J. Korean Soc. Food Nutr.
23 (3), 466 ~ 471 (1994)

Further Screening for Antioxidant Activity of Vegetable Plants and Its Active Principles from *Zanthoxylum schinifolium*

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Abstract

The antioxidant activity of methanol extracts of thirty plants was tested using the method of 1,1-diphenyl-2-picryl hydrazyl (DPPH) reactivity. Four methanol extracts from *Zingiber officinale, Piper nigrum, Zanthoxylum schinifolium* and *Capsicum annuum* were found to be the most effective on DPPH radical scavenging activity. The next effective ones were *Perilla frutescens, Sedum sarmentosum, Raphnus sativas, Arctium lappa, Beta vulgaris, Brassica oleracea* var. *acephala, Brassica juncea* in order, and the others did not show a considerable activity. The methanol extract obtained from the seed coats of *Zanthoxylum schinifolium* was fractionated with several solvents. The interphase materials exhibited the strongest antioxidant activity and was further purified by silica gel and Sephadex LH-20 column chromatography. Two active principles were isolated and identified as quercetin-3-O-α-L-rhamnopyranoside (quercitrin) and quercetin 3-O-α-D-galactopyranoside (hyperoside) by ultraviolet (UV), proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR). Its antioxidative activity was a little higher than that of L-ascorbic acid.

Key words : antioxidant activity, plant extracts, hyperoside, quercitrin, *Zanthoxylum schinifolium*

INTRODUCTION

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) has been used to prevent lipid peroxidation, but their applications as food additives are confined to a narrow range because of undesirable effects on the human body[1-6]. However phenolic antioxidants such as BHA, BHT, TBHQ, propyl gallate are still used partly as food antioxidants because of their excellent effects and low cost. Therefore, the development of alternative natural antioxidants has been strongly desired, and the natural antioxidants such as α-tocopherol and L-ascorbic acid have attained a high degree of consumer acceptance because of their safety. The antioxidant activities of α-tocopherol and L-ascorbic acid are, however, lower than those of synthetic antioxidants such as BHA and BHT. Hence, there is a pressing need to find safe, economic antioxidants with high antioxidant activity to replace these synthetic chemicals with the natural sources. Especially, the antioxidant compounds present in edible plants have recently been considered as reasonable food additives. To date, although a large number of reports have appeared in the literature concerning antioxigenic activity of foods[7-15], a little data exist regarding antioxigenic principles isolated from foods[16-20].

In the previous paper[20], we studied the antioxidative activity of the plants and marine algae and found (+)-catechin along with flavonoids as active principles from *Prunus davidiana*. In this report, we further extends the screening results of thirty vegetables and isolation of the antioxidant principles from the methanol extract of *Zanthoxylum schinifolium* seed coats used as a spice in Korea.

MATERIALS AND METHODS

Instruments

The mps were taken on a Thomas Hoover 6406-H apparatus and are uncorrected. The infra red (IR) spectra were determined in KBr tablets on a Varian Tech-

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tron Model 635 spectrophotometer and the ultra violet (UV) spectra were run with CE 599 Universal automatic scanning spectrophotometer. The ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.5 MHz) recorded with a Brucker-AM 300 spectrometer in DMSO-d_6 containing TMS as an internal standard and chemical shifts are given as δ(ppm). Optical rotations were measured on Rudolph Autopol III automatic polarimeter.

Materials

Korean pepper, *Zanthoxylum schinifolium* was gathered from Mugori, Sacheon-Gun, Kyung Sang Nam Do on August 1993. All other samples were purchased at a local market in Pusan at the same time.

1,1-diphenyl-2-picryl hydrazyl (DPPH), L-ascorbic acid, BHA and BHT were reagent grade, purchased from Sigma. All other reagents were of the highest grade commercially available.

Measurement of antioxidant or radical scavenging activity[21]

An 4ml of methanol solution of test extracts at various concentrations (2.5~120g/ml) was added to a solution of DPPH (1.5×10^{-4}M) in MeOH (1ml), and the reaction mixture was shaken vigorously. After storage at room temperature for 30 minutes in air, the remaining DPPH was determined by spectrophotometry at 520nm. The radical scavenging activity (%) of each sample was expressed by the ratio of lowering of the absorption of DPPH, relative to the absorption of DPPH solution in the absence of test sample (control). The mean values were obtained from duplicate experiments.

Extraction and fractionation

Commercially dried seed coats of *Zanthoxylum schinifolium* (680g) were extracted with hot MeOH under reflux as shown in Scheme 1. The MeOH extracts was partitioned with hexane, $CHCl_3$, EtOAc, BuOH, and H_2O successively.

