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Free Radical Scavengers from the Heartwood of *Juniperus chinensis*

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The antioxidant activity of *Juniperus chinensis* (Cupressaceae) was determined by measuring the radical scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl). The methanolic extract of *J. chinensis* heartwood showed the strong antioxidant activity. The antioxidant activity of *n*-BuOH soluble fraction was stronger than that of the others, and the fraction was subjected to purification by repeated silica gel and Sephadex LH-20 column chromatography. Quercetin, naringenin, taxifolin, aromadendrin and isoquercitrin were isolated from the *n*-BuOH fraction. Their structures were elucidated by physico-chemical and spectroscopic studies.

Key words: *Juniperus chinensis*, Antioxidant activity, Quercetin, Naringenin, Taxifolin, Aromadendrin, Isoquercitrin, DPPH radical

INTRODUCTION

The dried heartwood of *Juniperus chinensis* Linnaeus. (Cupressaceae) has been used as a traditional medicine for the remedies of cold, urinary infection, urticaria, dysentery, hemorrhage, leukorrhea and rheumatic arthritis in Korea and China (But *et al.*, 1997). This plant has been known to produce various compounds such as sesquiterpenoids (Shin *et al.*, 1999, Kuo *et al.*, 1996, Kuo *et al.*, 1994a, Kuo *et al.*, 1992a), diterpenoids (Lee *et al.*, 2001; Lee *et al.*, 1995; Kuo *et al.*, 1994b; Fang *et al.*, 1993a; Fang *et al.*, 1993b; Kuo *et al.*, 1992b), lignans (Muranaka *et al.*, 1998; Fang *et al.*, 1992) and other terpenes (Fang *et al.*, 1996). Recently, Ali *et al.* (1996) reported that crude extract of this plant showed antitumor activities. From those literatures, we knew that terpenoids were main components of *J. chinensis*. However, there are only a few reports about bioactive components of this plant. In our research for antioxidative components from natural plants, the methanolic extract of the heartwood of *J. chinensis* was found to exhibit significant activity, based on the scavenging activity of the stable DPPH free radical. The active compounds were isolated from the methanolic extract of the heartwood of *J. chinensis* and structures of these compounds were determined by physico-chemical and spectral evidences.

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MATERIALS AND METHODS

General

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. The EI/MS (70 eV) were determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). 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 $FeCl_3$ and dil. H_2SO_4, followed by heating. 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). Ascorbic acid and BHA (butylated hydroxyanisole) were obtained from Sigma Chemical Co.

Plant materials

J. chinensis were collected in May, 1999 at Moak mountain, Chonbuk, Korea. A voucher specimen (WSU-99-005) is deposited at the herbarium of the college of pharmacy, Woosuk University.

Extraction and isolation

The air-dried plant material (500 g) was extracted at room temperature for 7 days (×2) with MeOH. The resultant MeOH extract (90 g) followed by the successive solvent partition to give n-hexane (18 g), $CHCl_3$ (10 g), *n*-BuOH (35 g) and H_2O soluble fractions. The *n*-BuOH soluble fraction was chromatographed over silica gel column using a solvent system of *n*-hexane-$CHCl_3$-MeOH (23:10:1) as an eluent to give five subfractions (B1~B5), subfraction B1 (6.0 g) was rechromatographed on silica gel column ($CHCl_3$-EtOAc, 60:1) and purified by Sephadex LH-20 (MeOH-$CHCl_3$, 4:1) to yield **1** (20 mg). Subfraction B2 (3.5 g) was rechromatographed on silica gel column with $CHCl_3$-EtOAc (50:1) to give two fractions (B21, B22). Fraction B21 was recrystallized with MeOH to yield **2** (12 mg). Fraction B22 was applied over silica gel column chromatography (*n*-hexane-$CHCl_3$-MeOH, 8:10:1) to yield **3** (9 mg). Subfraction B3 (2.0 g) was rechromatographed on silica gel column (CH_2Cl_2-acetone-EtOAc, 1:5:1) and purified by Lobar A column ($CHCl_2$-MeOH-H_2O, 10:10:1) to give **4** (10 mg). Subfraction B4 (1.5 g) was rechromatographed on silica gel column ($CHCl_3$-MeOH-H_2O, 40:10:1) and purified by Sephadex LH-20 (MeOH) to give **5** (7 mg).

Quercetin (1) as a yellow powder from MeOH which showed physical and spectral data virtually identical to those reported in the literature (Shen *et al.*, 1993).

Naringenin (2) Isolated as a white needle (MeOH) which showed physical and spectral data virtually identical to those reported in the literature (Chien *et al.*, 1979; Shen *et al.*, 1993).

