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Chemical and Antimicrobial Properties of Essential Oils from Three Coniferous Trees *Abies koreana*, *Cryptomeria japonica*, and *Torreya nucifera*

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Three coniferous essential oils were extracted from *Abies koreana*, *Cryptomeria japonica*, and *Torreya nucifera* by hydrodistillation. The chemical composition of each oil was analyzed by GC-MS, and their antimicrobial activities were tested against two bacteria and one yeast strains. Forty-seven compounds were identified from *A. koreana* oil, 39 from *C. japonica*, and 59 from *T. nucifera*. Main components of the essential oils were limonene (23.5%), bornyl acetate (17.9%), α-pinene (11.1%), and camphene (10.2%) in *A. koreana*, kaurene (26.3%), γ-eudesmol (19.0%), elemol (6.9%), and sabinene (5.1%) in *C. japonica*, limonene (13.5%), δ-cadinene (10.5%), α-bisabolol (10.2%), and α-copaene (7.7%) in *T. nucifera*. Among the three coniferous trees tested, the essential oils of *A. koreana* exhibited higher and broader antimicrobial activity against the tested organisms than those of *C. japonica* and *T. nucifera*.

Key words: *Abies koreana, antimicrobial, coniferous, gas chromatography-mass spectrometry, volatile compound*

Essential oils are the concentrates of the volatile compounds, which comprise terpenoids, hydrocarbons, alcohols, and aldehydes from the herbal plants. They have been regarded as useful antibacterial, antifungal, antiviral, antioxidizing, and anticancer agents [Burt, 2004; Edris, 2007]. They were also used in the production of pharmaceutics, food flavorings, cosmetics, and fragrances by the industries.

Investigations on the compositions of the essential oils of firs and coniferous trees have been performed recently, including *Abies koreana* Wils., *Abies alba*, *A. nephrolepsis*, softwood leaves, and *Pinus* species [Baran *et al.*, 2007; Duquesnoy *et al.*, 2007; Hong *et al.*, 2004; Kurose *et al.*, 2007; Su *et al.*, 2006; Yang *et al.*, 2002]. However, little information is available on the antimicrobial and antifungal activities of the essential oils extracted from the coniferous trees.

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Abbreviations: MICs, minimum inhibitory concentrations

In our search for the available and useful essential oil sources, among the coniferous trees, *A. koreana*, *Cryptomeria japonica*, and *Torreya nucifera*, native to the Jeju Island, were selected. *A. koreana* (Korean fir) is a member of the family Pinaceae with cone shape and is one of the fragrant, alpine conifer trees, endemic to the high mountains in Southern Korea including Mt. Dukyu, Mt. Chiri, and Mt. Halla [Lee, 1996]. *A. koreana* has been reported to have antibacterial, cytotoxic, and memory-enhancing effects [Kim *et al.*, 2001; Kim *et al.*, 2006; Jeong *et al.*, 2007]. The Japanese cedars, *C. japonica* (Taxodiaceae), were artificially planted in Jeju. It has been reported that various parts of *C. japonica* have antibacterial, antifungal, and antitermiticidal activities [Cha *et al.*, 2007]. The Japanese torreya (*Torreya nucifera*), an evergreen tree indigenous to Japan, is a member of the family Taxaceae. Although studies have been performed on the composition of the essential oils of the coniferous trees, few have been done on the Japanese torreya seed oil used as edible oil, hair oil, light, and medicine [Endo *et al.*, 2006].

In this study, the antimicrobial activities of the essential oils isolated from three coniferous trees were investigated using the disc diffusion method and the minimal inhibitory concentration (MIC) assay. The chemical compositions of the essential oils isolated from the coniferous trees were also investigated.

Materials and Methods

Plant materials and preparation of essential oils. The needles of *A. koreana*, *C. japonica*, and *T. nucifera* were collected in June, 2007. This research was carried out using the samples of hydrodistilled oils. The needles of trees were kept in a deep-freezer before use. The needles of trees were distilled for 6 h using simultaneous steam distillation apparatus to obtain the essential oil. Anhydrous sodium sulphate was used to absorb the little water contained in the essential oil.

GC/MS analysis. Analyses were carried out by GC/MS using an Agilent Technologies 6890 N equipped with a 5973 network mass selective detector, split/splitless injector, autosampler, and column HP-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was programmed from 60 to 200°C at 2°C/min. The carrier gas was helium at a flow rate of 1 mL/min. The GC-MS was carried out on a 6890 N mass spectrometer operating in the EI mode at 70 eV. The oils were analyzed individually by injecting 1 mL of 1 : 5 (v/v) solution of the essential oil in dichloromethane. The identification of the chemical constituents was based on the comparisons of their retention times and mass spectra with those obtained from the authentic sample Wiley (ver.7.0) libraries spectra, and literatures [Adams, 1995].

