Arch. Pharm. Res. Vol.20, No.5, pp. 496-500, 1997

Antioxidant Effect of *Salvia miltiorrhiza*

Hye Sook Kang, Hae Young Chung[1], Jee Hyung Jung[1], Sam Sik Kang[2] and Jae Sue Choi

Department of Food and Life Science, Pukyong National University, Pusan 608-737, Korea, [1]Department of Pharmacy, Pusan National University, Pusan 609-735, Korea and [2]Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

(Received June 30, 1997)

A strong antioxidant activity, which was measured by the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical, was detected in the methanol extract of *Salvia miltiorrhiza* Bunge (Labiatae). By activity-directed fractionation, compounds 1 and 2 were isolated as antioxidant principles of *S. miltiorrhiza.* Compounds 1 and 2 were identified as dimethyl lithospermate and 3-(3,4-dihydroxyphenyl)lactamide, respectively, on the basis of spectral data. The radical scavenging effect of compounds 1 and 2 on DPPH radical exceeded that of L-ascorbic acid which is a well known antioxidant. These two compounds also showed prominent inhibitory activity against free radical generation in dichlorofluorescein (DCF) method and cytoprotective effect against *t*-BHP in cultured liver cell.

Key words : *Salvia miltiorrhiza,* dimethyl lithospermate, 3-(3,4-dihydroxyphenyl)lactamide, antioxidant activity

INTRODUCTION

Tanshen or Danshen, the rhizome of *Salvia miltiorrhiza* Bunge (Labiatae) is a wild herbal plant which has been used in traditional Chinese medicine to treat coronary heart disease, particularly angina pectoris and myocardial infarction (Tang *et al.,* 1992). The chemical components of *S. miltiorrhiza* have been studied extensively over the last 60 years. More than 25 orange-red crystalline pigments, known as the tanshinones, have been isolated from this herb, and many of these showed physiological activity (Baillie and Thomson, 1968, Fang *et al.,* 1976, Hayashi *et al.,* 1971, Takiura and Koizumi, 1962).

Some of the clinical effects of tanshen could be, to some extent, related to its possible antioxidant activity. A recent paper described the isolation of seven quinones from tanshen and an investigation of their antioxidant activity. It was found that dihydrotanshinone, tanshinone I, methylenetanshinquinone, cryptotanshinone, tanshinone IIB, and danshenxinkun B act as antioxidants in heated lard, whereas tanshinone IIA has no antioxidant properties (Zhang *et al.,* 1990, Weng and Gordon, 1992). They extended the investigation of the antioxidant properties of quinones from tanshen in heated fat by including dehydrorosmariquinone, miltirone I, and rosmariquinone, and also they proposed the mechanism by which the quinones act as antioxidants. The antioxidative effect of three water soluble-components, salvianolic acid A, salvianolic acid B, and rosmarinic acid isolated from *S. miltiorrhiza* have also been investigated (Huang and Zhang, 1992). All three components were found to inhibit both NADPH-vitamin C and Fe^{++}-cysteine induced lipid peroxidation in rat brain, liver and kidney microsomes *in vitro.* In addition, the three compounds lowered the production of superoxide anion radical in a xanthine-xanthine oxidase system.

We previously screened various plants for the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical to discover a new source of natural antioxidants, and reported that the MeOH extract of *S. miltiorrhiza* exhibits a strong antioxidative effect (Choi *et al.,* 1993). In this work, we report the isolation and characterization of the active principles and their antioxidant activity.

Correspondence to: Jae Sue Choi, Department of Food and Life Science, Pukyong National University, Pusan 608-737, Korea

MATERIALS AND METHODS

Instruments

Melting points are uncorrected. The UV spectrum was taken with a Shimadzu 202 UV spectrophotometer in MeOH solution and the IR spectrum on a JASCO IR-2 spectrometer in KBr disc. FAB-MS was obtained with a JMS-SX 102 mass spectrometer using a direct inlet system and glycerol was used as a matrix. ^1H- and ^{13}C-NMR spectra were taken with a Varian

UNITY-500 spectrometer. The chemical shifts were referenced to residual solvent peaks (2.5 ppm in ^{1}H-NMR, 39.5 ppm in ^{13}C-NMR), and were recorded in values. Multiplicities of ^{1}H- and ^{13}C-NMR signals are indicated as s (singlet), d (doublet), and t (triplet). Column chromatography was done with silica gel (Merck, 70~230 mesh). TLC was carried out on precoated Merck Kieselgel 60 F_{254} plate (0.25 mm) and spots were detected under UV light using 50% H_2SO_4 reagent.

