Natural Product Sciences
11(4) : 229-232 (2005)

Antioxidant Constituents from *Portulaca oleracea*

Bong-Rak Choi[1,2], Seong Su Hong[1], Xiang Hua Han[1], Ji Sang Hwang[1], Min Hee Lee[1,3], Jae Doo Hur[3], Bang Yeon Hwang[1], and Jai Seup Ro[1,*]

[1]*College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Bioland Ltd., Chonan 330-863, Korea*
[3]*Crown Pharmaceutical Co. Ltd., Anyang 430-817, Korea*

Abstract – The repeated column chromatographic separation of the EtOH extract of *Portulaca oleracea* afforded seven compounds. The structures of these isolates were identified as bergapten (**1**), umbelliferone (**2**), daidzein (**3**), genistein (**4**), protocatechuic acid (**5**), ferulic acid (**6**), and gallic acid (**7**) by the analysis of physico-chemical and spectral data. Their antioxidant effect on free radical scavenging was evaluated in the DPPH assay.
Keywords – *Portulaca oleracea*, Portulacaceae, DPPH assay, antioxidant effect

Introduction

Portulaca oleracea L. (Portulacaceae), a warm-climate annual plant, is grown in many areas of the world. *P. oleracea* has been used as an edible plant, but also as a traditional herbal medicine for diuretic, antipyretic, anti-asthma, anti-inflammatory, and antitussive (Jung and Shin, 1989). Recent pharmacological studies have shown that the extract of this plant exhibits gastric antiulcerogenic effects (Karimi *et al.*, 2004), bronchodilatory effect on asthmatic airways (Malek *et al.*, 2004), wound healing activity (Rashed *et al.*, 2003), antimicrobial activity (Lim and Kim, 2001), antifungal activity (Oh *et al.*, 2000), muscle relaxant activity (Habtemariam *et al.*, 1993; Okwuasaba *et al.*, 1987; Parry *et al.*, 1988), antifertility (Verma *et al.*, 1982), hepatoprotective (Lim and Suh, 2000) and zoospore motility inhibitory activity (Mizutani *et al.*, 1998).

Previous phytochemical studies on *Portulaca* species have resulted in the isolation of noradrenaline, dopamine, glutamic acid, aspartic acid, alanine, ferulic acid, *p*-coumaric acid, salicylic acid, vanillic acid, *p*-hydroxy-benzoic acid, umbelliferone, β-carotene, and portuloside A (Lee *et al.*, 2003; Sakai *et al.*, 1996; Park and Kim, 1988; Liu *et al.*, 2000).

Although the crude extract and solvent fractions were found to exhibit significant DPPH radical scavenging effect (Lee *et al.*, 2003), the active constituents have not been identified from this plant. Bioassay-guided fractionation of the EtOH extract of the whole plants of *P. oleracea* led to the isolation of bergapten (**1**), umbelliferone (**2**), daidzein (**3**), genistein (**4**), protocatechuic acid (**5**), ferulic acid (**6**), and gallic acid (**7**). The isolation, structure determination, and antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay of these isolates are reported in this paper.

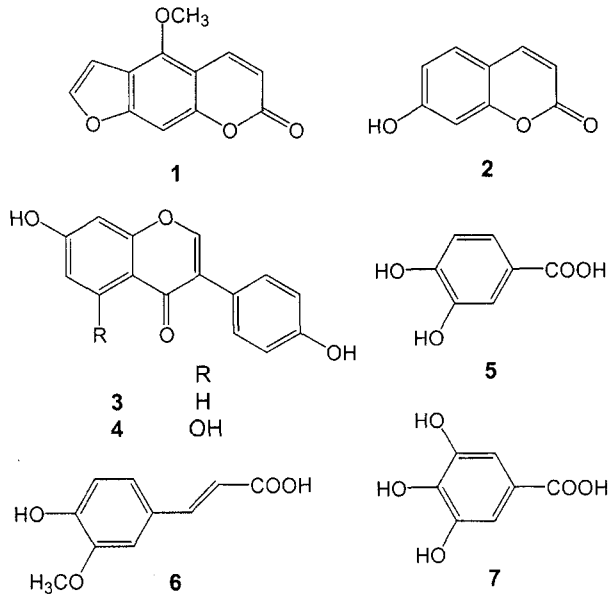

Fig. 1. Structures of the isolated compounds **1**- **7** from the whole plants of *Portulaca oleracea*.

