Natural Product Sciences
14(3) : 202-205 (2008)

Free Radical Scavengers from the Fruits of *Paeonia suffruticosa*

Sa Im Lee[1], Jae-Heon Yang, Nam-In Baek[2], Sung-Hoon Kim[3], Chong Hyeon Cho[4], Byung-Hyun Park[5], Byeong Suk Chae[1], and Dae Keun Kim[1,*]

[1]*College of Pharmacy, Woosuk University, Samrye 565-701, Korea*
[2]*Graduate School of Biotechnology & Plant Metabolism Research Center, KyungHee University, Suwon 449-701, Korea*
[3]*College of Oriental Medicine, KyungHee University, Seoul 131-701, Korea*
[4]*Jeollabuk-do Agricultural Research and Extension Servieces, Iksan 570-140, Korea*
[5]*Department of Biochemistry, Medical School, Chonbuk National University, Jeonju 561-756, Korea*

Abstract – The antioxidant activity of *Paeonia suffruticosa* (Paeoniaceae) was determined by measuring the radical scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl). The methanolic extract of *P. suffruticosa* showed the strong antioxidant activity. The antioxidant activity of ethyl acetate soluble fraction was stronger than that of the others (IC_{50}, 1.2 μg/mL), and the fraction was subjected to purification by repeated silica gel and Sephadex LH-20 column chromatography. Suffruticosol A, suffruticosol B, methyl gallate, and gallic acid, were isolated from the ethyl acetate soluble fraction. Their structures were elucidated by physico-chemical and spectroscopic studies.
Keywords – *Paeonia suffruticosa*, antioxidant activity, methyl gallate, gallic acid, suffruticosol A, suffruticosol B, DPPH radical

Introduction

The root cortex of *Paeonia suffruticosa* Andrews (Paeoniaceae) has been used as a traditional medicine for the remedies of women's diseases, blood circulation disorder, bleeding and fever in Korea, China and Japan (Kimura *et al*., 1996). This plant has been known to produce various compounds from root cortex such as monoterpene glycosides (Yoshikawa *et al*., 2000; Matsuda M. *et al*., 2001; Ryu *et al*., 2001; Wang *et al*., 2005a; Lin *et al*., 1996), paeonol glycosides (Yoshikawa *et al*., 1992; Li *et al*., 2004), tannins (Takechi *et al*., 1982; Lin *et al*., 1998), and phenolic compounds (Wang *et al*., 2005b). And Sarker *et al*. (1999) reported resveratrol trimers from the seeds of *P. suffruticosa*. In our search for antioxidative components from natural plants, the methanolic extract of the fruits of *P. suffruticosa* was found to exhibit significant activity, based on the scavenging activity of the stable DPPH free radical. The active compounds were isolated from the ethyl acetate soluble fraction of the fruits of *P. suffruticosa* methanolic extract. Structures of these compounds were determined by physico-chemical and spectral evidences.

*Author for correspondence
Fax: +82-63-290-1812; E-mail: dkkim@mail.woosuk.ac.kr

Experimental

General – ^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. The UV spectra were recorded on Shimadzu UV-1601 UV-Visible spectrophotometer. TLC was carried out on Merck precoated silica gel F_{254} plates and silica gel for column chromatography was Kiesel gel 60 (230 - 400 mesh, Merck). Spots were detected under UV and by spraying with 10% H_2SO_4 in ethanol followed by heating at 100 - 120 °C for 3 min. And Sephadex LH-20 was used for column chromatography (Pharmacia, 25 - 100 μm). Column for LPLC was Lobar A (Merck Lichroprep Si 60, 240 - 10 mm). Preparative HPLC was carried out on a Jaigel GS310 column (Japan). All other chemicals and solvents were of analytical grade and used without further purification. Ascorbic acid and BHA (butylated hydroxyanisole) were obtained from Sigma Chemical Co.

Plant materials – The fruits of *P. suffruticosa* were collected in August, 2006 at Wanju, Chonbuk, Korea. A voucher specimen (WSU-06-010) is deposited at the herbarium of the college of pharmacy, Woosuk University.