Plant materials

The rhizome of *S. miltiorrhiza* was purchased from a commercial supplier, in 1995 and authenticated by Prof. H. J. Chi of the Natural Products Research Institute, Seoul National University. A voucher specimen has been deposited in the Herbarium of the Natural Products Research Institute.

Extraction, fractionation and isolation

The powdered rhizome (17 kg) of *S. miltiorrhiza* was refluxed with MeOH for three hr. (9L×3). The total filtrate was concentrated to dryness *in vaccuo* at 40 °C to render the MeOH extract (2.05 kg), and this extract was suspended in distilled H_2O and partitioned with CH_2Cl_2 (330 g), EtOAc (160 g), *n*-BuOH (160 g) and H_2O (1.35 kg) in sequence. The CH_2Cl_2 (32 g) fraction was chromatographed on a Si gel column eluting with CH_2Cl_2-MeOH (gradient) to give a total of 11 subfractions. The fraction 2 (3.5 g) and the fraction 3 (4.0 g) were chromatographed on a Si gel column eluting with CH_2Cl_2 to give tanshinone IIA (**4**, 300 mg) and tanshinone I (**3**, 330 mg), respectively. The fraction 4 (2.5 g) was chromatographed on a Si gel column eluting with CH_2Cl_2-MeOH (10:1) to give cryptotanshinone IIA (**5**, 100 mg). The EtOAc and *n*-BuOH fraction showed strong scavenging activity against DPPH radical; Thus, the EtOAc (66 g) fraction was chromatographed on a Si gel column using EtOAc-MeOH (gradient) as solvent to yield 12 subfractions. The fraction 3 (12 g) was further chromatographed on a Si gel column eluting with CH_2Cl_2-MeOH (5:1) to give dimethyl lithospermate (**1**, 240 mg). 145 g of the *n*-BuOH fraction was first subjected to Amberlite IR-120 Plus ion exchange column chromatography (500 mL) with H_2O to give neutral and acidic fractions, respectively. The neutral and acidic fraction (45 g) was subjected to MCI-gel CHP20P column chromatography (300 mL) with H_2O containing increasing proportions of MeOH (0→100%, stepwise elution with 10% increase at each step) to give five fractions. The second of which (4.82 g) was separated by MCI-gel CHP20P using H_2O as solvent to yield 3-(3,4-dihydroxyphenyl)lactamide (**2**, 80 mg). The chemical structures of these compounds are shown in Fig. 1.

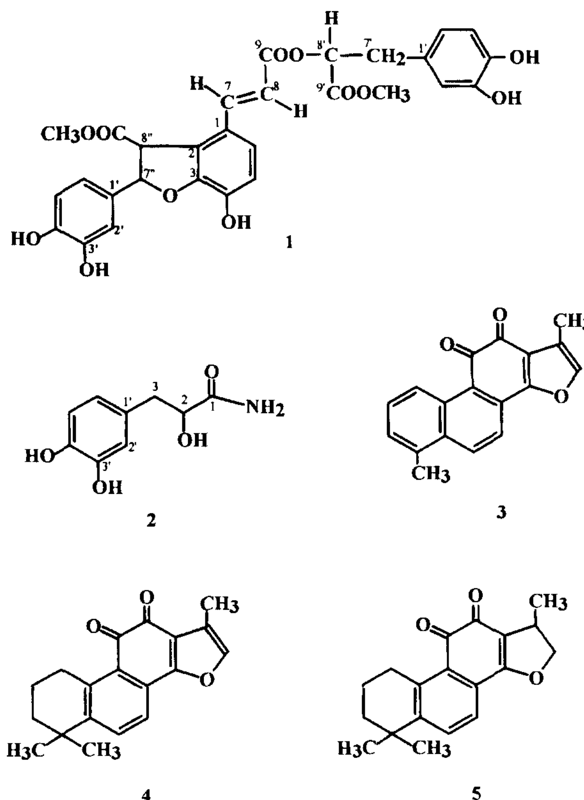

Fig. 1. Structures of isolated compounds.