Taxifolin (3) Isolated as a yellowish powder which showed physical and spectral data virtually identical to those reported in the literature (Lundgren *et al.* 1988; Shen *et al.*, 1993).

Aromadendrin (4) Isolated as a yellow needle (MeOH) which showed physical and spectral data virtually identical to those reported in the literature (Chien *et al.* 1979; Reisch *et al.*, 1984).

Isoquercitrin (5) Isolated as a yellow powder from MeOH which showed physical and spectral data virtually identical to those reported in the literature (Kim *et al.*, 1999).

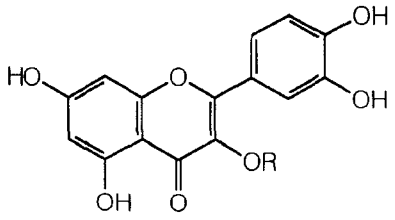
DPPH radical scavenging effect

MeOH solutions (4 ml) of flavonoids at various concentrations (1-14 μg/ml) were added to a solution of DPPH in MeOH (1.5×10^{-4} M, 1 ml) and the reaction mixture were shaken vigorously. After storing these mixtures for 30 minutes at room temperature, the remaining amounts of DPPH were determined by colorimetry at 520 nm (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.

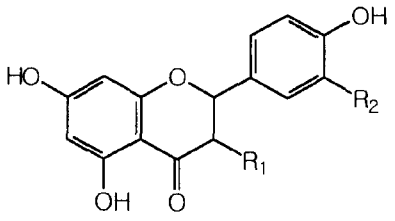
RESULTS AND DISCUSSION

After screening of various plant extracts for their scavenging activity on DPPH radical, a MeOH extract of *J. chinensis* heartwood was found to be potent at a concentration of 18.5 μg/ml (IC_{50}). Activity-guided fractionation of the *n*-BuOH soluble fraction of the heartwood of *J. chinensis* led to the isolation of five flavonoids. The five flavonoids were identified as quercetin (**1**, Shen *et al.*, 1993), naringenin (**2**, Chien *et al.*, 1979; Shen *et al.*, 1993), taxifolin (**3**, Lundgren *et al.* 1988; Shen *et al.*, 1993), aromadendrin (**4**, Chien *et al.* 1979; Reisch *et al.*, 1984) and isoquercitrin (**5**, Kim *et al.*, 1999). Their structures (Fig. 1) were elucidated by the comparison of UV, IR and NMR data with those reported literature.

The DPPH radical scavenging effect of the methanolic extract and its solvent partitioned fractions from *J. chinensis* are shown in Table I. The IC_{50} values of the methanolic extract, and *n*-BuOH fraction obtained from the methanolic extract were calculated as a concentration of 18.5 and 10.8 μg/ml, respectively, while the *n*-hexane,



1 R = H
5 R = Glc.



	R_1	R_2
2	H	H
3	OH	OH
4	OH	H

Fig. 1. Structures of compounds **1-5**

Table I. Scavenging effects of methanol extract and its subsequent fractions from the heartwood of *J. chinensis* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Fractions	IC_{50} (μg/ml)*
MeOH ext.	18.5
n-Hexane fr.	> 40.0
$CHCl_3$ fr.	> 40.0
n-BuOH fr.	10.8
H_2O	> 40.0
BHA	9.9

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

Table II. Scavenging effects of the compounds 1-5 from the heartwood of *J. chinensis* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Compounds	IC_{50} (μg/ml)*
Quercetin (**1**)	2.3
Naringenin (**2**)	> 120.0
Taxifolin (**3**)	10.8
Aromadendrin (**4**)	> 120.0
Isoquercitrin (**5**)	7.2
Ascorbic acid	3.6
BHA	9.9

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

$CHCl_3$ and H_2O soluble fractions showed a poor activity (>40.0 μg/ml). The radical scavenging effect of five compounds obtained from *J. chinensis* was also shown in Table II. Among five isolated compounds, the *ortho*-dihydroxylated aromatic components **1**, **3** and **5** exhibited higher scavenging activity on DPPH with IC_{50} values of 2.3, 10.8 and 7.2 μg/ml, respectively. However, **2** and **4** showed poor activities in comparison with reference antioxidants such as ascorbic acid and BHA. These result suggest that the radical scavenging effect in the original methanolic extract of *J. chinensis* was partially attributable to **1**, **3** and **5**. It was reported that naturally occurring phenolic compounds having catechol moiety, have antioxidant activity (Yoshida *et al.*, 1989). The present study indicates that the methanolic extract of *J. chinensis*, *n*-BuOH fraction and isolated flavonoid components may be useful for the treatment of various oxidative damage.

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