*Author for correspondence
Fax: +82-43-268-2732; E-mail: jsroh@chungbuk.ac.kr

Experimental

Plant material – The whole plants of *P. oleracea* were collected from the herb garden at Chungbuk National University, Cheongju, Korea, in August 2001 and identified by emeritus professor Kyong Soon Lee, a plant taxonomist at Chungbuk National University. The voucher specimen (CBNU 01032) was deposited at the Herbarium of College of Pharmacy, Chungbuk National University.

General experimental procedures – Melting points were measured on Buchi model B-540 without correction. UV and IR spectra were obtained on a JASCO UV-550 and JASCO FT/IR 300E spectrometer, respectively. NMR spectra were recorded on a Bruker AMX-500 MHz NMR spectrometer. EI-MS spectra were recorded with Hewlett-Packard MS-5988. Column chromatography was carried out on Sephadex LH-20 (25-100 μM, Pharmacia), Lichroprep. RP18 (particle size 40-63 μM, Merck), and thin layer chromatography (TLC) using a pre-coated silica gel 60 F_{254} (0.25 mm, Merck). Spots were detected under UV lamp and by spraying with $FeCl_3$ and vanillin-H_2SO_4 followed by heating. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were analytical grade.

Extraction and isolation – The pulverized and dried whole plants of *P. oleracea* (5 kg) were extracted with 80% aq. EtOH at room temp. The EtOH extract (410 g) was suspended in water (3 L) and then partitioned successively with CH_2Cl_2 (3 L×3), EtOAc (3 L×3), and H_2O (3 L×3). The CH_2Cl_2-soluble fraction (56 g) was subjected to column chromatography on silica gel (9×25 cm) eluted with CH_2Cl_2-MeOH gradient system to obtain seven fractions (POC-1 – POC-7). Repeated column chromatotraphy of fraction POC-1 on silica gel column (n-hexane; EtOAc = 50 : 1) and RP-18 flash column (20% aq. MeOH) afforded bergapten (**1**, 34 mg). Fraction POC-6 was further applied to RP-18 flash column chromatography (50% aq. MeOH) to give umbelliferone (**2**, 25 mg). The most active EtOAc-soluble fraction (47 g) was subjected to column chromatography on silica gel (9×25 cm) eluted with n-hexane-EtOAc gradient system to yield six fractions (POE-1 – POE-6). Fraction POE-2 was further applied to a silica gel column chromatography eluting with $CHCl_3$-MeOH gradient system to obtain six subfractions (POE-2A – POE-2F). Fraction POE-2B was further subjected to Sephadex LH-20 column chromatography (MeOH) to yield daidzein (**3**, 19 mg). Fraction POE-2D was subjected to RP-18 column chromatography (MeOH; H_2O = 2 : 1) to give genistein (**4**, 23 mg). POE-4 was further applied to a silica gel column chromatography eluted with n-hexane-EtOAc gradient system affording five subfractions (POE-4A – POE-4E). Fraction POE-4B was subjected to RP-18 column chromatography (30% MeOH) to give protocatechuic acid (**5**, 34 mg). Ferulic acid (**6**, 68 mg) was isolated from fraction POE-4D by Sephadex LH-20 column chromatography (MeOH). Repeated column chromatography of fraction POE-5 on silica gel column (n-hexane; EtOAc = 50 : 1) and Sephadex LH-20 column (MeOH) afforded gallic acid (**7**, 107 mg).

Bergapten (1) – White amorphous powder; mp: 188°C; IR ν_{max} cm^{-1}: 1729 (unsaturated six-membered lactone), 1617, 1461 (C = C), 1210 (asymmetric C-O-C); EI-MS *m/z*: 216 $[M]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.13 (1H, d, *J* = 10.0 Hz, H-4), 7.58 (1H, d, *J* = 2.5 Hz, H-2'), 7.09 (1H, s, H-8), 7.01 (1H, d, *J* = 2.5 Hz, H-3'), 6.26 (1H, d, *J* = 10.0 Hz, H-3), 4.26 (3H, s, -OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 161.2 (C-2), 158.3 (C-7), 152.6 (C-9), 149.5 (C-5), 144.7 (C-2'), 139.2 (C-4), 112.7 (C-6), 112.5 (C-3), 106.3 (C-10), 105.0 (C-3'), 93.7 (C-8), 60.0 (OCH_3).

