J. Microbiol. Biotechnol. (2008), **18**(1), 74–79

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Chemical Composition and Biological Activities of Essential Oils Extracted from Korean Endemic Citrus Species

Baik, Jong Seok[1], Sang-Suk Kim[2], Jung-A Lee[3], Tae-Heon Oh[1], Ji-Young Kim[3], Nam Ho Lee[1], and Chang-Gu Hyun[3]*

[1]*Department of Chemistry, Cheju National University, Jeju 690-756, Korea*
[2]*SkinCure Cosmetics Inc., JeJu Bio-Industry Development Center R-103, JeJu 690-121, Korea*
[3]*Laboratory of Microbiology and Biotechnology, Jeju Biodiversity Research Institute (JBRI), JeJu 697-943, Korea*

Received: March 11, 2007 / Accepted: June 6, 2007

The aim of this study was to analyze the chemical composition of 14 kinds of citrus oils and to test their biological activities. Citrus essential oils were obtained by steam distillation from immature fruits collected from Jeju Island and were analyzed using gas chromatograph (GC)-flame ionization detectors (FID) and GC-MS. Limonene (55.4% to 91.7%), myrcene (2.1% to 32.1%), α-pinene (0.6% to 1.6%) and linalool (0.4% to 6.9%) were the major components in most citrus species. To evaluate *in vitro* antibacterial activity, all essential oils were tested against *Propionibacterium acnes* and *Staphylococcus epidermidis*. Nine out of fourteen citrus oils exhibited antibacterial activity against *P. acnes*, but not against *S. epidermidis*. The effects of the citrus oils on DPPH radical scavenging, superoxide radical anion scavenging, nitric oxide radical, and cytotoxicity were also assessed. Three essential citrus oils, Joadeung, Dongjunggyul, and Bujiwha, exhibited potent inhibitory effects on nitric oxide production. Two essential oils, Dongjunggyul and Joadeung, showed potent free radical scavenging activities in the DPPH assay. For future applications in cosmetic products, we also performed MTT assays in a human dermal fibroblast cell line. The majority of the essential oils showed no cytotoxicity. The results indicate that citrus essential oils can be useful natural agents for cosmetic application.

Keywords: Antibacterial activity, antioxidant activity, chemical composition, *Citrus*, essential oils, MTT

Jeju Island is located about 100 kilometers south of the Korean mainland; the mean annual temperature is around 15°C. Citrus cultivation in Korea is limited to Jeju Island and the southern coast of Korea. Furthermore, citrus is the most cultivated fruit in Jeju Island. Citrus peel, called "Jin-Pi", has been used as a traditional medicine for severe dermatitis, atopic dermatitis, recovery of fatigue, and as a digestant. A variety of aromatic plant materials are gathered and cultivated as a source of essential oils, many of which are of considerable commercial importantce. Among them, citrus fruits are one of the most valuable materials. For this reason, many studies have explored the chemical composition and antimicrobial activities of *Citrus* species such as *C. bergamia* and *C. hallabong* [3, 12, 14]. However, their antibacterial activities against acne-causing pathogen, antioxidant activity, and cytotoxicity of human normal fibroblast cell line for application to cosmetic materials have not been reported.

Throughout the world, there are many *Citrus* species, including hybrids, and these citrus fruits are regarded as important products. However, no studies have compared the profile of volatile ingredients of citrus oils from Jeju Island, although they are popular fruits in Korea. The volatile compounds have numerous applications and are in high demand in the food, perfumery, cosmetic, pharmaceutical, and wine industries. In our search for commercially useful essential oils, we selected *Citrus* species as the plants for study. The aims of this work were to assess the chemical composition and biological properties of essential oils isolated from the peel of 14 *Citrus* species collected from Jeju Island.

The essential oil from citrus peel was analyzed to determine the chemical components. Fourteen kinds of immature citrus fruits harvested in July 2005 were obtained from the National Institute of Subtropical Agriculture in Jeju (Republic of Korea). The essential oils of *Citrus* species peels were extracted by hydrodistillation as described by Simard *et al.* [13].