Tanshinone I(3): Orange needles; Mp 241°C; ^{1}H-NMR ($CDCl_3$) 9.17 (1H, d, *J*=8.0 Hz), 8.13 (1H, d, *J*= 8.0 Hz), 7.60 (1H, d, *J*=8.0 Hz), 7.20~7.30 (3H, m), 2.60 (3H, s), 2.25 (3H, s); EI-MS (*m/z*) 276 (M^+), 248 (M^+-CO), 191; ^{13}C-NMR ($CDCl_3$) δ 118.69 (C-1), 130.61 (C-2), 128.32 (C-3), 135.16 (C-4), 123.20 (C-5), 132.86 (C-6), 124.76 (C-7), 129.57 (C-8), 126.90 (C-9), 133.60 (C-10), 183.39 (C-11), 175.57 (C-12), 121.74 (C-13), 161.12 (C-14), 142.01 (C-15), 120.46 (C-16), 8.76 (C-17), 19.80 (C-18).

Tanshinone IIA (4): Reddish-orange needles; Mp 216°C; ^{1}H-NMR ($CDCl_3$): δ 7.42 (2H, ABq, *J*=8.0 Hz), 7.10 (1H, q, *J*=2.0 Hz), 3.15 (2H, br t), 2.25 (3H, d, *J*= 2.0 Hz), 1.70 (4H, m), 1.30 (6H, s); EI-MS (*m/z*) 294 (M^+), 279 (M^+-CH_3), 261; ^{13}C-NMR ($CDCl_3$) δ 29.86 (C-1), 19.11 (C-2), 37.85 (C-3), 34.65 (C-4), 150.11 (C-5), 132.90 (C-6), 124.78 (C-7), 128.36 (C-8), 126.48 (C-9), 144.43 (C-10), 183.59 (C-11), 173.32 (C-12), 118.70 (C-13), 161.68 (C-14), 140.05 (C-15), 120.21 (C-16), 8.76 (C-17), 31.82 (C-18 & C-19).

Cryptotanshinone (5): Orange needles; MP 191°C; ^{1}H-NMR ($CDCl_3$) δ 7.42 (2H, ABq, *J*=8.0 Hz), 4.83 (1H, t, *J*=9.2 Hz), 4.31 (1H, dd, *J*=9.2 and 6.0 Hz), 3.55 (1H, m), 3.17 (2H, br t), 1.65 (4H, m), 1.40 (3H, d, *J*=6.8 Hz), 1.28 (6H, s); EI-MS (*m/z*) 296 (M^+), 268 (M^+-CO), 253; ^{13}C-NMR ($CDCl_3$) δ 29.58 (C-1), 19.00 (C-2), 37.73 (C-3), 34.76 (C-4), 143.57 (C-5), 132.48

(C-6), 122.43 (C-7), 128.30 (C-8), 126.19 (C-9), 152.28 (C-10), 184.16 (C-11), 175.59 (C-12), 118.21 (C-13), 170.66 (C-14), 81.38 (C-15), 34.54 (C-16), 18.74 (C-17), 31.85 (C-18), 31.80 (C-19).

