of *Sorbus commixta* Hedl. Molecules 14: 5323～5327.
10. Jayaprakasam, B., D. Gunasekar, K. V. Rao, A. Blond, and B. Bodo. Androechin. 2001. A new chalconeglucoside from *Andrographis Echioides.* J. Asian Nat. Prod. 3; 43～48.
11. Zhang, X. F., T. M. Hung, P. T. Phuong, T. M. Ngoc, B. S. Min, K. S. Song, Y. H. Seong, and K. H. Bae. 2006. Anti-inflammatory activity of flavonoids from *Populus davidiana.* Arch. Pharm. Res. 29: 1102～1108.
12. Ogawa, Y., H. Oku, E. Iwaoka, M. Iinuma, and K. Ishiguro. 2006. Allergy-preventive phenolic glycosides from *Populus sieboldii.* J. Nat. Prod. 69: 1215～1217.
13. Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, and N. Vidal. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97(4): 654～660
14. Si, C. L. 2006. Studies on the Chemical Constituents and Antioxidative Activities of *Paulownia coreana Uyeki.* Ph. D. Thesis.