Isolation of active principles

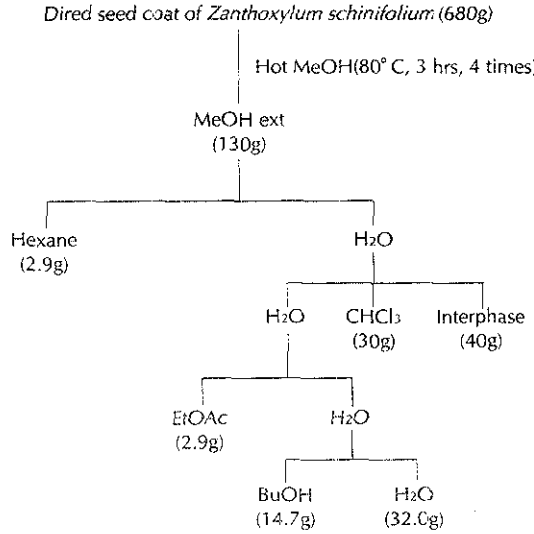
The interphase materials was subjected to chromatography using SiO_2 (EtOAc : MeOH=gradient) and Sephadex LH-20 (MeOH) columns to yield compounds 1 and 2 in the order of elution.

Compound 1(quercitrin)

mp 178-180°C, $[\alpha]_D^{25}$-108 (c=0.1, MeOH) ; UV λ MeOH, nm (log ε) ; 260 (4.15), 305 (sh, 3.80), 355 (4.02) ; λ MeOH + NaOMe ; 275 (4, 24), 332 (3.86), d 400 (4.11) ; λ MeOH + $AlCl_3$ 278 (4, 24), 305 (sh, 3.71), 440 (4.16) ; λ MeOH + $AlCl_3$ + HCl 275 (4.16), 305 (sh, 3.75), 360 (3.88), 405 (3.91) ; λ MeOH + NaOAc 275 (4.17), 325 (sh, 3.88), 370 (3.95) ; λ MeOH + NaOAc + H_3BO_3, 265 (4.24), 300 (sh,3.77), 370 (4.06) ; ^{1}H-NMR (DMSO-d_6, TMS)δ ; 12.60 (1H, brs, C_5-OH), 7.15 (1H, d, J=2.0, H-2'), 7.11 (1H, dd, J=2.0 and 8.5, H-6;467'), 6.72 (1H, d, J=8.5, H-5'), 6.23 (1H, d, J=2.0, H-8), 6.05 (1H, d, J=2.0, H-6), 5.11 (1H, s, anomeric), 0.80 (3H, d, J=6.0, Me of rhamnose) ; ^{13}NMR (DM SO-d_6, TMS) ; see Table 4

Compound 2(hyperoside)

mp 253-4°C, $[\alpha]_D^{25}$-70 (c=0.1, MeOH), IR KBr (cm^{-1}) ; 3200 (OH), 1650 (C=O), 1600, 1540, 1500 (C=C), 1075, 1050 (C-O), 1015, 990, 928, 880, 855, 818, 785 ; UV λ MeOH nm (log ε) ; 256 (4.10), 268 (sh, 4.00), 295 (sh, 3.69), 360 (4.02) ; λ MeOH + NaOMe 272(4.15), 330(3.73), 410(4.11) ; λ MeOH + $AlCl_3$ 272 (4.16), 302 (3.64), 333 (3.50), 431(4.14) ; λ MeOH + $AlCl_3$ + HCl 268 (4.14), 300 (3.76), 360(3.94),



Scheme 1. Extraction and fractionation of *Z. shinifolium*.

398 (3.96) ; λ MeOH + NaOAc 272 (4.10), 325 (3.81), 372 (3.95) ; λ MeOH + NaOAc 272 (4.10), 325 (3.81), 372 (3.95) ; λ MeOH + NaOAc + H_3BO_3 260 (4.16), 296 (3.60), 380 (4.06) ; ^{1}H-NMR (DMSO-d_6, TMS)δ ; 12.60 (1H, brs, OH), 10.77 (1H, brs, OH), 9.80 (1H, brs, OH), 9.03 (1H, brs, OH), 7.66 (1H, dd, J=2.0 and 8.5, H-6'), 7.53 (1H, d, J=2.0, H-2'), 6.84 (1H, d, J=8.5, H-5'), 6.40 (1H, d, J=2.0, H-8), 6.19 (1H, d, J= 2.0, H-6), 5.36 (1H, d, J=7.0, anomeric) ; ^{13}C-NMR(DMSO-d_6, TMS)δ ; see Table 4