Antimicrobial activity assay. The bacterial strains, *Escherchia coli* ATCC 25922 and *Staphyllococcus epidermidis* KCTC 3958, and the yeast strain, *Candida albicans* KCTC 7965, were used in this study. The bacterial strains were cultured overnight at 37°C in LB agar and Cornebacterium agar. The yeast was cultured at 30°C overnight in yeast malt agar.

Antimicrobial activities of the essential oil against bacteria and yeast strains were determined by the paper disc assay and the broth dilution method. The disc diffusion method was employed for the determination of antimicrobial activities of the essential oils. Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells per mL) was spread on to the solid media plates. Filter paper discs (8 mm in diameter) were impregnated with 40 mL of the oil and placed on the inoculated plates. These plates were incubated at 37°C for 24 h for bacteria and at 30°C for yeast. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicates.

The minimum inhibitory concentrations (MICs) of the essential oils were determined for the using the broth dilution method. The antibacterial activities were determined after incubation at 37°C for 24 h (bacteria) or at 30°C for 24 h (candida) using the dilution techniques. MICs were determined as the lowest concentration of visible growth in the broth. Turbidity of the broth indicates the microorganism growth (OD_{600}).

Results and Discussion

Chemical composition of the essential oils. The yield of essential oils from *A. koreana*, *C. japonica*, and *T. nucifera* were 0.53 ± 0.10, 0.84 ± 0.06, and 0.37 ± 0.04% (v/w), respectively. The chemical components analyzed by GC-MS are presented in Table 1.

Forty-seven compounds were identified from the essential oil of *A. koreana*, of which 11 are monoterpene hydrocarbons (55.7%), 8 oxygenated monoterpenes (21.3%), 20 sesquiterpene hydrocarbons (18.2%), 6 oxygenated sesquiterpenes (2.1%), and 2 others (2.2%). The most abundant compound was limonene (23.5%), followed by bornyl acetate (17.9%), α-pinene (11.1%), camphene (10.2%), β-himachalene (4.2%), β-myrcene (3.4%), γ-selinene (3.1%), γ-gurjunene (2.4%), β-eudesmene (2.3%), β-pinene (2.1%), and other minor constituents.

The essential oil of *C. japonica* consisted of 39 compounds including 13 monoterpene hydrocarbons (17.8%), 6 oxygenated monoterpenes (5.9%), 9 sesquiterpene hydrocarbons (3.3%), 8 oxygenated sesquiterpenes (41.8%), 2 diterpenes (26.6%), and 1 of 2-hexanal (0.3%). The most abundant compound was kaurene (26.3%), followed by γ-eudesmol (19.0%), α-eudesmol (7.9%), elemol (6.9%), β-eudesmol (6.0%), sabinene (5.1%), 4-terpineol (4.6%), α-pinene (3.0%), γ-terpinene (2.2%), and other minor constituents.

Fifty-nine compounds were identified from the essential oil of *T. nucifera* oil, more than those of *A. koreana* and *C. japonica*. Among the compounds, 13 monoterpene hydrocarbons (32.1%), 6 oxygenated monoterpenes (1.0%), 23 sesquiterpene hydrocarbons (34.6%), 9 oxygenated sesquiterpenes (15.3%), 7 diterpenes (3.0%), and 1 of 2-hexanal (0.2%). Both monoterpene and sesquiterpene hydrocarbons were more abundant than the oxygenated derivatives, constituting 32.1 and 34.6% of the total volatile compounds, respectively. The most abundant compound was limonene (13.5%), followed by δ-cadinene (10.5%), α-Bisabolol (10.2%), α-copaene (7.7%), δ-3-carene (7.1%), α-pinene (5.3%), β-farnesene (2.7%), caryophyllene (2.6%), α-terpinolene (2.3%), α-humulene (2.2%), and other minor constituents.