The volatile fraction yield differed according to the test samples; the yield ranged from 0.6% in Hagyul to 3.5% in Gamja (Table 1). Table 2 shows the volatile fraction

*Corresponding author
Phone: 82-64-720-2811; Fax: 82-2-64-720-2801;
E-mail: cghyun@jejuhidi.or.kr

Table 1. Traditional names of Jeju endemic *Citrus* species and content of essential oils in their peel.

Scientific names	Common names	Content of essential oils (%)
C. natsudaidai Hayata	Hagyul	0.6
C. tamurana Hort. ex Tanaka	Ilhyangha	2.1
C. tangerina Hort. ex Tanaka	Pyungyul	1.4
(*C. unshiu*×*C. sinensis*)×*C. reticulata*	Bujiwha	1.4
C. grandis	Dangyuja	0.8
C. aurantium L.	Jigak	0.9
C. aurantium L.	Joadeung	0.9
C. erythrosa	Dongjunggyul	0.9
C. natsudaidai Hayata	Gamwhagyul	1.2
C. benikoji	Gamja	3.5
C. pseudogulgul	Sadugam	0.7
C. paradisi Mac.×*C. tangerina* Hort. ex Tanaka	Seminole	2.5
C. sinensis	Chunggyun	1.0
C. platymamma	Byunggyul	1.4

composition in terms of components and classes of compounds. Fifty compounds were identified in citrus peel oils, accounting for more than 97% of the volatile compounds. Limonene (55.4% to 91.7%), and myrcene (2.1% to 32.1%) were the major components in most *Citrus* species. Other chemical components included α-pinene (0.6% to 1.6%), linalool (0.4% to 6.9%), β-pinene (0.24% to 2.0%), and α-terpinolene (0.07% to 0.54%).

Citrus oil was also tested for antibacterial activity against *P. acnes* and *S. epidermidis*. The antimicrobial activities of essential oils extracted from many plants have been recognized, albeit empirically, for centuries; only recently have such properties been confirmed [8]. The botanical source, provenance of the plant, harvest time or development stage, extraction technique, use of fresh or dried plant material, test microorganism(s), and antimicrobial

Table 2. Chemical composition (%) of 14 kinds of citrus peel essential oils.

Components	Composition (%)													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
α-Thujene ($C_{10}H_{16}$)	0.21	0.27	–	–	–	–	–	–	–	–	0.26	0.21	–	–
α-Pinene ($C_{10}H_{16}$)	1.32	1.24	0.54	1.04	0.75	0.73	0.74	0.64	1.34	0.89	1.50	1.55	0.74	0.65
Sabinene ($C_{10}H_{16}$)	–	2.28	–	–	–	–	–	–	–	–	–	–	–	–
β-Pinene ($C_{10}H_{16}$)	0.77	–	1.76	0.60	0.25	0.71	0.69	2.00	0.82	1.35	1.04	1.06	0.24	0.94
β-Myrcene ($C_{10}H_{16}$)	3.42	3.57	32.10	3.39	22.65	2.61	2.82	25.27	3.0	2.81	2.86	4.43	2.06	22.17
dl-Limonene ($C_{10}H_{16}$)	82.43	74.48	55.38	88.46	68.08	87.30	87.5	67.66	78.4	91.68	80.15	71.28	91.48	72.01
α-Ocimene ($C_{10}H_{16}$)	–	–	–	–	–	–	0.41	0.43	–	–	–	–	0.27	–
(E)-β-Ocimene ($C_{10}H_{16}$)	0.48	–	0.34	1.02	0.48	0.73	–	–	0.79	0.21	0.56	0.17	0.25	0.67
γ-Terpinene ($C_{10}H_{16}$)	6.83	6.55	0.16	0.76	1.63	–	0.12	0.12	6.47	0.11	8.03	6.6	–	–
Linalool oxide ($C_{10}H_{18}O_2$)	0.35	–	–	–	0.64	0.31	0.14	0.13	–	0.72	0.20	–	–	0.04
1-Octanol ($C_8H_{18}O$)	–	0.15	–	0.02	–	–	0.08	–	–	–	–	–	0.01	–
2-Allyltoluene ($C_{10}H_{12}$)	–	–	–	–	–	–	–	–	0.05	–	–	0.09	–	–
α-Terpinolene($C_{10}H_{16}$)	0.38	0.34	0.1	0.21	0.08	0.06	0.08	0.06	0.40	0.04	0.32	0.54	0.07	0.07
Linalool ($C_{10}H_{18}O$)	0.48	4.41	3.28	1.73	1.47	2.88	3.06	0.87	4.92	0.54	0.76	6.91	0.44	1.33
p-Mentha-1,5,8-triene ($C_{10}H_{14}$)	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–
cis-7-Methylenebicyclo[3.3.0]octan-2-one ($C_9H_{12}O$)	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–
β-Terpineol ($C_{10}H_{18}O$)	0.08	–	–	–	–	0.16	0.09	–	–	–	–	0.25	0.11	0.11
4-(1-Methylethenyl)cyclohex-2-enone ($C_9H_{12}O$)	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–