Acid hydrolysis of 1 and 2

Ten mg of samples was refluxed with 5% H_2SO_4 (30ml) for 5 hr. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to afford quercetin as yellow needles, mp 312-5. It was confirmed by direct comparisons with an authentic samples (TLC and mmp). The filtrate was neutralized with $BaCO_3$, filtered and concentrated. L-rhamnose from **1** and D-galactose from **2** was identified by TLC (precoated cellulose, pyridine : EtO Ac : HOAc : H_2O=36 : 36 : 7 : 21, Rf 0.65 and 0.40)

RESULTS AND DISCUSSION

Antioxidant activity of vegetable plants

To find the antioxidant materials from plants , the free radical scavenging activity was evaluated by the

Table 1. Free radical scavenging effects of several edible plants on DPPH

English name	Scientific name	Part	50% reduc.[a](mg)
Burdock	*Arctium lappa*	seed	160.2
Chard	*Beta vulgaris*	seed	61.6
Kale	*Brassica oleracea* var. *acephala*	seed	186.2
Korean cabbage	*Brassica campestris* var. *pekinensis*	seed	210.2
lettuce	*Lactuca sativa*	seed	323.0
Black pepper	*Piper nigrum*	seed	52.8
Korean peper	*Zanthoxylum shinifolium*	seed coat	59.0
Redish rod	*Raphnus sativas*	seed	148.7
Leaf mustard	*Brassica juncea*	seed	194.0
Mallow	*Malva verticillata*	seed	480<
Spinach	*Spinacia oleracea*	seed	267.2
Sedum	*Sedum sarmentosum*	leaf	148.0
Crown daisy	*Chrysanthenum coronarium*	leaf	347.9
Amaranth	*Amaranthus margostanus*	leaf	480<
Wild dropwort	*Oenanthe stolonifera*	leaf	338.0
	Isodon japonicus	leaf	246.2
Green perilla	*Perilla frutescens*	leaf	264.3
	Perilla frutescens	seed	145.0
Parsley	*Petroselium sativum*	leaf	480<
Onion	*Allium cepa*	rhizome	480<
Garlic	*Allium sativum*	semen	480<
Leek	*Allium tuberosum*	leaf & stem	480<
Large Geen onion	*Allium fistulosum*	leaf & stem	480<
Ginger root	*Zingiber officinale*	rhizome	49.6
Butterbur	*Petasites japonicus*	leaf	206.8
	Saururus chinensis	leaf	460.0
Jujube	*Zizypus jujuba*	seed	480<
Red peper	*Capsicum annuum*	seed	84.6
	Youngia sonchifolia	leaf & stem	220.0
Dandelion	*Taraxacum platycarpum*	leaf	302.6
Doraji (Root of Chiness bellflower)	*Platycodon grandiflorum*	root	480<
	BHT		9.5
	L-ascorbic acid		8.1

[a] Amount required for 50% reduction of DPPH after 30min

scavenging effect of DPPH radical. The control intensity (absence of sample extracts) was taken as 100%, and the percentage intensity was calculated. The concentration of each fraction for 50% free radical inhibition is shown in Table 1.

As shown in Table 1, some plant extracts such as *Perilla frutescens, Sedum sarmentosum, Raphnus sativas, Arctium lappa, Beta vulgaris,* and *Brassica juncea* exhibited somewhat high scavenging effects on DPPH. The most effective ones were *Zingiber officinale, Piper nigrum, Zanthoxylum schinifolium,* and *Capsicum annuum*. These results suggest that these spices contained a certain antioxidant(s).

Antioxidant activity and active principles of *Zanthoxylum schinifolium*

The present study was also carried out to investigate the active principles in MeOH extract of *Zanthoxylum schinifolium*, whose seed coat showed marked antioxidant activity. The MeOH extract of *Zanthoxylum schinifolium* seed coat was partitioned by hexane, $CHCl_3$, EtOAc, BuOH, interphase materials and water successively. And then, these solvent-soluble fractions were measured free radical scavenging effect on DPPH. As the result of them, interphase materials showed the strongest antioxidant activity (Table 2). This fraction was further purified to obtain active compounds 1 and 2 by repeated silica gel and gel filtration column chromatography. The two compounds 1 and 2 was identified as quercitrin and hyperoside, respectively. Table 3 summarized the radical scavenging results of isolated compounds on DPPH. Their antioxidant effects were a little higher than those of ascorbic acid.

