Radical-Scavenging Activities of Citrus Essential Oils and Their Components: Detection Using 1,1-Diphenyl-2-picrylhydrazyl

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Thirty-four kinds of citrus essential oils and their components were investigated for radical-scavenging activities by the HPLC method using 1,1-diphenyl-2-picrylhydrazyl (DPPH). To examine the oils' relative radical-scavenging activities compared with that of a standard antioxidant, Trolox was employed. All of the essential oils were found to have scavenging effects on DPPH in the range of 17.7−64.0%. The radical-scavenging activities of 31 kinds of citrus essential oils were comparable with or stronger than that of Trolox ($p < 0.05$). The oils of Ichang lemon (64.0%, 172.2 mg of Trolox equiv/mL), Tahiti lime (63.2%, 170.2 mg of Trolox equiv/mL), and Eureka lemon (61.8%, 166.2 mg of Trolox equiv/mL) were stronger radical scavengers than other citrus oils. Citrus volatile components such as geraniol (87.7%, 235.9 mg of Trolox equiv/mL), terpinolene (87.4%, 235.2 mg of Trolox equiv/mL), and γ-terpinene (84.7%, 227.9 mg of Trolox equiv/mL) showed marked scavenging activities on DPPH ($p < 0.05$).

Keywords: *Radical-scavenging activity; citrus essential oil; antioxidant; 1,1-diphenyl-2-picrylhydrazyl*

INTRODUCTION

In recent years many researchers have focused on natural antioxidants such as ascorbic acid and α-tocopherol rather than synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Synthetic antioxidants have been widely used in the food industry for extending the shelf life of foods that are susceptible to lipid oxidation. However, there are some arguments about the safety and adverse effects of synthetic antioxidants as food additives. Inatani et al. (1983) reported that synthetic antioxidants had an abnormal effect on enzyme systems. Natural antioxidants found in dietary plants may play an important role in the prevention of cardio- and cerebrovascular diseases, carcinogenesis, and various chronic diseases (Khanum et al., 1999). Although the exact mechanisms of these diseases have not been completely identified, there is much evidence for an inverse relation between dietary plant intake and the occurrence of these diseases and mortality from them (Willett, 1994). These diseases are considered to be free radical-induced diseases (Aruoma et al., 1993). Therefore, one important strategy in the prevention of these diseases is to neutralize or scavenge free radicals: an effective therapeutic agent would be expected to be one that manifests radical-scavenging activity.

Many aromatic plants, especially citrus fruits, and their essential oils are used as flavoring agents in a wide range of food, beverage, and confectionery products and fragrance applications. They have been known to support various biological activities such as antimicrobial (Baratta et al., 1998; Lis-Balchin et al., 1998; Griffin et al., 1999) and antioxidant properties (Frankel et al., 1996; Schwarz and Ernst, 1996). Some antioxidants play an important role in preventing free radical-induced diseases such as cancer (Cerutti, 1985). There is increasing interest in the radical-scavenging activities of some natural antioxidants, especially those found in edible plants, which may play a role in preventing various chronic diseases. Recent works have shown that some fruits (Unno et al., 1997), herbs (Cao et al., 1996; Gadow et al., 1997), and vegetables (Yamaguchi et al., 1999) have radical-scavenging capacity. However, little is known about the radical-scavenging activities of the essential oils from edible plants. Furthermore, there is apparently no information in the literature on the radical-scavenging activities of citrus essential oils.

In this study, the radical-scavenging activities of citrus essential oils and their components were investigated. The objective of this study was to evaluate this functional property of citrus volatile flavor by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method.

MATERIALS AND METHODS

Chemicals. DPPH was obtained from Wako Pure Chemical Industries (Osaka, Japan), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Polyoxyethylene sorbitan monolaurate (Tween 20) was obtained from Sigma Chemical Co. (St. Louis, MO). Tris(hydroxymethylamino)methane (Tris) and all of the solvents used for HPLC were of the highest purity of Nacalai Tesque Inc. (Kyoto, Japan). The water used in this experiment was purified with a Milli-Q system (Millipore, Tokyo, Japan).