Dimethyl lithospermate (1): Yellow amorphous hygroscopic powder; $[\alpha]^{21}$=+164.3° (c 0.07, MeOH); positive-ion FAB-MS (*m/z*) 567 (MH^+); UV (MeOH) λ_{max} (log ε) 252.6 (4.57), 286.4 (4.52), 304.6 (4.51), 332.6 (4.47) nm; IR ν_{max} 3384.6 (OH), 2954.6 (-CH), 1723.4 (ester), 1610.2, 1509.1 (aromatic ring), 1444.1 ($-CH_2$), 1263.6, 1161.9, 1113, 978.4, 866.8, 809.3 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.60 (1H, d, *J*=15.9 Hz), 7.30 (1H, d, *J*=8.4 Hz), 6.82 (1H, d, *J*=8.4 Hz), 6.72 (1H, dd, *J*=1.8 and 7.7 Hz), 6.72 (1H, d, *J*=7.8 Hz), 6.68 (1H, d, *J*=1.8 Hz), 6.64 (1H, d, *J*=7.8 Hz), 6.63 (1H, d, *J*=1.8 Hz), 6.50 (1H, dd, *J*=1.8 and 7.7 Hz), 6.36 (1H, d, *J*=15.9 Hz), 5.84 (1H, d, *J*=4.8 Hz), 5.14 (1H, dd, *J*=4.8 Hz), 4.53 (1H, d, *J*=4.5 Hz), 3.65 (3H, s, $-OCH_3$), 3.67 (3H, s, $-OCH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.83, 170.03, 165.88, 147.19, 145.84, 145.51, 145.12, 144.25, 142.62, 131.04, 126.69, 126.05, 122.24, 121.13, 120.15, 117.37, 117.20, 116.61, 115.54, 115.08, 113.05, 86.33, 72.94, 54.89, 52.50, 52.01, 40.28.

3-(3,4-dihydroxyphenyl)lactamide (2): Yellow amorphous powder; negative ESI-MS (*m/z*) 196 (MH^-); UV (MeOH) λ_{max} (log ε) 280.2 (3.44); IR ν_{max} 3132.2 (-OH), 1580.7, 1528.2 (aromatic ring), 1456.0 ($-CH_2$), 1276.4, 1115.5 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 6.80 (1H, d, *J*=8.1 Hz, H-5'), 6.78 (1H, d, *J*=1.8 Hz, H-2'), 6.68 (1H, dd, *J*=1.8 and 8.1 Hz, H-6'), 4.11 (1H, dd, *J* =8.1 and 3.9 Hz, H-2), 2.94 (1H, dd, *J*=14.3 and 3.9 Hz, H-3), 2.71 (1H, dd, *J*=14.3 and 8.1 Hz, H-3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 181.62 (C-1), 145.61 (C-3'), 144.32 (C-4'), 132.62 (C-1'), 123.40 (C-6'), 118.84 (C-2'), 117.62 (C-5'), 75.02 (C-2), 41.29 (C-3).

DPPH radical scavenging effect

The DPPH radical scavenging effect was measured according to the method first employed by M. S. Blois (Blois, 1958). Four milliliters of MeOH solution of varying sample concentrations was added to 1.0 ml DPPH methanol solution (1.5×10^{-4} M). After storing at room temperature for 30 min, the absorbance of this solution was determined at 520 nm using a spectrophotometer and the remaining DPPH was quantified. The results were calculated by taking the means of all triplicate values.

Assay for the free radical generation

Liver cells (AC_2F) were incubated for 24 hrs. in serum free media in a CO_2 incubator at 37°C until confluent, and the cells were transferred to multiwell plates with about 10^5 cells/well and cultured with or without a suspension of compounds **1-5** (2, 10 or 50 μM), then incubated with 12.5 μM DCFH-DA at 37°C for 30 min. Fluorescence was monitored on a spectrofluorometer, with excitation wavelength at 460 nm, and emission wave length at 530 nm.

In vitro cytoprotective assay

Liver cells (AC_2F) were cultured in DMEM with 20% fetal calf serum, in a CO_2 incubator at 37°C until confluent. Then the cells were transferred to multiwell plates with about 10^5 cells/well and cultured with or without a suspension of compounds **1-5** (2, 10 or 50 μM) in the media for one hr. After the incubation, the viable cell number was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Results are presented as means±S.D. of three determinations.