Extraction and isolation – The plant material (300 g) was extracted three times with MeOH at room temperature, and then filtered. The filtrate was evaporated

in vacuo to give a dark red residue. The resultant methanolic extract (15 g) was subjected to successive solvent partitioning to give ethyl acetate (5.2 g), *n*-BuOH (2.5 g) and H_2O soluble fractions. Each fraction was tested for the radical scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl). Among these fractions, the ethyl acetate soluble fraction showed the most significant free radical scavenging effect on DPPH. The ethyl acetate soluble fraction was subjected to chromatography on a Sephadex LH-20 column, and eluted with methanol to give six fractions (E1 - E6). Fraction E1 was chromatographed on a silica gel column using $CHCl_3$-MeOH-H_2O (30 : 10 : 1) as an eluent to give eight subfractions (E11 - E18). E15 and E16 were purified on Sephadex LH-20 (MeOH) column to give compound **1** (30 mg). E18 was purified on Sephadex LH-20 (MeOH) column to give compound **2** (17 mg). Fraction E5 was chromatographed on a silica gel column using $CHCl_3$-MeOH (6 : 1) as an eluent to give four subfractions (E51 - E54). E53 was chromatographed by Lobar A column with $CHCl_3$-EtOAc-MeOH (8 : 1 : 1), and purified by Sephadex LH-20 (MeOH) to give compound **3** (13 mg). E54 was purified on a JAI-GS310 column (MeOH) to give compound **4** (20 mg).

Suffruticosol B (1) – Brownish amorphous powder (MeOH); UV λ_{max} (MeOH) 280, 221; ^{1}H-NMR, (400 MHz, CD_3OD) δ: 7.58 (2H, d, *J* = 8.8 Hz, H-2", 6"), 6.92 (2H, br d, 2', 6'), 6.91 (2H, d, *J* = 8.8 Hz, H-3", 5"), 6.50 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.29 (2H, d, *J* = 8.8 Hz, H-3, 5), 6.27 (2H, d, *J* = 8.8 Hz, H-2, 6), 6.24 (1H, s, H-10), 6.23 (1H, s, H-14), 6.22 (1H, s, H-12'), 6.18 (1H, d, *J* = 2.2 Hz, H-12"), 6.15 (1H, t, *J* = 2.2 Hz, H-12), 5.95 (1H, d, *J* = 2.4 Hz, H-14"), 5.86 (1H, d, *J* = 11.2 Hz, H-7"), 5.09 (1H, d, *J* = 11.2 Hz, H-8"), 4.23 (1H, d, *J* = 11.2 Hz, H-7'), 4.10 (1H, m, H-8'), 4.09 (1H, s, H-8), 3.81 (1H, d, *J* = 6.0 Hz, H-7); ^{13}C-NMR, (100 MHz, CD_3OD) δ: 160.18 (C-11'), 159.36 (C-11, 13), 159.10 (C-4"), 158.38 (C-13"), 157.15 (C-11"), 156.13 (C-4'), 156.03 (C-4), 155.72 (C-13'), 147.47 (C-9'), 147.46 (C-9), 142.38 (C-9"), 135.52 (C-1), 133.80 (C-1'), 133.04 (C-2', 6'), 130.92 (C-1"), 130.51 (C-2", 6"), 129.47 (C-2, 6), 123.57 (C-14'), 122.86 (C-10"), 118.49 (C-10'), 116.52 (C-3", 5"), 115.17 (C-3, 5), 114.72 (C-3', 5'), 107.36 (C-10, 14), 104.98 (C-12"), 103.72 (C-14"), 101.48 (C-12), 96.26 (C-12'), 91.10 (C-7"), 63.07 (C-7), 56.87 (C-8), 49.10 (C-8"), 47.81 (C-8'), 46.46 (C-7').