The major components and their percentages were

Table 1. Chemical composition of the essential oils of the three valuable conifers in Jeju Island

Components[a]	Formula	Area (%)		
		A. koreana	*C. japonica*	*T. nucifera*
Monoterpenes				
Tricyclene	$C_{10}H_{16}$	2.30	0.10	tr[b]
α-Thujene	$C_{10}H_{16}$	-[c]	0.57	-
α-Pinene	$C_{10}H_{16}$	11.11	2.96	5.34
α-Fenchene	$C_{10}H_{16}$	-	-	0.68
Camphene	$C_{10}H_{16}$	10.16	0.61	-
β-Pinene	$C_{10}H_{16}$	2.07	-	0.67
Sabinene	$C_{10}H_{16}$	-	5.06	0.22
β-Myrcene	$C_{10}H_{16}$	3.41	1.26	1.24
1-Phellandrene	$C_{10}H_{16}$	0.06	0.13	tr
δ-3-Carene	$C_{10}H_{16}$	1.74	0.62	7.08
α-Terpinene	$C_{10}H_{16}$	tr	1.42	0.09
dl-Limonene	$C_{10}H_{16}$	23.49	1.89	13.52
γ-Terpinene	$C_{10}H_{16}$	0.10	2.15	0.20
α-Terpinolene	$C_{10}H_{16}$	1.27	0.93	2.33
α-Ocimene	$C_{10}H_{16}$	-	0.10	0.74
		55.71	17.8	32.11
Oxygenated Monoterpenes				
Linalool	$C_{10}H_{18}O$	0.21	0.06	0.10
p-Menth-2-en-l-ol	$C_{10}H_{18}O$	-	0.10	-
Camphor	$C_{10}H_{16}O$	-	-	0.06
α-Camphenal	$C_{10}H_{16}O$	0.07	-	-
L-Borneol	$C_{10}H_{18}O$	1.27	-	-
4-Terpineol	$C_{10}H_{18}O$	0.09	4.63	0.29
α-Terpineol	$C_{10}H_{18}O$	0.41	0.29	0.22
β-Citronellol	$C_{10}H_{20}O$	-	-	0.25
Fenchyl acetate	$C_{12}H_{20}O_2$	0.97	-	-
Bornyl acetate	$C_{12}H_{20}O_2$	17.87	0.71	0.08
Sabinyl acetate	$C_{12}H_{18}O_2$	-	0.06	-
Geranyl acetate	$C_{12}H_{20}O_2$	0.42	-	-
		21.31	5.85	1.00
Sesquiterpenes				
δ-Elemene	$C_{15}H_{24}$	-	tr	0.17
α-Longipinene	$C_{15}H_{24}$	-	-	0.48
α-Ylangene	$C_{15}H_{24}$	-	-	0.06
Isoledene	$C_{15}H_{24}$	-	-	0.06
α-Copaene	$C_{15}H_{24}$	-	-	0.06
(-)-Alloaromadendrene	$C_{15}H_{24}$	-	-	tr
β-Elemene	$C_{15}H_{24}$	0.25	0.17	0.12
α-Gurjunene	$C_{15}H_{24}$	0.13	-	0.14
Junipene	$C_{15}H_{24}$	0.19	-	-
α-Chamigrene	$C_{15}H_{24}$	0.13	-	-
Caryophyllene	$C_{15}H_{24}$	1.50	0.07	2.64
β-Cubebene	$C_{15}H_{24}$	-	-	1.18
α-Guaiene	$C_{15}H_{24}$	0.12	-	-
α-Selinene	$C_{15}H_{24}$	0.06	-	
γ-Elemene	$C_{15}H_{24}$	-	-	0.08
β-Farnesene	$C_{15}H_{24}$	0.15	0.11	2.66

Table 1. (Continued)