Table 2. Effects of several fraction of the methanol extract from *Zanthoxylum shinifolium* on DPPH

Fractions	50% reduc.[a] (mg)
Hexane	375.2
Interphase materials	9.1
$CHCl_3$	140.8
EtOAc	10.2
BuOH	34.0
H_2O	91.6
L-ascorbic acid	14.7

[a] Amount required for 50% reduction of after 30min.

Table 3. Effects of isolated compounds from *Z. shinifolium* on DPPH

Compounds	50% reduc.[a] (mg)
Quercitrin	6.50
Hyperoside	8.45
L-ascorbic acid	14.7

[a] Amount required for 50% reduction of DPPH after 30min.

Antioxidant activities of various flavonoids are well known. As to flavonoids, the relationship between the position of the hydroxyl groups and the antioxidant activity has been discussed[22]. Quercetin is an effective flavonols in the same ways as morin, kaempferol, and luteolin[23]. Quercitrin and hyperoside isolated from the methanol extract of *Z. schinifolium* also may be usable as an antioxidant component. The findings of the present study indicate that the methanolic extract of *Z. schnifolium* seed coats and its components (quercitrin and hyperoside) may be useful for antioxidant.

Structure elucidation of active principles

The interphase materials were subjected to chromatography using SiO_2 and Sephadex LH-20 to yield compounds 1 and 2 in the order of increasing polarity.

Compound 1, mp 178-180° C and compound 2, mp 254-6° C, showed positive Mg + HCl and Molisch tests. Acid hydrolysis of each compound afforded as the aglycone, quercetin, mp 315-6 and as the sugar, L-rhamnose from compound 1 and D-galactose from compound 2. The ^{1}H-NMR and ^{13}C-NMR (Table 4) spectra showed only one anomeric proton signal, in-

Table 4. ^{13}C-NMR chemical shift of compounds 1 and 2

Carbon No.	1	2	Carbon No.	1	2
2	157.2	156.2	3′	145.1	144.7
3	134.2	133.5	4′	148.3	148.3
4	1771.7	177.4	5′	115.6	115.7
5	161.3	161.1	6′	121.0	121.0
6	98.6	98.5	1″	101.7	101.6
7	164.1	164.0	2″	70.0[a]	71.1
8	93.5	93.4	3″	70.3[a]	73.0
9	156.4	156.2	4″	71.2	67.6
10	103.9	103.6	5″	70.5[a]	75.7
1′	120.7	121.6	6″	17.4	60.0
2′	115.4	115.1			

[a] assignments may reversed

dicating the presence of one mole of sugar in each compound.

The UV spectrum of each compound, exhibiting band I peak at 355-360nm, was very similar to those reported for a number of 3-hydroxy substituted flavonols[24]. A bathochromic shift of band I in the presence of $AlCl_3$ or $AlCl_3$+HCl and of band II in the presence of NaOAc indicated the presence of free 5-hydroxyl and 7-hydroxyl groups. And also a bathochromic shift with NaOMe, without a decrease in intensity, showed the presence of a free 4'-hydroxyl group. It was thus, suggested that the sugar might be attached to 3-hydroxyl group. The ^{13}C-NMR spectrum of each compound confirmed this suggestion. The configuration and conformation of sugar moiety was determined by the J value of the anomeric proton signal. Compounds 1 and 2 were, therefore, identified as quercetin 3-O-α-L-rhamnopyranoside (quercitrin) and quercetin 3-O-β-D-galactopyranoside (hyperoside), respectively.

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(Received May 16, 1994)

식용식물의 항산화 효과 검색과 산초의 항산화 성분

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요 약

30여종의 식용식물 메탄올 엑스에 대한 항산화 효과를 1,1-diphenyl-2-picrylhydrazyl (DPPH)을 사용하여 검색하였다. 생강, 산초, 후추, 고추 메탄올 엑스에서 DPPH radical을 소거하는 효과가 가장 강하게 나타났으며 기타, 들깨, 돌나물, 머위, 쑥갓, 방아, 돌미나리, 배추씨에서는 그 효과가 다소 미약하였다. 산초 종피의 여러 용매 추출 분획물 중에서 interphase 분획물이 free radical 소거 효과가 가장 현저하였으며 interphase 분획물을 silica gel 및 Sephadex column chromatography 하여 분리한 quercitrin과 hyperoside는 산초 종피의 항산화 활성성분들로 밝혀졌으며 이들은 L-ascorbic acid 보다 그 효과가 다소 높았다.