Table 2. Continued.

Components	Composition (%)													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Citronella ($C_{10}H_{18}O$)	–	0.30	0.30	–	0.21	–	–	1.08	–	0.16	–	0.10	–	–
1-Terpinen-4-ol ($C_{10}H_{18}O$)	0.40	1.68	–	1.28	0.22	0.22	0.19	0.23	–	0.25	0.84	1.64	0.58	0.32
α-Terpineol ($C_{10}H_{18}O$)	0.92	0.98	0.52	0.56	0.66	1.75	1.53	0.38	1.14	0.40	0.79	2.50	–	0.73
P-Menth-1-en-9-al ($C_{10}H_{16}O$)	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
Cyclooctane (C_8H_{16})	–	–	0.12	–	–	–	0.19	–	–	–	–	–	–	–
δ-4-Carene ($C_{10}H_{16}$)	0.08	–	0.24	–	0.04	0.25	–	–	0.08	–	0.17	0.03	0.03	–
Decanal ($C_{10}H_{20}O$)	0.64	–	–	–	–	–	–	–	–	–	–	–	0.52	–
cis-Ocimene ($C_{10}H_{16}$)	–	0.37	–	–	–	–	–	–	–	–	–	–	–	–
dl-Carvone ($C_{10}H_{14}O$)	–	–	–	0.06	0.06	-	0.08	–	0.03	0.04	0.05	0.04	0.04	–
Z-Citral ($C_{10}H_{16}O$)	0.08	0.23	0.9	–	0.18	0.64	0.10	–	0.04	0.03	0.11	–	0.09	0.26
Methylthymylether ($C_{11}H_{16}O$)	–	–	–	–	–	–	–	–	–	–	–	0.32	–	–
Perilla aldehyde ($C_{10}H_{14}O$)	–	–	–	–	–	–	–	–	–	0.03	–	0.30	–	–
8-Methylene-bicyclo[5.1.0]octane (C_9H_{14})	–	–	–	0.08	–	–	–	–	–	–	0.23	–	–	–
E-Citral ($C_{10}H_{16}O$)	–	0.25	1.14	–	0.13	–	–	0.09	0.05	0.04	0.14	0.19	0.12	0.27
Linalyl acetate ($C_{12}H_{20}O_2$)	–	–	–	–	–	0.33	0.41	–	–	–	–	–	–	–
2,4-Mesitylenediamine ($C_9H_{14}N_2$)	–	–	0.08	–	–	0.11	0.11	0.14	–	0.07	–	–	–	–
Carvacrol ($C_{10}H_{14}O$)	–		–	–	–	–	–	–	–	–	–	–	–	–
Thymol ($C_{10}H_{14}O$)		–	–	–	–	–	–					0.67		
α-Terpinene ($C_{10}H_{16}$)		–		–	–	–	–		0.05					
Citronellyl propionate ($C_{13}H_{24}O_2$)	–	–	–	0.02	–	–	–							
Bornylene ($C_{10}H_{16}$)	–	–	–	–	–	–	–			0.03				
Neryl acetate ($C_{12}H_{20}O_2$)	0.25	–	0.46	–	–	0.15	0.18	0.07	0.05	–	0.05	–	0.17	0.04
Geranyl acetate ($C_{12}H_{20}O_2$)	–		1.76	–	0.23	0.39	0.37	0.14	0.07	–	0.08	–	–	0.04
Elemene ($C_{15}H_{24}$)	–	–	0.05	–	–	–	–	0.08	0.02	0.02	–	–	–	–
1,10-Decanediol ($C_{10}H_{22}O_2$)	–	–	–	0.06	–	–	–	–	–	–	–	–	–	–
γ-Cadinene ($C_{15}H_{24}$)	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04
Epi-bicyclosesquiphellandrene ($C_{15}H_{24}$)	–	–	–	–	0.29	–	–	–	–	–	–	–	–	–
δ-Cadinene ($C_{15}H_{24}$)	–	–	–	–	0.05	–	–	–	–	0.04	0.03	–	–	–
Farnesol ($C_{15}H_{26}O$)		–	–	–	–	–	0.10	–	–	–	–	–	–	–
α-Sinensal ($C_{15}H_{22}O$)		–		–	–	–	–	–	0.11	–	–	–	0.08	–
Tridecanoic acid ($C_{13}H_{26}O_2$)		–	–	–	–	–	–	–	–	–	0.07	–	–	–
Tetradecanoic acid ($C_{14}H_{28}O_2$)		–	–	–	0.18	–	–	–	–	–	–	–	–	–
(E,Z)-1,5-Cyclodecadiene ($C_{10}H_{16}$)		–	–	–	0.18	–	–	–	–	–	–	–	–	–