Statistics

The data were analyzed for statistical significance using student's t-test. Differences at a *p* value of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

As indicated above, after screening of various plant extracts for their scavenging activity on DPPH radical (Choi *et al.*, 1993), a MeOH extract of *S. miltiorrhiza* was found to be most potent at a concentration of 27.4 μg/ml and was further fractionated into CH_2Cl_2-, EtOAc-, *n*-BuOH-, and H_2O-soluble fractions. These fractions were assayed for DPPH radical scavenging activity. As can be seen in Table I, The EtOAc- and *n*-BuOH fractions showed a significant scavenging activity on DPPH radical at concentrations of 2.4 and 15.6 μg/ml, respectively, while the CH_2Cl_2-soluble fraction exhibited weak scavenging activity. The H_2O-soluble fraction showed no activity at concentrations over 80 μg/ml. Therefore, the EtOAc-and *n*-BuOH-soluble fractions were subjected to further chemical analysis and, after column chromatographic separation, the two compounds (**1 & 2**) were isolated. The CH_2Cl_2-soluble fraction was also chromatographed to yield

Table I. The radical scavenging effect of the methanol extract and its fractions of *S. miltiorrhiza* on DPPH radical

Samples	50% reduc.[a] (μg/ml)
MeOH ext.	27.4
CH_2Cl_2 fr.	50.7
EtOAc fr.	2.4
BuOH fr.	15.6
H_2O fr.	>80.0
L-Ascorbic acid	2.0
BHT	2.4

[a]Amount required for reduction of DPPH after 30 min. Values are means of three experiments

Table II. The radical scavenging effect of compounds **1-5** on DPPH radical

Sample	50% reduc. (μM)
1	1.17
2	6.32
3	1301.4
4	1141.3
5	939.0
L-ascorbic acid	11.36
BHT	11.98

[a]Amount required for reduction of DPPH after 30 min. Values are means of three experiments

quinones (**3-5**) because quinones are reported as having antioxidant activity as indicated above.

3-(3,4-dihydroxyphenyl)lactamide (**2**), hygroscopic powder, was recognized as a phenolic compound from a positive reaction to iron chloride. The IR spectrum of **2** showed a broad hydroxyl and aromatic ring at 3132.2 and 1580.7, respectively. The ^{1}H-NMR spectrum of **2** showed both aromatic (δ 6.78, δ 6.80, and δ 6.68) and aliphatic signals (δ 4.11, δ 2.94, and δ 2.71). These data and ^{13}C-NMR (see materials and methods) indicated that **2** was a phenylpropanoid. Thus the structure of **2** was recognized to be 3-(3,4-dihydroxyphenyl)latamide by the comparison of NMR spectral data with those reported in the literature (Nagatsu *et al.*, 1995). Although the compound was first isolated from roasted perilla seeds by Nagatsu *et al.*, this is the first example of its occurrence from natural sources. The other three quinones, tanshinone I, tanshinone IIA, and cryptotanshinone, and dimethyllithospermate have already been reported from this plant (Kakisawa *et al.*, 1969, Kohda *et al.*, 1989).

The radical scavenging effect of compounds (**1-5**) on DPPH radical were tested. As shown in Table II, the IC_{50} of the two compounds (**1** and **2**) showed scavenging activity on DPPH radical at concentrations of 1.17 and 6.32 μM, respectively. These radical scavenging activities were more potent than that of L-ascorbic acid or BHT which are well known antioxidant. However, quinone compounds (**3-5**) were found to be inactive even at the higher concentration. The DPPH stable radical loses its characteristic purple color when supplied with electrons or hydrogen ions. The capacity of the tested samples to donate electrons can be estimated from the degree of their loss of color (Blois, 1958).

Dimethyl lithospermate was only reported as an adenylate cyclase inhibitor (Khoda *et al.,* 1989), and 3-(3,4-dihydroxyphenyl)lactamide was recently reported as an antioxidant from roasted perilla seeds (Nagatsu *et al.*, 1995). As dimethyl lithospermate and 3-(3,4-dihydroxyphenyl)lactamide have an catechol moiety, the antioxidative potency of these compounds may be attributable to this moiety. The antioxidative potency of phenolic acids are inter-related. These compounds react with the free radicals formed during autoxidation, and generate a new radical which is stabilized by the resonance effect of the aromatic nucleus (Cuvelier *et al.*, 1992). The higher radical scavenging property of catechol phenolic acids is probably due to a superior stability of radical derived from catechol compared to that of phenoxyl radical (Ruiz-Larrea *et al.*, 1994). The present work indicate that dimethyl lithospermate and 3-(3,4-dihydroxyphenyl)lactamide may be useful for the treatment of oxidative damage.