Suffruticosol A (2) – Brownish amorphous powder (MeOH); UV λ_{max} (MeOH) 280, 221; ^{1}H-NMR, (400 MHz, CD_3OD) δ: 7.10 (2H, d, *J* = 8.8 Hz, H-2", 6"), 6.95 (2H, d, *J* = 8.8 Hz, H-2, 6), 6.68 (2H, d, *J* = 8.8 Hz, H-3", 5"), 6.48 (2H, d, *J* = 8.8 Hz, 2', 6'), 6.37 (2H, d, *J* = 8.8 Hz, H-3, 5), 6.25 (1H, d, *J* = 2.2 Hz, H-12"), 6.19 (1H, d, *J* = 0.8 Hz, H-12'), 6.12 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.06 (1H, t, *J* = 2.2 Hz, H-12), 5.98 (2H, d, *J* = 2.2 Hz, H-10, 14), 5.93 (1H, d, *J* = 1.8 Hz, H-14"), 5.68 (1H, d, *J* = 11.2 Hz, H-7"), 4.23 (1H, d, *J* = 3.2 Hz, H-7'), 4.74 (1H, s, H-8), 4.35 (1H, d, *J* = 11.2 Hz, H-8"), 3.93 (1H, m, H-8'), 3.68 (1H, d, *J* = 8.0 Hz, H-7); ^{13}C-NMR, (100 MHz, CD_3OD) δ: 160.16 (C-11'), 159.26 (C-11, 13), 158.92 (C-4"), 156.66 (C-13"), 156.48 (C-4), 155.12 (C-13'), 154.94 (C-11"), 154.48 (C-4'), 148.39 (C-9), 144.65 (C-9'), 141.78 (C-9"), 135.53 (C-1), 133.92 (C-1'), 130.84 (C-1"), 130.74 (C-2', 6'), 130.72 (C-2, 6), 130.45 (C-2", 6"), 126.94 (C-10"), 123.01 (C-14'), 117.26 (C-10'), 116.24 (C-3", 5"), 115.44 (C-3, 5), 114.16 (C-3', 5'), 106.84 (C-10, 14), 105.94 (C-14"), 101.93 (C-12"), 101.37 (C-12), 96.22 (C-12'), 91.48 (C-7"), 61.04 (C-7), 54.55 (C-8), 48.78 (C-8"), 48.58 (C-8'), 39.75 (C-7').

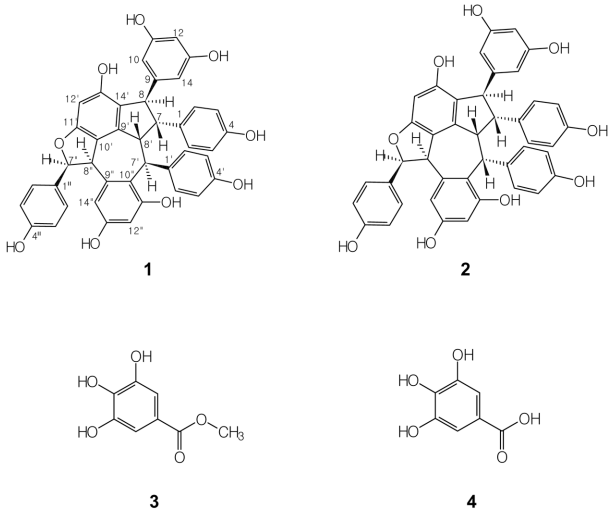

Fig. 1. Structures of compounds **1** - **4**.

Methyl gallate (3) – Brownish amorphous powder (MeOH); ^{1}H-NMR, (400 MHz, CD_3OD) δ: 7.04 (2H, s, H-2, 6), 3.80 (3H, s, OCH_3); ^{13}C-NMR, (100 MHz, CD_3OD) δ: 169.02 (COO), 146.46 (C-3, 5), 139.72 (C-4), 121.46 (C-1), 110.06 (C-2, 6), 52.25 (OCH_3).

Gallic acid (4) – Brownish amorphous powder (MeOH); ^{1}H-NMR, (400 MHz, CD_3OD) δ: 7.04 (2H, s, H-2, 6); ^{13}C-NMR, (100 MHz, CD_3OD) δ: 171.36 (COO), 146.29 (C-3, 5), 139.11 (C-4), 123.41 (C-1), 110.30 (C-2, 6).