Citrus Essential Oils and Authentic Compounds. Thirty-three kinds of citrus fruit and one kind of kumquat were used in this study. Thirty-two fruits were obtained from the Kochi Prefectural Fruit Tree Experimental Station and the

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Table 1. Sample of *Citrus* Genus

no.	variety	common name	species[a]
1	*C. junos* Sieb. ex Tanaka	yuzu	*C. ichangensis*
2	*C. junos* Sieb. ex Tanaka	yuzu (Korea)	*C. ichangensis*
3	*C. junos* Sieb. ex Tanaka	mukakuyuzu or seedless yuzu	*C. ichangensis*
4	*C. inflata* Hort. ex Tanaka	mochiyuzu	*C. ichangensis*
5	*C. sudachi* Hort. ex Shirai	sudachi	*C. ichangensis*
6	*C. yuko* Hort. ex Tanaka	yuko	*C. ichangensis*
7	*C. Wilsonii* Tanaka	Ichang lemon	*C. ichangensis*
8	*C. sphaerocarpa* Tanaka	kabosu	*C. ichangensis*
9	*C. tagumasudachi* Hort. ex Tanaka	naoshichi	*C. ichangensis*
10	*C. aurantifolia* Swingle	Mexican lime	*C. aurantifolia*
11	*C. latifolia* Tanaka	Tahiti lime	*C. aurantifolia*
12	*C. bergamia* Risso var. *Fantastico*	bergamot (Italy)	*C. aurantifolia*
13	*C. bergamia* Risso var. *Balotin*	bergamot	*C. aurantifolia*
14	*C. limon* Burm. f. cv. *Eureka*	Eureka lemon	*C. limon*
15	*C. limon* Burm. f. cv. *Lisbon*	Lisbon lemon	*C. limon*
16	*C. grandis* Osbeck forma *Tosa*	Tosa-buntan	*C. grandis*
17	*C. grandis* Osbeck forma *Banhakuyu*	banhakuyu	*C. grandis*
18	*C. paradisi* Macfadyen	grapefruit	*C. paradisi*
19	*C. hassaku* Hort. ex Y. Tanaka	hassaku	*C. paradisi*
20	*C. natsudaidai* Hayata	natsudaidai	*C. paradisi*
21	*C. aurantium* Linn. var. Cyathifera Y. Tanaka	daidai	*C. aurantium*
22	*C.* sp.	kiyookadaidai	*C. aurantium* (tentative)
23	*C. neo-aurantium* Tanaka	konejime	*C. aurantium*
24	*C. aurantium* Linn. forma *Kabusu*	kabusu	*C. aurantium*
25	*C. sinensis* Osbeck forma *Valencia*	Valencia orange	*C. sinensis*
26	*C. sinensis* Osbeck var. Sanguinea Tanaka forma *Tarocco*	Tarocco orange	*C. sinensis*
27	*C. iyo* Hort. ex Tanaka	Iyokan	*C. sinensis*
28	*C. tamurana* Hort. ex Tanaka	Hyuganatsu	*C. sinensis*
29	*C. ujukitsu* Hort. ex Shirai	ujukitsu	*C. sinensis*
30	*C. unshiu* Marcov. forma *Miyagawa-wase*	unshumikan or Satsuma mandarin	*C. reticulata*
31	*C. unshiu* Marcov. forma *Imamura*	unshumikan or Satsuma mandarin	*C. reticulata*
32	*C. reticulata* Blanco cv. *F-2426*	ponkan	*C. reticulata*
33	*C. ozu* Hort. ex Y. Tanaka	ozu	unidentified
34	*Fortunella japonica* Swingle[b]	kinkan or kumquat	*Fortunella japonica*

[a] Classified by Swingle. [b] Another genus in the Rutaceae family comprising the *Citrus* genus.