The essential oils of *Citrus* species peels were extracted by hydrodistillation as described by Simard *et al.* [13]. Briefly, approximately 400 g of fresh citrus peel was immersed in 3.5 l of distilled water in a 5-l three-neck flask. Steam distillation was carried out for 12 h at atmospheric pressure. Gas chromatographic analyses were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a polar Supelcowax column (30 m× 0.25 mm×0.25 μm), an apolar DB-1HT column (30 m×0.25 mm), and a split-splitless injection port (split mode). The temperature program was 40°C for 5 min, ramped to 210°C at 10°C/min and held at 250°C for 28 min. The compounds were identified by their retention indices on both columns and by GC-MS using a Hewlett-Packard MSD 5972 mass spectrometer at 70 eV coupled to an HP 5890GC equipped with a DB-1HT column (30 m×0.32 mm×0.1 μm). The retention indices and mass spectra of each compound were compared with those in the literature. A, Hagyul; B, Ilhyangha; C, Pyungyul; D, Bujiwha; E, Dangyuja; F, Jigak; G, Joadeung; H, Dongjunggyul; I, Gamwhagyul; J, Gamja; K, Sadugam; L, Seminole; M, Chunggyun; N, Byunggyul.

Table 3. Minimal inhibitory concentration (MIC) of the essential oils of *Citrus* species against *Propionibacterium acnes*

Bacterial species	MIC (μl/ml)													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Propionibacterium acnes	1.25	2.5	>50	2.5	5	2.5	5	20	>50	5	>50	10	>50	>50
Staphylococcus epidermidis	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

Two Gram-positive bacterial species, *P. acnes* ATCC 6919 and *S. epidermidis* KCTC 3958, which are involved in acne, were selected as test microorganisms according to their pathological capacity. *P. acnes* ATCC 6919 was cultured at 37°C for 48 h in GAM broth (Nissui, Japan) under anaerobic conditions, and *S. epidermidis* KCTC3958 was cultured at 37°C for 24 h with *Corynebacterium* media before the assay. The minimal inhibitory concentration (MIC) was estimated by the broth dilution method. The GAM and *Corynebacterium* broths used for sample dilution were also supplemented with 5% Tween-80 (Merck, Germany) to enhance volatile oil solubility. The MIC was taken as the lowest volatile fraction concentration that prevented bacterial growth when determined visually after 24 h incubation at 37°C. A, Hagyul; B, Ilhyangha; C, Pyungyul; D, Bujiwha; E, Dangyuja; F, Jigak; G, Joadeung; H, Dongjunggyul; I, Gamwhagyul; J, Gamja; K, Sadugam; L, Seminole; M, Chunggyun; N, Byunggyul.

methodology are all factors that influence the antimicrobial activity [2, 4, 6, 15] and must therefore be taken into account whenever antimicrobial assays of these oils are performed.