Recently, 2',7'-dichlorofluorescin diacetate (DCFH-DA) has been used as a probe of reactive oxygen species (ROS) such as $\cdot O_2$ and H_2O_2 etc. Liposoluble DCFH-DA becomes water-soluble dichlorofluorescin (DCFH) as a results of the activities of mitochondrial esterase or hydrolysis, then it is oxygenized to dichlorofluorescein (DCF) which has strong fluorescence. Therefore, this method is useful to measure changes of ROS (Label *et al.*, 1990). Compounds **1** and **2** significantly inhibited the generation of free radicals of hepatocyte, as shown in Table III. Compound 1 showed greater inhibitory action than compound 2. This indicated that structural modifications influence inhibition. Compound 1 has two more catechol moieties than compound 2. Compounds with more than one catechol moiety increase the resonance stability, and this stability may have influenced the inhibitory effect of the compound. However, quinone compounds (**3-5**) were found to be inactive at the same concentration.

Organic hydroperoxides such as *tert*-butyl hydroperoxide (*t*-BHP) have been widely utilized to study the effects of oxidative stress on cells because it is not

Table III. The effect of compounds **1-5** on free radical generation of hepatocyte (AC_2F)[a]

conc.	comp.				
	1	2	3	4	5
Control	53.66±1.60	50.00±1.51	50.37±1.14	50.37±1.14	53.66±1.60
2 μM	24.12±0.91**	30.85±3.20*	53.83±0.71	55.93±2.30	54.89±1.50
10 μM	9.73±0.87***	18.59±3.53**	61.07±0.69	62.78±0.75	59.53±0.53
50 μM	5.08±0.23***	11.62±1.23**	63.78±1.22	61.92±1.73	63.73±1.44

[a]Hepatocytes were incubated in serum free media and preparated with various concentration of each components. After preincubation for 1 hr., 12.5 μM DCFH-DA were added and change in fluorescence was measured.
Values are means±S.D. of three experiments. Statistical significance : *p<0.05, **p<0.01 ***p<0.001 vs control group.

Table IV. The cytoprotective effect of compounds **1-5** against 2×10^{-4} M *tert*-butyl hydroperoxide (*t*-BHP) in cultured liver cell

conc.	comp.				
	1	**2**	**3**	**4**	**5**
Normal	199.52±6.43	199.52±6.43	199.52±6.43	199.52±6.43	199.52±6.43
Control	100.00±6.85	100.00±6.85	100.00±6.85	100.00±6.85	100.00±6.85
2 μM	133.49±8.50*	107.00±4.18	127.32±1.71	90.96±4.44	99.80±1.87
10 μM	168.88±1.09**	129.40±4.05*	137.29±4.80*	103.14±3.54	107.07±2.32
50 μM	171.73±7.85**	168.44±5.88**	131.35±2.67*	101.77±1.87	91.75±6.01

Values are means±S.D. of three experiments. Statistical significance: * $p<0.05$, ** $p<0.01$ vs control group

metabolized by catalase, which is often a contaminant in homogenate preparations. *t*-BHP is metabolized to methyl, *tert*-butoxyl, and *tert*-butylperoxyl radicals by mitochondrial cytochrome *c* or cytochrome *c* ,, which causes fatal cell damage as a result of lipid peroxidation, protein oxidation, and nucleic acid lesion, *etc.* (Christoper *et al.*, 1992). As shown in Table IV, compounds 1 and 2 showed the cytoprotective effect on *t*-BHP in cultured liver cells, while other quinone compounds (**3-5**) showed no significant cytoprotective activity even at a higher concentration of 50 μM. These results indicate that compounds 1 and 2, dimethyllithospermate and 3-(3,4-dihydroxyphenyl)-lactamide may protect the liver cell from oxidative stress such as *t*-BHP through strong free radical-scavenging activity. Investigation of further antioxidant principles is now in progress.