Ministry of Agriculture, Forestry and Fisheries, Okitsu Branch Experimental Station in Shizuoka. Korean yuzu fruit was obtained from the Chindo Yuzanara Farm in Korea. Bergamot (*Fantastico*) was collected in Reggio Calabria in Italy. The citrus fruit classification used here is based on the taxonomy of Swingle (1943), as shown in Table 1. The essential oil samples were prepared by means of the cold-pressing method described by Sawamura and Kuriyama (1988) and kept at −25 °C until analyzed.

Twenty-one authentic reagents were used in this study. α-Pinene, terpinolene, *d*-limonene, α-terpineol, citronellol, decanol, and citral (a mixture of neral and geranial) were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). β-Pinene, *p*-cymene, geraniol, linalol, octanol, octanal, decanal, geranyl acetate, and citronellal were obtained from Wako Pure Chemical Industries. Myrcene and terpinen-4-ol were obtained from Aldrich Chemical Co. α-Terpinene was obtained from Sigma Chemical Co., and γ-terpinene was obtained from Extrasynthèse S.A. (Genay, France). Nootkatone was provided by Ogawa & Co., Ltd. (Tokyo, Japan).

Measurement of Radical-Scavenging Activities. The antioxidant activity of citrus essential oils and authentic compounds were determined by a modification of the DPPH radical-scavenging method of Yamaguchi et al. (1998). Each sample (10 μL) was mixed with 900 μL of 100 mM Tris-HCl buffer (pH 7.4), 40 μL of ethanol, and 50 μL of 0.5% (w/w) Tween 20 solution and then added to 1 mL of 0.5 mM DPPH in ethanol (250 μM in the reaction mixture). Tween 20 was used as an oil-in-water emulsifiers (Frankel et al., 1996; Sirendi et al., 1998). The control sample was prepared using water instead of essential oils or authentic compounds. Trolox, a hydrophilic carboxylic acid derivative of α-tocopherol, was employed as a standard antioxidant to examine the radical-scavenging activities. Fifty microliters of 1 mM Trolox in ethanol was added to the reaction mixture (final concentration of 25 μM). The mixture was shaken with a mechanical shaker and left to stand for 30 min at room temperature in the dark room. DPPH is a stable free radical and has a dark violet color. It has a maximum absorption at 517 nm, and the peak of the DPPH radical is decreased in the presence of a hydrogen donor, that is, a free radical-scavenging antioxidant. Thus, we investigated the free radical-scavenging effects of citrus essential oils by evaluating the decrease in the peak height of the DPPH radical at 517 nm. The radical-scavenging activity, expressed as milligrams of Trolox equivalent per milliliter of each sample, was calculated by means of the following equation (Khanum et al., 1999):

$$\text{mg of Trolox equiv} = (A - B)/(A - C) \times 25/1000 \times 250.29/1000 \times 1000/10 \times D$$

where A is the peak height of the control, B is the peak height of the sample, C is the peak height of Trolox, 250.29 is the molecular weight of Trolox, and D is the dilution factor.

All tests and analyses were run in triplicate and averaged. Results were tested by one-way analysis of variance ($p < 0.05$) using the Statistical Analysis System (SAS Institute Inc., 1996) software package. Significant differences between means were determined by Duncan's multiple-range tests.

HPLC Analysis. The determinations of DPPH radical-scavenging activities of citrus essential oils and their components were carried out by HPLC. The HPLC consisted of a Jasco PU-1580 pump and a Toyo Soda UV-8000 UV detector set at 517 nm. Analyses were performed in a Cosmosil 5C18 column (4.6 mm i.d. × 150 mm, Nacalai Tesque, Inc., Japan) at 37 °C, and 70% methanol was used as the mobile phase at a flow rate of 1 mL/min.