Umbelliferone (2) – White amorphous powder; mp: 201°C; UV λ_{max} (MeOH) nm: 205, 214, 241, 255, 325; IR ν_{max} cm^{-1}: 3160 (OH), 1680 (α-pyrone C = O), 1620, 1560 (aromatic C = C), 1380, 1190, 1130 (C-O); EI-MS *m/z*: 162 $[M]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 9.65 (s, OH), 7.85 (1H, d, *J* = 9.5 Hz, H-4), 7.50 (1H, br d, *J* = 8.4 Hz, H-5), 6.84 (1H, dd, *J* = 2.2, 8.4 Hz, H-6), 6.74 (1H, br d, *J* = 2.2 Hz, H-8), 6.15 (1H, d, *J* = 9.5 Hz, H-3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 161.6 (C-7), 160.7 (C-2), 155.7 (C-9), 144.3 (C-4), 129.6 (C-5), 114.5 (C-3), 114.5 (C-6), 113.3 (C-10), 102.5 (C-8).

Daidzein (3) – Pale yellow powder; mp: 330°C; UV λ_{max} (MeOH) nm: 238, 249, 259, 303; IR ν_{max} cm^{-1}: 3400 (OH), 1645 (C = O), 1520, 1470 (C = C); EI-MS *m/z*: 254 $[M]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 9.60 (1H, br s, -OH), 8.29 (1H, s, H-2), 7.95 (1H, d, *J* = 8.8 Hz, H-5), 7.37 (2H, d, *J* = 8.7 Hz, H-2', 6'), 6.92 (1H, dd, *J* = 8.8, 2.2 Hz, H-6), 6.85 (1H, d, *J* = 2.2 Hz H-8), 6.80 (2H, d, *J* = 8.7 Hz, H-3', 5'); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 178.0 (C-4), 164.9 (C-7), 160.1 (C-9), 159.2 (C-4'), 155.0 (C-2), 132.0 (C-2', 6'), 129.1 (C-5), 126.2 (C-1'), 124.8 (C-3), 118.8 (C-10), 116.9 (C-6), 116.8 (C-3', 5'), 103.8 (C-8).

Genistein (4) – Pale brown powder; mp: 301-302°C; IR ν_{max} cm^{-1}: 3400 (OH), 2930 (C-H), 1645 (α, β-unsaturated C = O), 1625, 1520, 1470 (aromatic C = C); EI-MS *m/z*: 270 $[M]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.09 (1H, s, H-2), 7.41 (2H, d, *J* = 8.7 Hz, H-2', 6'), 6.89 (2H, d, *J* = 8.7 Hz, H-3', 5'), 6.38 (1H, d, *J* = 2.2 Hz, H-8), 6.26

(1H, d, J = 2.2 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 184.0 (C-4), 163.0 (C-7), 161.3 (C-5), 156.8 (C-2), 156.8 (C-9), 156.8 (C-4'), 134.1 (C-2', 6'), 126.9 (C-3), 126.5 (C-1'), 118.9 (C-3', 5'), 104.6 (C-10), 98.6 (C-6), 93.7 (C-8).

Protocatechuic acid (5) – White amorphous powder; mp: 196-198°C; IR ν_{max} cm^{-1}: 3200 (OH), 1650 (aromatic ring), 1595, 1460; EI-MS *m/z*: 154 [M]$^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.42 (1H, br d, J = 8.0 Hz, H-6), 7.37 (1H, br s, H-2), 6.88 (1H, br d, J = 8.0 Hz, H-5); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 169.6 (C-7), 150.5 (C-4), 145.4 (C-3), 122.4 (C-1), 122.2 (C-6), 117.1 (C-5), 115.7 (C-2).

Ferulic acid (6) – White needle crystal; mp: 174-177 °C; UV λ_{max} (MeOH) nm: 212, 232, 295, 321; IR ν_{max} cm^{-1}: 3448 (OH), 1690 (COOH), 1638, 1515, 1465 (C = C); EI-MS *m/z*: 194 [M]$^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.52 (1H, d, J = 15.9 Hz, H-7), 7.31 (1H, d, J = 1.3 Hz, H-2), 7.11 (1H, dd, J = 8.1, 1.3 Hz, H-6), 6.82 (1H, d, J = 8.1 Hz, H-5), 6.50 (1H, d, J = 15.9 Hz, H-8), 3.85 (3H, s, -OCH$_3$); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 168.0 (C-9), 149.1 (C-3), 147.9 (C-4), 144.5 (C-7), 125.8 (C-1), 122.8 (C-6), 115.6 (C-8), 115.5 (C-5), 111.2 (C-2), 55.7 (-OCH$_3$).

Gallic acid (7) – Colorless needle crystal; mp: 266-268°C; IR ν_{max} cm^{-1}: 3300 (OH), 1700 (COOH), 1020 (C-O); EI-MS *m/z*: 170 [M]$^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.12 (2H, s, H-2, 6); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 167.3 (C-7), 145.3 (C-3, 5), 138.3 (C-4), 121.1 (C-1), 109.6 (C-2, 6).