ACKNOWLEDGEMENT

This work was partly supported by a research grant from the G-7 project program.

REFERENCES CITED

Baillie, A. C. and Thomson, R. H., Naturally occurring quinones. Part XI. The tanshinones. *J. Chem. Soc.* (*C*), 48-52 (1968).

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

Choi, J. S., Lee, J. H., Park, H. J., Kim, H. K., Young, H. S. and Mun, S. I., Screening for antioxidant activity of plant and marine algae and its active principles from *Prunus davidiana. Kor. J. Pharmacogn.*, 24, 299-303 (1993).

Christoper, H. K., Daniel, F. C., Gray, W. W. and William, A. P., *t*-Butyl hydroperoxide-induced radical production in rat liver mitochondria. *Free Radical, Biology and Medicine,* 12, 381 (1992).

Cuvelier, M-E., Richard, H. and Berset, C., Comparison of the antioxidative activity of some acid-phenols: Structure-activity relationship. *Biosci. Biotech. Biochem.*, 56(2), 324-325 (1992).

Fang, C. N., Chang, P. L. and Hsu, T. P., The antibacterial components of dan-shen. *Acta Chim. Sin.*, 34, 197-209 (1976).

Hayashi, T., Handa, T., Ohashi, M. and Kakisawa, H., The structure of salviol, a new phenolic diterpene. *J. Chem. Soc. Chem. Commun.,* 541-542 (1971).

Huang, Y. S. and Zhang, J. T., Antioxidative effect of three water-soluble components isolated from *Salvia miltiorrhiza in vitro. Acta Pharmaceutica Sinica,* 27, 96-100 (1992).

Kakisawa, H., Hayashi, T. Okazaki, I. and Ohashi, M., Isolation and structures of new tanshinones. *Tetrahedron Lett.*, 3231-3234 (1968).

Kohda, H., Takeda, O., Tanaka, S., Yamasaki, K., Yamashita, A., Kurokawa, T. and Ishibashi, S., Isolation on inhibitors of adenylate cyclase from danshen, the root of *Salvia miltiorrhiza. Chem. Pharm. Bull.*, 37, 1287-1290 (1989).

Label, C. P. and Bondy, S. C., Sensitive and rapid quantitation of oxygen reactive species formation in rat synaptosomes. *Neurochem. Int.*, 17, 435-441 (1990).

Nagatsu, A., Tenmaru, K., Matsuura, H., Murakami, M., Kobayashi, T., Okuyama, K. and Sakakibara, J., Novel antioxidants from roasted perilla seed, *Chem. Pharm. Bull.*, 43, 887-889 (1995).

Ruiz-Larrea, M. B., Leal, A. M., Liza, M., Lacort, M. and de Groot, H., Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. *Steroids*, 59, 383-388 (1994).

Shanghai Science and Technological Publisher, *The Dictionary of Chinese Drugs* (Zhong Yao Da Ci Dian), Vol. 3, Shougakukan, Tokyo, pp.1729-1730, 1985

Takiura, K. and Koizumi, K., Uber die bestandteile der Chinesischen dorge tanshin IV. Zurkenntnis der konstitution des tanshinones II-A. *Chem. Pharm. Bull.*, 10, 112-116 (1962).

Tang, W. and Eisenbrand, G., *Chinese Drugs of Plant Origin,* Springer-Verlag, New York, pp. 891, 1992.

Weng, X. C. and Gordon, M. H., Antioxidant activity of quinones extracted from tanshen (*Salvia miltiorrhiza* Bung). *J. Agric. Food Chem.*, 40, 1331-1336 (1992).

Zhang, K. Q., Bao, Y., Wu, P., Rose, R. T. and Ho, C. T., Antioxidative components of tanshen (*Salvia miltiorrhiza Bung*). *J. Agric. Food Chem.*, 38, 1194-1197 (1990).