J Food Sci Nutr
Vol 13, p 241 ~244 (2008)
DOI: 10.3746/jfn.2008.13.3.241

J Food Science and Nutrition

Antioxidative Substance Isolated from the Leaf of *Zanthoxylum schinifolium*

– Research Note –

Chang-Ho Jeong and Ki-Hwan Shim†

Division of Applied Life Sciences, and Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju 660-701, Korea

Abstract

Methanol extracts were prepared from *Z. schinifolium* leaf and successively fractionated with chloroform, butanol, and water. The butanol fraction exhibited the highest antioxidative activities. Therefore the butanol fraction was purified and a chemical structure was identified by ^{1}H-^{13}C-NMR spectra, and FT-IR. The isolated antioxidative substance was identified as quercitrin.

Key words: *Z. schinifolium*, antioxidative activities, quercitrin

INTRODUCTION

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide anion radical ($\cdot O_2^-$), and hydroxyl radical (·OH) are generated from the autoxidation of lipids, as well as reactive nitrogen species (RNS) (1,2). Formations of these excess ROS and RNS by UV irradiation, smoking and drug metabolism are likely to damage several cellular components such as lipids, proteins, nucleic acid, and DNAs through oxidative nitration processes (3). In addition these reactive species cause inflammation or lesions on various organs and are associated with various degenerative diseases, including cancer, aging, arteriosclerosis, and rheumatism (4-8). Plants contain a wide variety of chemicals that have potential antioxidant activity. The best known phytochemical antioxidants are traditional nutrients, such as β-carotene, ascorbic acid, and α -tocopherol. However, there is growing evidence that a significant portion of the antioxidant capacity of many food plants is due to compounds other than the traditional vitamins (9). Recently, researchers have sought to isolate powerful and nontoxic natural antioxidants from edible plants, not only to prevent human disease resulting from autoxidation and lipid peroxidation, but also to replace synthetic antioxidants (10). Therefore, the research into the determination of the natural antioxidant source is very important to promote public health.

Zanthoxylum schinifolium (Korean name: *Sancho*) is an aromatic medicinal plant belonging to the Rutaceae family and distributed in Korea, China, Japan, and Taiwan (11-14).

The chemical constituents of *Z. schinifolium*, especially from the fruit, have been studied extensively. The known compounds in *Z. schinifolium* have been identified as aurapten, collinin, epoxyaurapten, hydrangetin, umbelliferone, acetoxycollinin, aesculetin dimethylether, norchelerythrine, dictamnine, skimmianine, and friedelin (11,12). Choi et al. (15) investigated antimicrobial activity and the essential oils composition of *Z. piperitum* and *Z. schinifolium*. However there is little information available about the antioxidative activities of extracts from *Z. shinifolium* leaf.

The objectives for this study were to investigate the antioxidative activities of extracts from *Z. schinifolium* leaf. In addition, a compound was isolated and identified from the active butanol fraction of *Z. schinifolium* leaf.

MATERIALS AND METHODS

Instruments

IR spectra were obtained with a Hitachi 270-50 spectrophotometer, ^1H- and ^{13}C-NMR along with 2D-NMR data were obtained with a Brucker AM 500 (^1H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz) spectrometer in methanol-d_4.

Extraction and isolation

Z. schinifolium leaf (300 g) belonging to the family Rutaceae, were collected from a plantation at Jinju, Korea in June 2007. They were ground in a blender, extracted three times with methanol (1.5 L) each for 3 days at room temperature. The combined methanol solution was concentrated and dried under reduced pressure at a temperature no higher than 45°C. The methanol extract (37.40 g) was sequentially extracted with chloroform (18.34 g), butanol (5.07 g) and water extract (13.99