Gas Chromatography (GC) and GC−Mass Spectrometry (MS). Most quantitative analysis of the major volatile components of cold-pressed citrus oils was based on the data from Sawamura et al. (1999). A Shimadzu GC-14A equipped with a flame ionization detector and a Shimadzu GC-MS QP-5000 were used for quantitative determination and identification of the volatile components, and the relative peak area

Table 2. Volatile Components[a] of *Citrus* Essential Oils

	sample[b]																
compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
α-pinene	1.8	1.9	1.8	0.3	1.6	3.3	0.7	3.3	0.6	1.0	3.6	3.2	1.6	1.3	2.3	2.6	1.1
β-pinene	0.7	0.8	0.7	0.1	0.5	1.8	*[c]	1.8	0.2	0.3	13.4	13.0	8.9	6.8	10.5	14.0	0.6
myrcene	2.2	1.1	2.1	20.6	1.3	1.3	0.3	0.7	18.5	1.8	1.4	1.3	0.9	0.7	1.6	1.4	1.2
α-terpinene	0.2	0.2	0.3	*	1.4	0.3	0	2.4	*	*	0.3	0.3	0.2	0.1	*	*	0.1
limonene	78.1	72.2	78.1	77.2	69.1	66.6	93.9	68.8	75.5	90.5	50.5	52.2	38.8	24.3	69.7	64.6	75.3
γ-terpinene	9.3	9.4	9.1	0.8	7.5	21.3	*	16.0	2.8	4.2	17.7	17.0	8.3	5.6	8.2	10.3	4.9
p-cymene	0.4	0.4	0.4	*	0.4	0.2	*	*	*	*	0.1	0.1	0.3	*	*	*	0.1
terpinolene	0.4	0.4	0.5	*	0.3	0.9	*	0.7	0.1	0.2	0.7	0.7	0.3	0.2	0.3	0.4	0.4
octanal	*	0.1	*	0	*	0.1	0	0	0.2	0.1	*	*	0	*	*	0.1	0.2
citronellal	*	0	*	*	*	*	*	0	0	*	0.1	0.1	0	0	0.1	*	0.2
decanal	*	0.1	*	*	0.1	0.1	0	0	0	0.3	*	0.1	0.1	*	*	*	0.4
linalol	1.8	4.7	1.8	*	0.3	0.2	0.1	0.2	0.1	0.1	*	0.2	4.2	18.2	0.2	0.2	0.2
octanol	0	0	0	0	*	0.1	0	0	0	0	0	*	0	0	0	0	0
linalyl acetate	0	*	0	0	0	0	0	0	*	*	0	0	32.1	39.0	0	0	0
terpinen-4-ol	0	*	0	0	0	0.1	*	*	0	*	*	0	0.1	0.1	*	0	0.1
neral	*	0	0	*	0	0.1	*	*	*	*	1.0	1.2	0.2	0.2	1.1	0.6	0.1
α-terpineol	0.1	0.2	0.1	*	0.2	0.5	0.1	*	*	*	0.3	0.4	0.2	0.1	0.2	0.3	0.1
geranial	*	0	*	*	*	*	0	0	0	*	2.1	2.3	0.3	0	1.0	1.1	0.2
geranyl acetate	0	0	0	0	0	0	0	*	*	*	0.7	1.0	0.3	0.2	0.2	0.2	0.1
decanol	*	0	*	0	0	0	0	0	0	0	0	0	*	0	0	*	0
citronellol	0	0	0	0	0	0	*	0	0.1	0	*	0	0	*	*	*	*
nerol	0	*	0	0	0	0	*	*	*	*	0.1	0	*	0.1	*	*	*
geraniol	0	0	0	*	*	*	0	*	*	*	0.1	*	*	*	*	0.1	*
nootkatone	0	0	0	0	0	0	0	0	0	0	*	0	0.1	*	0	0	0.4