Assay of DPPH free radical scavenging activity – The antioxidant activities of isolated compounds **1-7** and each solvent fraction were assessed on the basis of the scavenging activity of the stable DPPH free radical by a previously described method (Blois, 1958; Hwang *et al.*, 2001). The reaction mixture containing tested samples and 100 mM DPPH ethanolic solution in 96-well plate was incubated 37°C for 30 min, and absorbances were measured at 517 nm. Percent inhibition by sample treatment was determined by comparison with a DMSO treated control group. IC_{50} values denote the concentration of sample required to scavenging 50% DPPH free radicals.

Results and Discussion

Bioassay-guided fractionation and separation of the EtOH extract of the whole plants of *P. oleracea* led to the isolation of seven known compounds. The structures of these isolates were identified as bergapten (**1**), umbelliferone (**2**), daidzein (**3**), genistein (**4**), protocatechuic acid (**5**), ferulic acid (**6**) and gallic acid (**7**) by comparison with authentic samples and the literatures (Krishnamurty *et al.*, 1980; Fujika *et al.*, 1999; Shin *et al.*, 1992; Cha *et al.*, 2000; Blade *et al.*, 1991; An *et al.*, 2005).

Table 1. DPPH free radical scavenging activities of the solvent fractions from the whole plants of *Portulaca oleracea*

Extract or fractions	Concentration (μg/ml)	Inhibition (%)
EtOH extract	250	73.5
CH_2Cl_2 fraction	200	39.3
EtOAc fraction	200	78.7
H_2O fraction	200	19.1

Table 2. DPPH free radical scavenging activities of the isolated compounds **1-7** from the whole plants of *Portulaca oleracea*

Compounds	IC_{50} (mM)[a]
Bergapten (**1**)	>1000
Umbelliferone (**2**)	>1000
Daidzein (**3**)	>1000
Genistein (**4**)	>1000
Protocatechuic acid (**5**)	17.9 ± 0.53
Ferulic acid (**6**)	20.2 ± 1.25
Gallic acid (**7**)	9.4 ± 0.19
Ascorbic acid[b]	57.3 ± 0.82

[a] Each value is the mean of at least three independent experiments ± SD.
[b] Ascorbic acid was used for positive control.

The antioxidant activity of these isolates was evaluated with the DPPH assay, using a free radical which shows a characteristic absorption at 517 nm. The purple color rapidly bleached when DPPH encountered any radical scavengers. As shown in Table 1, the EtOAc-soluble fraction showed strong DPPH radical scavenging activity. This observation is consistent with previous report that the EtOAc and BuOH fraction of this plant have shown to potent antioxidant activity (Lee *et al.*, 2003). Accordingly, the phenolic compounds, which were isolated from the EtOAc fraction of the whole plants of *P. oleracea* in the present study, showed strong DPPH radical scavenging activity.

Among these compounds, protocatechuic acid (**5**), ferulic acid (**6**), and gallic acid (**7**) exhibited strong antioxidant activity with IC_{50} values of 17.9, 20.2, and 9.4 μM, respectively, in comparison with reference antioxidant, ascorbic acid (IC_{50} 57.3 μM).

However, coumarins and isoflavonoids such as bergapten (**1**), umbelliferone (**2**), daidzein (**3**), and genistein (**4**) were considered to be inactive in this antioxidant system using DPPH radical scavenging activity (Table 2).

In conclusion, phenolic acid such as protocathechuic

acid, ferulic acid, and gallic acid might be active principles responsible for the antioxidant activity of the whole plant of *P. oleracea*.

Acknowledgements

This work was supported by the grant from Chungbuk National University in 2005.

References

An, R.B., Park, B.Y., Kim, J.H., Kwon, O.K., Lee, J., Min, B.S., Ahn, K.S., Oh, S.R., and Lee, H.K., Coumarins and chromones from *Angelica genuflexa*. *Nat. Prod. Sci.* **11**, 79-84 (2005).

Blade, A.M., Claeys, M., Pieters, L.A., Wray, V., and Vlietinck, A.J., Ferulic acid esters from stem bark of *Pavetta owariensis*. *Phytochemistry* **30**, 1024-1026 (1991).

Blois, M.S., Antioxidant determination by the use of a stable free radical. *Nature* **181**, 1199-1200 (1958).

Cha, B.C., Lee, S.B., Rhim, T.J., and Lee, K.H., Constituents of antioxidative activity and free radical scavenging effect from Galla Rhois (*Rhus javanica* Linne). *Kor. J. Pharmacogn.* **31**, 185-189 (2000).