†Corresponding author. E-mail: khshim@gnu.ac.kr
Phone: +82-55-751-5479, Fax: +82-55-753-4630

g) for a subsequent bioassay. The organic solvent extracts were concentrated to dryness *in vacuo* at 45°C, and the water extract was freeze dried. The butanol fraction (5.07 g) was chromatographed on a silica gel column (Merck 70～230 mesh, 500 g, 70 cm×5.5 I.D.) and successively eluted with a stepwise gradient of chloroform/methanol (99/1→0/1). Active fraction F10 (0.72 g) was combined and applied to a silica gel column, eluting with a dichloromethane-ethanol mixture of increasing polarity (49/1→1/1), to give six major subfractions (F10-1 through F10-6), based on a comparison of the TLC profiles after examination with shortwave UV light (254 nm) and by spray with 10% v/v sulfuric acid in water. F10-4 was purified on a Sephadex LH-20 resin column chromatograph using methanol. For further separation of the biologically active substance, a Waters Delta Prep 4000 HPLC was used. The column [300 mm×3.9 m PREP ODS column (Hewlett Packard)] was eluted with 30% methanol at a flow rate of 5 mL/min, and the elute was measured at 254 nm.

Compound 1 (102 mg) was isolated by recrystallization with MeOH. The homogeneity of 1 was demonstrated by TLC in a developing solvent system of chloroform/methanol (3:1) (Rf=0.32).

Compound 1 (Quercitrin). IR ν_{max} (KBr) cm^{-1}: 3378, 2932, 1651, 1607, 1505; ^{1}H-NMR (500 MHz, methanol-d_4) δ: 0.94 (d, J=6.0 Hz, 3H), 3.31 (dd, J=3.5, 2.0 Hz, 1H), 3.43 (m, 1H), 3.76 (brd, J=7.5 Hz, 1H), 4.22 (s, 1H), 5.34 (s, 1H), 6.13 (s, 1H), 6.28 (s, 1H), 6.90 (d, J=6.5 Hz, 1H), 7.27 (d, J=7.5 Hz, 1H), 7.33 (s, 1H); ^{13}C-NMR (125 MHz, methanol-d4) δ: 18.1, 72.3, 72.4, 72.6, 73.8, 96.1, 101.5, 103.9, 105.2, 116.8, 117.3, 123.2, 123.4, 136.4, 147.0, 150.5, 159.2, 159.3, 163.4, 170.2, 179.6.

DPPH free radical-scavenging activity

The effect of quecitrin on DPPH free radical was estimate according to the method of Blois (16). Quecitrin (0～200 μg/mL) in 4 mL of DMSO was added to an ethanol solution of DPPH (1 mM, 1 mL) The mixture was shaken and left to stand at room temperature for 30 min; the absorbance of the resulting solution was measured spectrophotometrically at 517 nm (UV-1201, Shimadzu, Tokyo, Japan). This activity is given as percent DPPH free radical scavenging that is calculated as % DPPH scavenging=[(control absorbance – extract absorbance) / (control absorbance)]×100.

Reducing power

Reducing power was estimated according to the method of Oyaizu (17). Briefly, extracts (0～250 μg/mL) in 1 mL of appropriate solvents were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Afterward, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm (UV-1201, Shimadzu, Tokyo, Japan).

Inhibition of lipid oxidation

The ferric thiocyanate (FTC) method (18) using the linoleic acid model system was used to measure inhibitory activity on lipid oxidation. Quercitrin dissolved in 0.12 mL of ethanol was added to a reaction mixture in a screw-cap test tube. Each reaction mixture consisted of 2.88 mL of 2.51% linoleic acid in ethanol and 9 mL of 40 mM phosphate buffer (pH 7.0). The known antioxidants, BHA and α-tocopherol, were used as a comparative standard to evaluated the antioxidant activity of quercitrin. The test tube was covered with aluminum foil to eliminate the influence of the light upon the lipid peroxidation and then placed in an incubator at 40°C. At daily intervals during an incubation period, a 0.1 mL aliquot of the mixture was diluted with 9.7 mL of 75% ethanol, followed by addition of 0.1 mL of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm (UV-1201, Shimadzu, Tokyo, Japan).