	sample[b]																
compound	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
α-pinene	1.1	0.3	0.5	1.2	1.2	0.5	0.9	0.6	0.5	0.5	1.2	1.4	1.4	1.0	1.2	1.2	0.4
β-pinene	0.6	0.3	*	0.4	0.3	0.7	1.2	0.9	*	*	0.3	0.8	0.4	0.2	0.3	0.3	*
myrcene	1.2	37.2	1.3	1.8	1.7	1.6	1.6	1.7	1.8	1.8	1.8	1.8	28.3	1.8	1.8	1.8	1.8
α-terpinene	0.1	0	0	0	0	0	*	0	0	0	0	*	0.1	0	0	0	0
limonene	75.3	58.5	83.1	89.8	90.2	94.7	85.8	92.0	95.8	96.6	88.2	84.5	59.2	90.6	89.7	89.9	96.7
γ-terpinene	4.9	0.1	0.1	5.6	4.9	0.1	3.4	0	*	*	5.4	7.5	7.6	3.5	4.6	4.6	0.1
p-cymene	0.1	0	0	*	0.1	0	*	0	0	0	0.1	*	0.1	0.2	0.2	0.1	*
terpinolene	0.4	*	*	0.2	0.2	*	0.1	*	*	*	0.2	0.3	0.3	0.2	0.2	0.2	*
octanal	0.2	0	0.5	0.1	0.3	0.1	0.1	0.2	0.3	0.1	0.1	*	*	0.1	*	0.2	*
citronellal	0.2	*	0.1	*	0	0	*	0	*	*	*	0.2	*	*	*	0.1	0
decanal	0.4	0.1	0.5	0.1	0.2	0.1	*	0.1	0.1	0.1	0.1	*	0	0.1	0.1	0.1	*
linalol	0.2	0.4	0.2	0.1	0.1	0.2	0.7	1.1	0.4	0.3	1.3	1.3	0.7	0.4	0.3	0.6	0.1
octanol	0	0	0	0	*	0	*	0	*	*	*	*	0	0	0	*	0
linalyl acetate	0	0	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0
terpinen-4-ol	0.1	0.2	0.2	*	*	0	*	0	*	0	*	*	*	*	*	0	0
neral	0.1	0.5	0	*	*	*	0.1	0.1	0.1	*	*	*	0.1	*	0	*	0
α-terpineol	0.1	0.1	0.1	0.1	0.1	*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	*
geranial	0.2	0.6	0.1	0.1	0	*	0.2	0.1	0.1	0.1	0	0	0	0	0	*	0
geranyl acetate	0.1	*	0.1	*	0.1	0.1	*	0.1	0	*	0.1	*	*	*	0.1	*	0.1
decanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
citronellol	*	0	*	*	0	*	*	0	*	*	*	*	0	*	*	*	0
nerol	*	0.1	0	0	0	*	0	0	*	*	0	0	0.1	0	0	*	0
geraniol	*	*	*	0	0	*	0	0	*	0	0	0	*	0	0	0	0
nootkatone	0.4	0.2	0.1	*	*	0	0	0	0	0	0	0	0	*	0	0	0

[a] Given in the relative peak area percent. [b] The sample number is coincident with that in Table 1. [c] *, <0.05%.

percent was used for data calculation. The analytical conditions were the same as those described by Njoroge et al. (1994).

RESULTS AND DISCUSSION

Radical-Scavenging Effects of Citrus Essential Oils. The radical-scavenging effects (percentage of quenched radical) were determined for 34 kinds of citrus essential oils. Citrus essential oils mixed with DPPH decolorized the DPPH due to its hydrogen-donating ability. All of the essential oils revealed scavenging effects on DPPH ranging from 17.7 to 64.0%, as shown in Figure 1. There was weak radical-scavenging effect in the essential oils of ozu, daidai, and Valencia orange (17.7−19.1%); the extent of the effect was weaker than that of Trolox ($p < 0.05$). On the other hand, 31 kinds of citrus essential oils exhibited scavenging effects ranging from 21.6 to 64.0%, and their effects were comparable with or stronger than that of Trolox ($p < 0.05$). On the basis of scavenging effects on DPPH, significant differences among these fruits were observed (Figure 1). Eureka lemon, Tahiti lime, and Ichang lemon showed the highest radical-scavenging effects (61.8−64.0%) followed by sudachi, yuko, kabosu, yuzu, and Mexican lime (57.3−58.9%). The radical-scavenging effects of Lisbon lemon and hassaku (46.6−48.4%) were significantly greater than those of Miyagawa-wase (Satsuma mandarin), natsudaidai, Imamura (Satsuma mandarin), mochiyuzu, and Korean yuzu (37.8−39.3%). The essential oils of Hyuganatsu, konejime, grapefruit, ponkan, ujukitsu, Iyokan, kabusu, naoshichi, Tosabuntan, Kiyookadaidai, and seedless yuzu showed scavenging effects on DPPH in the range from 27.7 to 34.1%. The essential oil of Ichang lemon showed the strongest radical-scavenging effect, as high as 64.0%. There was