DPPH radical scavenging effect – Ethanol solutions of test samples at various concentrations (0.1 - 200 μg/mL) were added to a solution of DPPH in ethanol (1.5×10^{-4} M) in 96 well plates. After storing these mixtures for 30 minutes at room temperature, the remaining amounts of DPPH were determined by

colorimetry at 520 nm on a microplate reader (Yoshida *et al*., 1989). And the radical scavenging activity of each compound was expressed by the ratio of the lowering of the DPPH solution in the absence of compounds. The mean values were obtained from triplicate experiments.

Results and Discussion

After screening of various plant extracts for their scavenging activity on DPPH radical, a methanolic extract of the fruits of *P. suffruticosa* was found to be potent at a concentration of 1.9 μg/mL (IC_{50}). Activity-guided fractionation of ethyl acetate soluble fraction of *P. suffruticosa* led to the isolation of four compounds.

NMR spectrum of **1** was very similar to that of **2**. The ^{1}H-NMR spectra of **1** and **2** revealed the presence of six sets of *ortho*-coupled aromatic hydrogens assignable to three 4-hydroxyphenyl groups, and three sets of 3,5-dihydroxyphenyl groups. And six other methane hydrogen signals were observed. The ^{13}C-NMR spectra of **1** and **2** exhibited signals for six phenyl ring systems, including nine oxygenated aromatic carbons, five methine carbons, and a peak deduced to be oxymethine. The main differences in ^{1}H-NMR spectra of **1** and **2** were splitting patterns and coupling constants for H-2', 6' (**1**, δ 6.92, br d; **2**, 6.48, d, J = 8.8 Hz) and H-7' (**1**, δ 4.23, d, J = 11.2 Hz; **2**, δ 4.23, d, J = 3.2 Hz). From these evidences, the structure of compound **1** and **2** were deduced to be resveratrol trimers. On the basis of the above data, com compound **1** and **2** were identified as suffruticosol B (**1**) and A (**2**), respectively, by comparison of spectral data with those reported in the literature (Sarker *et al*., 1998). Compounds **3** and **4** were identified as methyl gallate and gallic acid, respectively, by comparison with the reported data (Chung *et al*., 1999).

The DPPH radical scavenging effect of the methanolic extract and its solvent partitioned fractions from *P. suffruticosa* are shown in Table 1. The IC_{50} values of the methanolic extract, and ethyl acetate fraction obtained from the methanolic extract were calculated as a concentration of 1.9 and 1.2 μg/mL, respectively. The radical scavenging effect of four compounds isolated from ethyl acetate soluble fraction of *P. suffruticosa* was also shown in Table 2. Among four isolated compounds, **3** and **4** exhibited higher scavenging activity on DPPH with IC_{50} values of 0.4 and 0.2 μg/mL, respectively. However, stilbene trimer derivatives compounds **2** and **4** showed mild activities in comparison with reference antioxidants such as ascorbic acid and BHA. The present study indicates that the methanolic extract of *P. suffruticosa*, ethyl acetate soluble fraction and isolated compounds may be useful for the treatment of various oxidative damage.

Table 1. Scavenging effects of methanol extract and its subsequent fractions from the fruits of *P. suffruticosa* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Fractions	IC_{50} (μg/mL)*
MeOH ext.	1.9
EtOAC fr.	1.2
n-BuOH fr.	28.2
H_2O	6.8
BHA	2.5

*The values indicate 50% decrease of DPPH radical and are the means of triplicate data.

Table 2. Scavenging effects of the compounds 1 - 4 from the fruits of *P. suffruticosa* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Compounds	IC_{50} (μg/mL)*
Suffruticosol B (**1**)	31.7
Suffruticosol A (**2**)	49.8
Methyl gallate (**3**)	0.4
Gallic acid (**4**)	0.2
Ascorbic acid	1.1
BHA	2.8

*The values indicate 50% decrease of DPPH radical and are the means of triplicate data.