As shown in Table 3, the essential oil exhibited notable antibacterial activity against *P. acnes*. However, *S. epidermidis* were not susceptible to the essential oils at low concentrations. The lowest MIC value against *Propionibacterium* was shown by the Hagyul essential oil (1.25 μl/ml). The essential oils of Ilhyangha, Bujiwha, and Jigak also demonstrated remarkably low MIC values against *Propionibacterium* (2.5 μl/ml, respectively). On the other hand, five *Citrus* species, Pyunggyul, Gamwhagyul, Sadugam, Chunggyul, and Byunggyul, showed very weak activities.

Table 4. Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide (NO) by citrus essential oils.

Citrus species	DPPH IV_{50} (μl/ml)*	NO radical inhibition IV_{50} (μl/ml)
Ilhyangha	10.0	21.7
Pyungyul	19.6	22.3
Bujiwha	10.8	8.5
Dangyuja	10.6	10.9
Jigak	15.2	20.0
Joadeung	6.7	1.8
Dongjunggyul	5.7	2.0
Gamwhagyul	13.0	28.7
Seminole	11.6	>100
Chunggyun	19.3	27.8
Byunggyul	12.5	22.3

The effect of citrus essential oils on DPPH free radical was determined as previously described [9]. Briefly, 100 μl/ml of the essential oil was added to solution of 1.5×10^{-4} M DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 517 nm, and the radical scavenging activity was obtained from the equation:

Radical scavenging activity (%)
$= [(OD\ control - OD\ sample)/OD\ control] \times 100\%$

The effect of citrus essential oils on NO radical was determined as follow. The reaction mixture (3 ml) containing 15 mmol sodium nitroprusside (2 ml), phosphate buffer saline (0.5 ml), and various concentrations of essential oils was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture, containing nitrite, was removed, mixed with 1 ml of 0.33% sulfanilic acid reagent (in glacial acetic acid), and allowed to stand for 5 min to permit complete diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed, and allowed to stand at 25°C for 30 min. A pink chromophore formed in diffused light. The absorbance of the chromophore was determined spectrophotometrically (540 nm), and the amount of nitrite generated, in the presence or absence of citrus essential oils, was measured using a standard curve based on sodium nitrite solutions of known concentrations. *IV_{50} is a measure of volume used in this research. IV_{50} represents the volume of essential oils that is required for 50% inhibition of its target.

The capacity of citrus peel essential oils to scavenging DPPH, O_2^-, and NO was also measured (Table 4). The effect of citrus essential oils on DPPH free radical was determined as previously described [9, 11]. Eleven out of 14 essential oils exhibited dose-response curves for DPPH radical scavenging activity (Table 4). The most potent citrus peel essential oil was found to be Dongjunggyul, followed in order by Joadeung, Ilhyangha, Dangyuja, and Bujiwha. These results imply that these essential oils may contain constituents with strong proton-donating abilities.

The primary free radical in most biological systems is O_2^-. Although O_2^- itself is quite unreactive compared with other radicals, biological systems convert it into more reactive species (*e.g.*, OH^- radicals) [17]. The superoxide radical scavenging potential of the essential oils was analyzed using a xanthine/xanthine oxidase generating system coupled with NBT reduction as previously described [7]. Only Hagyul could inhibit the rate of NBT reduction in a concentration-dependent manner; the IV_{50} was 97.8 μl/ml. However, the essential oils weakly scavenged O_2^- compared with DPPH.

It is well known that nitric oxide has an important role in various types of inflammatory processes. In the present study, the essential oils were checked for their inhibitory effect on nitric oxide production. Of all tested compounds, ten kinds of citrus peel essential oils reduced the nitric oxide production in a dose-dependent manner. Joadeung, Dongjunggyul, and Bujiwha effectively reduced the nitric oxide production with low IV_{50} values. The IV_{50} values of Joadeung, Dongjunggyul, and Bujiwha were 1.8 μl/ml, 2.0 μl/ml, and 8.49 μl/ml, respectively.