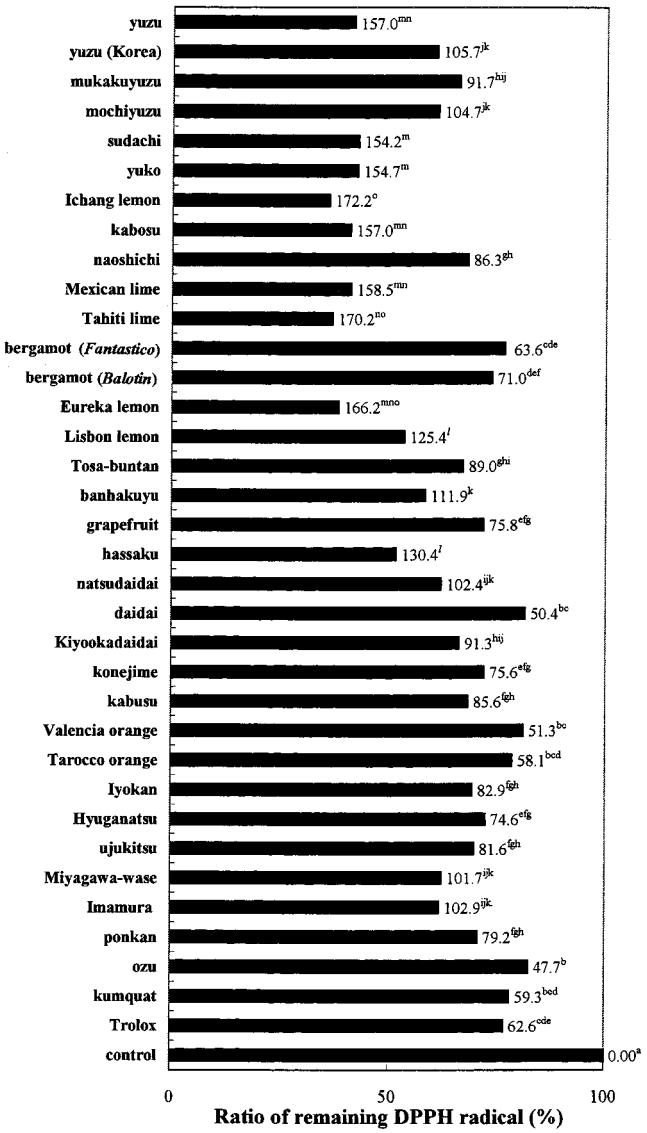

Figure 1. Scavenging effects of citrus essential oils on the DPPH radical. [a−o] Means of the radical-scavenging activity (mg of Trolox equiv/mL). Values with the same superscripts are not significantly different ($p < 0.05$).

no direct correlation among species of the *Citrus* and radical-scavenging effects on DPPH.

Radical-Scavenging Effects of Authentic Compounds. The radical-scavenging effects of 21 authentic compounds of citrus essential oils are shown in Figure 2. The radical-scavenging effects of α-terpinene, nootkatone, citronellal, citral, γ-terpinene, terpinolene, and geraniol were much higher than that of Trolox ($p < 0.05$). According to calculations based simply on the decrease of peak height of DPPH, γ-terpinene (84.7%), terpinolene (87.4%), and geraniol (87.7%) have a radical-scavenging effect 3.5 times as strong as that of Trolox. No significant differences ($p < 0.05$) were found for radical-scavenging effects among decanal, geranyl acetate, and the control. The radical-scavenging effects of linalol, citronellol, α-pinene, and octanal (18.7−22.4%) were higher than those of α-terpineol, octanol, myrcene, decanol, β-pinene, terpen-4-ol, *p*-cymene, and *d*-limonene (8.8−16.5%).