Acknowledgement

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (The Regional Research Universities Program/ Center for Healthcare Technology Development).

References

Chung, S.-C., Hwang, B. Y., Oh, G.-J., Kang, S. J., Kim, M.-J., Choi, W.-H., Lee, K. S., and Ro, J. S., Chemical components from the stem bark of *Rhus javanica* L. *Kor. J. Pharmacogn*. **30**, 295-300 (1999).

Kimura, T., But, P.P.H., Gue, J.-X., and Sung, C.K., International collation of traditional and folk medicine. Vol. 1. World scientific, Singapore, pp. 45-46 (1996).

Li, G., Seo, C.S., Lee, K.S., Kim, H.J., Chang, H.W., Jung, J.S., Song, D.K., and Son, J.K., Protective constituents against sepsis in mice from the root cortex of *Paeonia suffruticosa*. *Arch. Pharm. Res.* **27**, 1123-1126 (2004).

Lin, H.-C., Ding, H.-Y., and Wu, Y.-C., Two novel compounds from *Paeonia suffructicosa*. *J. Nat. Prod.* **61**, 343-346 (1998).

Lin, H.-C., Ding, H.-Y., Wu, T.-S., and Wu, P.-L., Monoterpene glycosides from *Paeonia suffruticosa*. *Phytochemistry* **41**, 237-242 (1996).

Matsuda, H., Ohta, T., Kawaguchi, A., and Yoshikawa, M., Bioactive constituents of chinese natural medicines. VI. Moutan cortex. (2): structures and radical scavenging effects of suffruticosides A, B, C, D,

and E and galloyl-oxypaeoniflorin. *Chem. Pharm. Bull.* **49**, 69-72 (2001).

Ryu, G., Park, E.K., Joo, J.H., Lee, B.H., Choi, B.W., Jung, D.S., and Lee, N.H., A new antioxidant monoterpene glycoside, alpha-benzoyloxypaeoniflorin from *Paeonia suffruticosa*. *Arch. Pharm. Res.* **24**, 105-108 (2001).

Sarker, S.D., Whiting, P., and Dinan, L., Identification and ecdysteroid antagonist activity of three resveratrol trimers (Suffruticososols A, B and C) from *Paeonia suffruticosa*. *Tetrahedron* **55**, 513-524 (1999).

Takechi, M. and Tanaka, Y., Antiviral substances from the root of paeonia species. *Planta Med.* **45**, 252-253 (1982).

Wang, S.J., Yang, Y.C., Li, S., and Shi, J.G., A new paeoniflorin derivative isolated from the root bark ethanol extract of *Paeonia suffruticosa*. *Zhongguo Zhong Yao Za Zhi*. **30**, 759-761 (2005a).

Wang, X., Cheng, C., Sun, Q., Li, F., Liu, J., and Zheng, C., Isolation and purification of four flavonoid constituents from the flowers of *Paeonia suffruticosa* by high-speed counter-current chromatography. *J. Chromatogr.* A. **1075**, 127-131 (2005b).

Yoshica, T., Mori, K., Hatano, T., Okumura, T., Uehara, L., Komagoe, K., Fujita, Y., and Okuda, T., Studies on inhibition mechanism of autooxidation by tannins and flavonoids. V. Radical scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem. Pharm. Bull.* **37**, 1919-1921 (1989).

Yoshikawa, M., Ohta, T., Kawaguchi, A., and Matsuda, H., Bioactive constituents of Chinese natural medicines. V. Radical scavenging effect of Moutan Cortex. (1): Absolute stereostructures of two monoterpenes, paeonisuffrone and paeonisuffral. *Chem. Pharm. Bull.* **48**, 1327-1331 (2000).

Yoshikawa, M., Uchida, E., Kawaguchi, A., Kitagawa, I., and Yamahara, J., Galloyl-oxypaeoniflorin, suffruticosides A, B, C, and D, five new antioxidative glycosides, and suffruticoside E, A paeonol glycoside, from Chinese moutan cortex. *Chem. Pharm. Bull.* **40**, 2248-2250 (1992).

(Accepted September 10, 2008)