As afore-mentioned, essential oil from citrus peel exhibited antibacterial and antioxidant activities. These activities

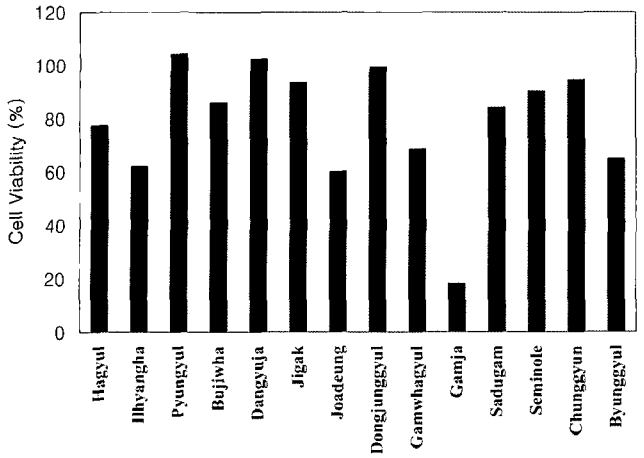

Fig. 1. Cell viabilities of human dermal fibroblasts treated with citrus essential oils.

Human dermal fibroblast cells were cultured in DMEM (Hyclone, UT, U.S.A.) containing 10% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified 95% air: 5% CO_2 atmosphere. Cells were seeded on 24-well plates and the essential oil treatment was supplemented 24 h after seeding. General viability of cultured cells was determined by the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to formazan. The MTT assay was performed after incubation of normal fibroblast cells with various concentrations of the essential oils for 24 h at 37°C in 5% CO_2 atmosphere. MTT (1 mg/ml in phosphate-buffered saline) was added to each well in a 1/10 volume of media. Cells were incubated at 37°C for 3 h, and dimethylsulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance was then measured at 570 nm using a spectrophotometer (Power Wave, Bio-tek Inc., VT, U.S.A.). The entire experiment was performed in triplicate, and results were confirmed by three independent experiments. Pyunggyul, Bujiwha, Dangyuja, Jigak, Dongjunggyul, Sadugam, Seminole, and Chunggyul showed relatively low cytotoxicities, with cell viability above 80% at a concentration of 10 μl/ml.

may be attributed to the presence of β-pinene, α-pinene, limonene, α-terpinene, and α-terpinolene found in citrus essential oils. Enantiomers of α-pinene, 2-β-pinene, and limonene have a strong antibacterial activity [10]. These chemical components exert their toxic effects against these microorganisms through disruption of bacterial membrane integrity [1, 16]. α-Pinene and β-pinene are able to destroy cellular integrity and thereby inhibit respiration and ion transport processes. They also increase membrane permeability in yeast cells and isolated mitochondria [1, 16]. This is strongly supported by the study on the effects of different essential oil components on membrane permeability in acne-inducing bacteria [5].

In the present study, we used human dermal fibroblast as an *in vitro* model to measure cytotoxic effects with the aid of the MTT assay. Among the 14 tested oils, 8 oils, Pyunggyul, Bujiwha, Dangyuja, Jigak, Dongjunggyul, Sadugam, Seminole, and Chunggyul, showed relatively low cytotoxicity, with viability above 80% at concentration of 10 μl/ml (Fig. 1).

In conclusion, this study indicates that essential oils from various citrus peels have potential as an anti-acne and antioxidant agent and may be useful in the pharmaceutical and cosmetic industries. Our results contribute to better valorization of these regional fruits endemic to Jeju Island, Republic of Korea. Several other biological tests to search for different biological activities of these oils may also be worthwhile.

Acknowledgments

This research was partially supported by the Program for the Training of Graduate Students in Regional Innovation and the Regional Technology Innovation Program (RTI04-02-07), which were conducted by the Ministry of Commerce Industry and Energy of the Korean Government.