Radical-Scavenging Activities of Citrus Essential Oils and Authentic Compounds. The radical-scavenging activities (milligrams of Trolox equivalent per milliliter) of citrus essential oils and their components were significantly different, as shown in Figures 1 and 2. A possible explanation for the difference in efficiencies found in this study may be the substantial variation in the composition of the citrus essential oils. Among 34 kinds of citrus essential oils, the radical-scavenging activity of Ichang lemon (172.2 mg of Trolox equiv/mL) was the highest, and Tahiti lime (170.2 mg of Trolox equiv/mL) and Eureka lemon (166.2 mg of Trolox equiv/mL) oils were also strong radical scavengers, as shown in Figure 1. Geraniol (235.9 mg of Trolox equiv/mL) had the highest hydrogen-donating capacity against the DPPH radical, whereas decanal (5.4 mg of Trolox equiv/mL) had the smallest (Figure 2). The family of *C. ichangensis* (samples 1−9, 86.3−157.0 mg of Trolox equiv/mL), *C. aurantifolia* (samples 10−13, 63.6−170.2 mg of Trolox equiv/mL), and *C. limon* (samples 14 and 15, 125.4−166.2 mg of Trolox equiv/mL) showed higher radical-scavenging activities. The higher efficiency may have been caused by the composition of essential oils having a higher content of terpenes with the exception of limonene and myrcene. Radical-scavenging activity was found to be high when the terpenes included a higher content of γ-terpinene and terpinolene. In Mexican and Tahiti limes and in Eureka and Lisbon lemons, the combined percent of neral and geranial ranged from 1.7 to 3.5%. It was also considered that the characteristic components of neral and geranial contributed to their scavenging effects in these samples. In *C. sinensis* (samples 25−29) and *C. reticulata* (samples 30−32), Valencia and Tarocco oranges, which had poor radical-scavenging activities, contained little of the more effective compounds such as γ-terpinene and terpinolene. However, Miyagawa-wase and Imamura showed

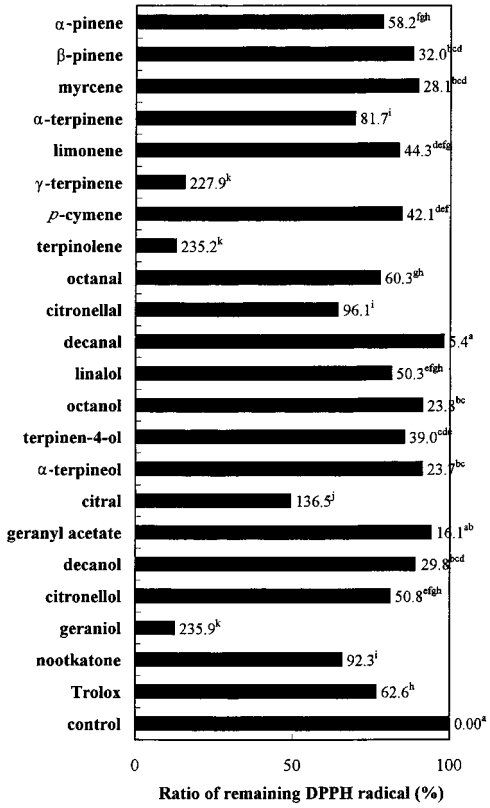

Figure 2. Scavenging effects of the authentic compounds relating to citrus essential oils on the DPPH radical. [a−k] Means of the radical-scavenging activity (mg of Trolox equiv/mL). Values with the same superscripts are not significantly different ($p < 0.05$).