Statistical analysis

The results for each group were expressed as mean ±SD. Data were analyzed by one way ANOVA between control and sample treated groups using SAS software (SAS Institute Inc., Cary, NC, USA). Significant differences were determined among groups at $p<0.05$.

RESULTS AND DISCUSSION

During the preliminary screening for antioxidative activities, we observed that the methanol extract of *Z. schinifolium* leaf showed significant antioxidative activities. In fractionation guided by antioxidative activities, the butanol fraction was purified by silica gel column chromatography and the isolated fraction was bioassayed. Finally, an active compounds was isolated. The chemical structure was identified as quercitrin through spectroscopic analysis including IR and NMR.

Scavenging effect on DPPH radical

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scav-

enging ability of various samples. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. Fig. 1 shows the dose-response curves for DPPH radical-scavenging activities of quercitrin. Quercitrin exhibited 67.58 and 85.49% radical scavenging activity at concentrations of 25 and 200 μg/mL, respectively (IC_{50}=12.9 μg/mL). However, BHA (IC_{50}=12.1 μg/mL) and α-tocopherol (IC_{50}=13.9 μg/mL) showed 90.64 and 92.83% radical scavenging activity at a concentration of 25 μg/mL, respectively. The free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts and food materials (19,20) exhibit an almost linear correlation between DPPH radical scavenging activity and concentration of polyphenolic compounds in various vegetable and fruits (21,22). Our results revealed that quercitrin isolated from *Z. schinifolium* leaf was one of the major free radical scavengers, acting possibly as a primary antioxidant.

Reducing power

In the reducing power assay, the presence of reductants (antioxidants) in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donation of an electron. The amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 2 shows the dose-response curves for the reducing powers of quercitrin. It was found that the reducing powers of quercitrin also increased with the increase of quercintrin concentrations. At 150, 200, and 250 μg/mL concentration, the quercitrin showed absorbances of 0.82, 0.98, and 1.19, respectively. The BHA and α-tocopherol, positive control, exhibited high reducing powers of 1.84 and 0.93 in 250 μg/mL, respectively. Many studies have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts (23,24). The quercitrin examined in this study demonstrated good reducing capacity thereby acting as an efficient reductone.

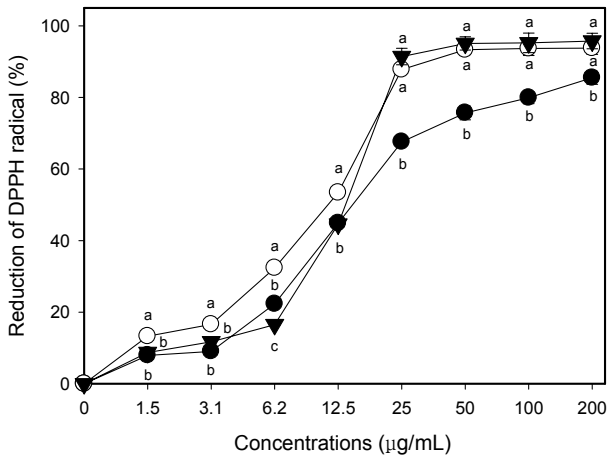

Fig. 1. DPPH radical scavenging activity of quercitrin isolated from *Z. schinifolium* leaf. ●: Quercitrin, ○: BHA, ▼: α-Tocopherol.

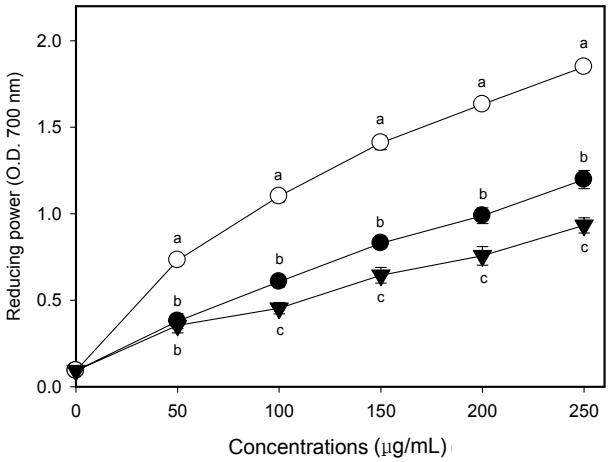

Fig. 2. Reducing power of quercitrin isolated from *Z. schinifolium* leaf. ●: Quercitrin, ○: BHA, ▼: α-Tocopherol.