References

1. Andrews, R. E., L. W. Parks, and K. D. Spence. 1980. Some effects of Douglas fir terpenes on certain microorganisms. *Appl. Environ. Microbiol.* **40:** 301–304.
2. Choi, G. J., J. C. Kim, K. S. Jang, H. K. Lim, I. K. Park, S. C. Shin, and K. Y. Cho. 2006. *In vivo* antifungal activities of 67 plant fruit extracts against six plant pathogenic fungi. *J. Microbiol. Biotechnol.* **16:** 491–495.
3. Choi, H. S. 2003. Character impact odorants of *Citrus* Hallabong [(*C. unshiu* Marcov×*C. sinensis* Osbeck)×C. reticulata Blanco] cold-pressed peel oil. *J. Agric. Food Chem.* **51:** 2687–2692.
4. Cosentino, S., C. I. G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi, and F. Palmas. 1999. *In-vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Lett. Appl. Microbiol.* **29:** 130–135.
5. Helander, I. M., H. L. Alakomi, L. K. Kyosti, T. Mattiala-andholm, I. Pol, E. J. Smid, G. M. Gorris, and A. von Wright. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem.* **46:** 3590–3595.
6. Janssen, A. M., J. Scheffer, and A. Svendsen. 1987. Antimicrobial activity of essential oils: A 1976–1986 literature review. Aspects of test methods. *Planta Med.* **53:** 395–398.
7. Kim, M. R., J. Y. Lee, H. H. Lee, D. K. Aryal, Y. G. Kim, S. K. Kim, E. R. Woo, and K. W. Kang. 2006. Antioxidative effects of quercetin-glycosides isolated from the flower buds of *Tussilago farfara* L. *Food Chem. Toxicol.* **44:** 1299–1307.
8. Lee, S. B., K. H. Cha, S. N. Kim, S. Altantsetseg, S. Shatar, O. Sarangerel, and C. W. Nho. 2007. The antimicrobial activity of essential oil from *Dracocephalum foetidum* against pathogenic microorganisms. *J. Microbiol.* **45:** 53–57.
9. Li, X., S. K. Kim, J. S. Kang, H. D. Choi, and B. W. Son. 2006. Radical scavenging hydroxyphenyl ethanoic acid derivatives from a marine-derived fungus. *J. Microbiol. Biotechnol.* **16:** 637–638.
10. Magwa, M. L., M. Gundidza, N. Gweru, and G. Humphrey. 2006. Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. *J. Ethnopharmacol.* **103:** 85–98.

11. Ozer, H., M. Sokmen, M. Gulluce, A. Adiguzel, F. Sahin, A. Sokmen, H. Kilic, and O. Baris. 2007. Chemical composition and antimicrobial and antioxidant activities of the essential oil and methanol extract of *Hippomarathrum microcarpum* (Bieb.) from Turkey. *J. Agric. Food Chem.* **55:** 937–942.
12. Sanguinetti, M., B. Posteraro, L. Romano, F. Battaglia, T. Lopizzo, E. De Carolis, and G. Fadda. 2007. *In vitro* activity of *Citrus bergamia* (bergamot) oil against clinical isolates of dermatophytes. *J. Antimicrob. Chemother.* **59:** 305–308.
13. Simard, S., J. M. Hachey, and G. J. Collin. 1988. The variations of essential oil composition during the extraction process. The case of *Thuja occidentalis* L. and *Abies balsamea* (L.) MILL. *J. Wood Chem. Tech.* **8:** 561–573.
14. Song, H. S., N. T. Lan Phi, Y. H. Park, and M. Sawamura. 2006. Volatile profiles in cold-pressed peel oil from Korean and Japanese Shiranui (*Citrus unshiu* Marcov.×*C. sinensis* Osbeck×*C. reticulata* Blanco). *Biosci. Biotechnol. Biochem.* **70:** 737–739.
15. Sung, W. S., H. J. Jung, I. S. Lee, H. S. Kim, and D. G. Lee. 2006. Antimicrobial effect of furaneol against human pathogenic bacteria and fungi. *J. Microbiol. Biotechnol.* **16:** 349–354.
16. Uribe, S., T. Ramirez, and A. Pena. 1985. Effects of β-pinene on yeast membrane functions. *J. Bacteriol.* **161:** 195–200.
17. Winterbourn, C. C. and A. J. Kettle. 2003. Radical-radical reactions of superoxide: A potential route to toxicity. *Biochem. Biophys. Res. Commun.* **305:** 729–736.