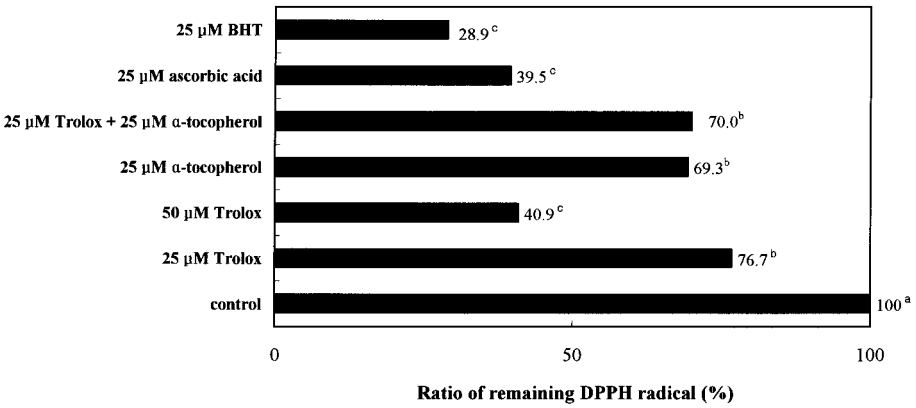

Figure 3. Scavenging effects of Trolox, α-tocopherol, ascorbic acid, and BHT on the DPPH radical. [a−c] Means of remaining DPPH %. Values with the same superscripts are not significantly different ($p < 0.05$).

stronger scavenging activities than Iyokan, Hyuganatsu, and ujukitsu ($p < 0.05$). Daidai, Valencia, and Tarocco oranges, ozu, and kumquat, in which γ-terpinene and terpinolene were almost 0%, form a group with the lowest DPPH radical-scavenging activity level. As those oils were mainly composed of limonene in a proportion of >90%, it is considered that limonene would not play the principal role in determining the scavenging activity for the radical. Kiyookadaidai, banhakuyu, ujukitsu, and mochiyuzu were exceedingly abundant in myrcene compared with others, 63.7, 37.2, 28.3, and 20.6%, respectively. However, there was no direct correlation between the myrcene content and the radical-scavenging activity. In each citrus essential oil, geraniol content was low; nevertheless, geraniol showed the highest radical-scavenging activity.

The relative effectiveness of antioxidants is dependent on the action type of antioxidants (free radical inhibitor, peroxide decomposer, metal inactivator, or oxygen scavenger) (Yagi, 1970, Yen and Duh, 1994), test system, emulsion system, concentration, oxidation time, method used (Frankel et al., 1994; Huang et al., 1996), and growth stage of plant samples (Baldwin, 1993). Under a wide range of conditions and test systems, the antioxidant activity of Trolox proved to be superior to that of α-tocopherol (Frankel et al., 1996). For example, Trolox is significantly more active than α-tocopherol in a bulk oil system, whereas in an oil-in-water emulsion α-tocopherol is significantly more active than Trolox (Frankel et al., 1994). Yamaguchi et al. (1998) reported, however, that α-tocopherol and Trolox had almost the same free radical-scavenging activities when determined by the DPPH−HPLC method, which means that neither lipophilicity nor hydrophilicity of the antioxidant affects the reaction with DPPH. This result is in agreement with our data, shown in Figure 3. As shown in Figure 3, ascorbic acid and BHT acted as more potent radical scavengers than α-tocopherol and Trolox at 25 μM. No significant difference was found between the radical-scavenging activities of 25 μM α-tocopherol and 25 μM Trolox. Moreover, the use of both α-tocopherol and Trolox did not show any synergistic effect. There was no significant difference among 25 μM ascorbic acid, 25 μM BHT, and 50 μM Trolox.

Antioxidants rein in free radicals by offering their own electrons. Free radicals can be produced from a number of sources such as cigarette smoke, pollution, pesticides, herbicides, and overexposure to sunlight. Free radicals are responsible for inflammation, compromised immune systems, and degenerative and other age-related diseases. Crowell (1997) and Gould (1997) reported that terpenoids such as carveol, limonene, sobrerol, and perillyl alcohol, which are found in plant essential oils, are effective in the treatment of breast, liver, and/or other cancers. These facts support our idea that citrus essential oils will protect against damaging free radicals and may be natural protectors against such diseases. Further work is required to determine the mechanism involved in the radical-scavenging activity of citrus essential oils. The present results support the view that citrus essential oils are highly bioavailable and may be active in the body as antioxidants and free radical scavengers.

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