Inhibitory effect of lipid oxidation

Antioxidant activity of quercitrin was determined by the FTC method. Quercitrin exhibited effective antioxidant activity at all concentrations. The effects of various amounts of quercitrin on peroxidation of linoleic acid emulsion are shown in Fig 3. The antioxidant activity of quercitrin increased with increasing concentration. The 1,000 μg/mL concentration of quercitrin showed higher antioxidant activity than that of 1,000 μg/mL concentration of α-tocopherol (70.35%). At 250, 500, and 1,000 μg/mL concentrations of quercitrin, the percentage of inhibition in the linoleic acid system were 7.59, 54.47,

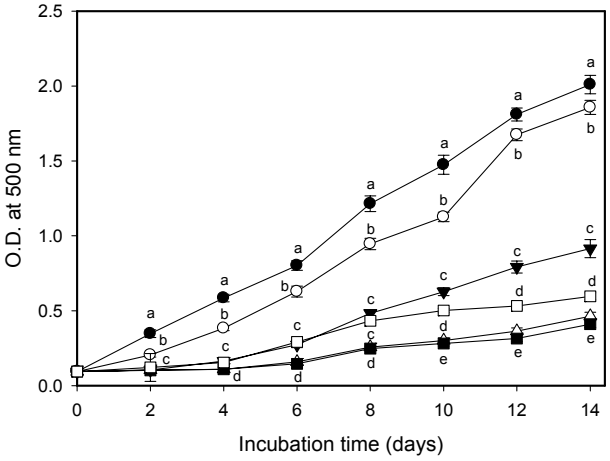

Fig. 3. Antioxidant activity of quercitrin isolated from *Z. schinifolium* leaf at the level of 250, 500, and 1,000 μg/mL. ●: Control, ○: 250 μg/mL, ▼: 500 μg/mL, △: 1,000 μg/mL, ■: BHA (1,000 μg/mL), □: α-Tocopherol (1,000 μg/mL).

and 76.94, respectively. The result shows that quercitrin is also a free radical inhibitor, particularly of the peroxy radical, which is the major propagator of the fatty acid autoxidation cascade, thus terminating the chain reaction. The antioxidant quercitrin was also isolated from *Z. schinifolium* leaf. Quercitrin is a well-known antioxidant substance found in many edible plants such as chestnut leaves (25) and Korean cherry (26).