Fujika, T., Furumi, K., Fujh, H., Okabe, H., Mihashi, K., Nakano, Y., Matsunaga, H., Katano, M., and Mori, M., Antiproliferative constituents from umbelliferae plants. *Chem. Pharm. Bull.* **47**, 96-100 (1999).

Habtemariam, S., Harvey, A.L., and Waterman, P.G., The muscle relaxant properties of *Portulaca oleracea* are associated with high concentrations of potassium ions. *J. Ethnopharmacol.* **40**, 195-200 (1993).

Hwang, B.Y., Kim, H.S., Lee, J.H., Hong, Y.S., Ro, J.S., Lee, K. S., and Lee, J.J., Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. *J. Nat. Prod.* **64**, 82-84 (2001).

Jung, B.S., and Shin M.K., Encyclopedia of Illustrated Korean Natural Drugs, Young Lim Sa, Seoul, 1989, pp. 350-351.

Karimi, G., Hosseinzadeh, H., and Ettehad, N., Evaluation of the gastric antiulcerogenic effects of *Portulaca oleracea* L. extracts in mice. *Phytother. Res.* **18**, 484-487 (2004).

Krishnamurty, H.G., and Prasad, J.S., Isoflavones of *Moghania macrophylla*. *Phytochemistry* **19**, 2797-2798 (1980).

Lee, H.J., Lee, B.J., Lee, D.S., and Seo, Y.W., DPPH radical scavenging effect and in vitro lipid peroxidation inhibition by *Portulaca oleracea*. *Korean J. Biotechnol. Bioeng.* **18**, 165-169 (2003).

Lim, J.P., and Suh, E.S., Hepatoprotective, diuretic and anti-inflammatory activities of the extract from *Portulaca oleracea* L. *Korean J. Medicinal Crop Sci.* **8**, 189-193 (2000).

Lim, M.K. and Kim, M.R., Antimicrobial activity of methanol extract from Soibirhym (*Portulaca oleracea*) against food spoilage disease microorganisms and the composition of the extract. *Korean J. Soc. Food Cookery Sci.* **17**, 565-570 (2001).

Liu, L., Howe, P., Zhou, Y.F., Xu, Z.Q., Hocart, C., and Zhan, R., Fatty acids and β-carotene in australian purslane (*Portulaca oleracea*) varieties. *J. Chromatogr. A.* **893**, 207-213 (2000).

Malek, F., Boskabady, M.H., Borushaki, M.T., and Tohidi, M., Bronchodilatory effect of *Portulaca oleracea* in airways of asthmatic patients. *J. Ethnopharmacol.* **93**, 57-62 (2004).

Mizutani, M., Hashidoko, Y., and Tahara, S., Factors responsible for inhibiting the motility of zoospores of the phytopathogenic fungus *Aphanomyces cochlioides* isolated from the non-host plant *Portulaca oleracea*. *FEBS Lett.* **438**, 236-240 (1998).

Oh, K.B., Chang, I.M., Hwang, K.J., and Mar, W., Detection of antifungal activity in *Portulaca oleracea* by a single-cell bioassay system. *Phytother. Res.* **14**, 329-332 (2000).

Okwuasaba, F., Ejike, C., and Parry, O., Effects of extracts of *Portulaca oleracea* on skeletal muscle in vitro. *J. Ethnopharmacol.* **21**, 55-63 (1987).

Park, J.S., and Kim, K.U., Identification of physiologically active compounds from Purslane (*Portulaca oleracea* L.). *Kor. J. Weed Sci.* **8**, 169-175 (1988).

Parry, O., Okwuasaba, F., and Ejike, C., Effect of an aqueous extract of *Portulaca oleracea* leaves on smooth muscle and rat blood pressure. *J. Ethnopharmacol.* **22**, 33-44 (1988).

Rashed, A.N., Afifi, F.U., and Disi, A.M., Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in Mus musculus JVI-1. *J. Ethnopharmacol.* **88**, 131-136 (2003).

Sakai, N., Inada, K., Okamoto, M., Shizuri, Y., and Fukuyama, Y., Portuloside A,A monoterpene glucoside, from *Portulaca oleracea*. *Phytochemistry* **42**, 1625-1628 (1996).

Shin, K.H., Kang, S.S., and Chi, H.J., Analysis of the coumarin constituents in Peucedanii Radix. *Kor. J. Pharmacogn.* **23**, 20-23 (1992).

Verma, O.P., Kumar, S., and Chatterjee, S.N., Antifertility effects of common edible *Portulaca oleracea* on the reproductive organs of male albino mice. *Indian J. Med. Res.* **75**, 301-310 (1982).

(Accepted November 13, 2005)