REFERENCES

1. Singh A. 1989. Chemical and biochemical aspects of activated oxygen: singlet oxygen, superoxide anion, and related species. In *CRC Handbook of free radical and antioxidant in Biomedicine*. Mequel J, Quintanilha AT, Weber H, eds. CRC press, Inc., Boca Raton, FL, USA. Vol 1, p 17-28.
2. Aruoma OI. 1996. Assessment of potential prooxidant and antioxidant actions. *J Am Oil Chem Soc* 73: 1617-1625.
3. Sawa T, Akaike T, Maeda H. 2000. Tyrosine nitration by peroxynitrite formed nitric oxide and superoxide generated by xanthine oxidase. *J Biol Chem* 275: 32467-32474.
4. Dreher D, Junod F. 1996. Role of oxygen free radicals in cancer development. *Eur J Cancer* 32A: 30-38.
5. Sohal RS. 2002. Role of oxidative stress and protein oxidation in the aging process. *Free Radic Biol Med* 33: 37-44.
6. Griffiths HR, Lunec J. 1996. The C1q binding activity of IgG is modified in vitro by reactive oxygen species: implication for rhematinoid arthritis. *FEBS Lett* 388: 161-164.
7. Squadrito GL, Pryor WA. 1998. Oxidative chemistry of nitric oxide: the role of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med* 25: 392-403.
8. Choi HR, Choi JS, Han YN, Bae SJ, Chung HY. 2002. Peroxynitrite scavenging activity scavenging activity of herb extracts. *Phytother Res* 16: 364-367.
9. Cao G, Sofic E, Prior R. 1996. Antioxidant capacity of tea and common vegetables. *J Agric Food Chem* 44: 3426-3431.
10. Branen AL. 1975. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J Am Oil Chem Soc* 52: 59-63.
11. Chang CT, Doong SL, Tsai IL, Chen IS. 1997. Coumarins and anti-HBV constituents from *Zanthoxylum schinifolium*. *Phytochemistry* 45: 1419-1422.
12. Chen IS, Lin YC, Tsai IL, Teng CM, Ko FN, Ishikawa T, Ishi H. 1995. Coumarins and anti-platelet aggregation constituents from *Zanthoxylum schinifolium*. *Phytochemistry* 39: 1091-1097.
13. Cho MG, Kim H, Chae YA. 2003. Analysis of volatile compounds in leaves and fruits of *Zanthoxylum schinifolium* Siebold et Zucc. and *Zanthoxylum piperitum* DC. By headspace SPME. *Korean J Med Crop Sci* 11: 40-45.
14. Ko YS, Han HJ. 2002. Chemical constituents Korean *chopi* (*Zanthoxylum peperitum*) and *sancho* (*Zanthoxylum schinifolium*). *Korean Food Sci Technol* 28: 19-27.
15. Choi SI, Chang KM, Lee YS, Kim GH. 2008. Antibacterial activity of essential oils from *Zanthoxylum piperitum* A.P. DC. And *Zanthoxylum schinifolium*. *Food Sci Biotechnol* 17: 195-198.
16. Blois MA. 1958. Antioxidant determination by the use of a stable free radical. *Nature* 181: 1199-1200.
17. Oyaizu M. 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr* 44: 307-315.
18. Nakatani N, Nikuzaki H. 1987. A new antioxidative glucoside isolated from Oregano (*Origanum vulgare* L.). *Agric Biol Chem* 51: 2727-2732.
19. Shih PW, Lai PL, Jen HWK. 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chem* 99: 775-783.
20. Kim JS, Moon GS, Kim HO, Lee YS. 2007. Antioxidant properties of ginseng (*P. ginseng* C.A. Meyer) extracts by organic solvent fractionation. *J Food Sci Nutr* 12: 267-272.
21. Robards K, Prenzeler PD, Tucker G, Swatsitang P, Glover W. 1999. Phenolic compounds and their role in oxidative process in fruits. *Food Chem* 66: 401-436.
22. Pyo YH, Lee TC, Logendra L, Rosen RT. 2004. Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris subspecies cycla*) extracts. *Food Chem* 85: 19-26.
23. Elzaawely AA, Xuan TD, Tawata S. 2005. Antioxidant and antibacterial activities of *Rumex japonicus* HOUTT. aerial parts. *Biol Pharm Bull* 28: 2225-2230.
24. Lee JG, Choi SI, Lee YS, Kim GH. 2008. Antioxidant and anti-inflammatory activities of ethanol extract from leaves of *Cirsium japonicum*. *Food Sci Biotechnol* 17: 38-45.
25. Choi YH, Kim JH, Kim MJ, Han SS, Rim YS. 2000. Antioxidative compounds in leaves of *Castanea crenata* S. et Z. *Korean J Medicinal Crop Sci* 8: 373-377.
26. Hwang HS, Kim JM, Jeon YJ, Song YA, Park HS. 2003. Flavonoids and antimicrobial activity of the ethanol extract of Korean cherry (*Prunus tomentosa* Thunberg). *J Korean Soc Food Sci Nutr* 32: 833-839.

(Received June 4, 2008; Accepted August 20, 2008)