Components[a]	Formula	Area (%)		
		A. koreana	*C. japonica*	*T. nucifera*
(+)-Aromadendrene	$C_{15}H_{24}$	-	-	0.06
α-Amorphene	$C_{15}H_{24}$	-	-	3.70
Germacrene-D	$C_{15}H_{24}$	-	0.30	1.60
β-Eudesmene	$C_{15}H_{24}$	2.31	-	-
γ-Selinene	$C_{15}H_{24}$	3.09	-	-
α-Muurolene	$C_{15}H_{24}$	-	0.28	0.06
cis-α-Bisabolene	$C_{15}H_{24}$	-	-	0.28
β-Bisabolene	$C_{15}H_{24}$	0.98	-	2.00
γ-Gurjunene	$C_{15}H_{24}$	2.41	-	-
γ-Cadinene	$C_{15}H_{24}$	-	0.21	-
7-epi-α-Selinene	$C_{15}H_{24}$	1.08	-	-
δ-Cadinene	$C_{15}H_{24}$	0.19	1.78	10.52
α-Cadinene	$C_{15}H_{24}$	-	-	0.05
Germacrene B	$C_{15}H_{24}$	0.28	-	0.90
trans-γ-Bisabolene	$C_{15}H_{24}$	0.52	-	-
Cis-α-Bisabolene	$C_{15}H_{24}$	0.08	-	-
Junipene	$C_{15}H_{24}$	0.36	-	-
α-Copaene	$C_{15}H_{24}$	-	-	7.71
Ledene	$C_{15}H_{24}$	0.18	-	-
Thujopsene	$C_{15}H_{24}$	-	-	0.07
β-Himachalene	$C_{15}H_{24}$	4.18	-	-
Viridiflorene	$C_{15}H_{24}$	-	0.42	-
		18.19	3.34	34.62
Oxygenated Sesquiterpenes				
α-Humulene	$C_{15}H_{24}O$	0.66	0.05	2.24
Elemol	$C_{15}H_{26}O$	-	6.87	-
Palustrol	$C_{15}H_{26}O$	-	-	0.22
α-Bisabolol	$C_{15}H_{26}O$	0.67	-	10.20
Junipercamphor	$C_{15}H_{26}O$	0.07	0.40	-
Farnesol	$C_{15}H_{26}O$	-	-	0.18
tau-Cadinol	$C_{15}H_{26}O$	-	-	1.67
β-Eudesmol	$C_{15}H_{26}O$	-	5.98	-
α-Eudesmol	$C_{15}H_{26}O$	-	7.86	-
Nerolidol	$C_{15}H_{26}O$	0.26	0.06	0.35
Caryophyllene oxide	$C_{15}H_{24}O$	0.09	-	0.17
Ledol	$C_{15}H_{26}O$	-	-	0.05
α-Eudesmol	$C_{15}H_{26}O$	-	1.59	-
γ-Eudesmol	$C_{15}H_{26}O$	0.32	19.02	-
Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	-	-	0.21
		2.07	41.83	15.29
Diterpenes				
Isopimaradiene	$C_{20}H_{32}$	-	0.32	0.13
Kaurene	$C_{20}H_{32}$	-	26.31	0.12
Phytol	$C_{20}H_{40}O$	-	-	0.59
Dehydroabietal	$C_{20}H_{28}O$	-	-	0.38
Ferruginol	$C_{20}H_{30}O$	-	-	1.24
Methyl dehydroabietate	$C_{21}H_{30}O_2$	-	-	0.29
4-Epidehydroabietol	$C_{20}H_{30}O$	-	-	0.24
		0.00	26.63	2.99

Table 1. (Continued)

Components[a]	Formula	Area (%)		
		A. koreana	*C. japonica*	*T. nucifera*
Others				
2-Hexenal	$C_6H_{10}O$	0.34	0.26	0.23
Santene	C_9H_{14}	1.84	-	-
		2.18	0.26	0.23
Total		99.46	95.71	86.24
Grouped components				
Monoterpene hydrocarbons (%)		55.71	17.80	32.11
Oxygenated monoterpenes (%)		21.31	5.85	1.00
Sesquiterpenes hydrocarbons (%)		18.19	3.34	34.62
Oxygenated sesquiterpenes (%)		2.07	41.83	15.29
Diterpenes (%)		0.00	26.63	2.99
Others (%)		2.18	0.26	0.23
Total identified (%)		99.46	95.71	86.24

a: Components were analyzed on the Hp-5 column
b: Trace (<0.05%)
c: Not detected

Table 2. Antimicrobial activity of the coniferous essential oils by the diffusion method

Microorganisms	Inhibition zones (mm)[a]		
	Abies koreana	*Cryptomeria japonica*	*Torreya nucifera*
Escherichia coli	14.5 ± 1.32	-[b]	-
Staphylococcus epidermidis	27.30 ± 1.53	21.17 ± 1.04	11.67 ± 0.28
Candida albicans	34.0 ± 2.83	11.5 ± 2.12	-

a: Average value (triplicate) ± standard eviation.
b: Not detected.

Table 3. Determination of MIC (Minimum Inhibitory Concentration) of the essential oils

Microorganisms	MIC (mg/mL)		
	Abies koreana	*Cryptomeria japonica*	*Torreya nucifera*
Escherichia coli	2.05 ± 0.51	>26.16	>25.95
Staphylococcus epidermidis	3.81 ± 0.50	18.60 ± 2.01	>20.76 ± 2.44
Candida albicans	2.05 ± 0.51	7.56 ± 1.00	>25.95

limonene (23.5%) and bornyl acetate (17.9%) in *A. koreana,* kaulene (26.3%) and γ-eudesmol (19.0%) in *C. japonica*; and limonene (13.5%) and δ-cadinene (10.5%) in *T. nucifera.*

A. koreana is distributed in the alpine regions of southern Korean peninsula and Jeju Island. Our results on the main components of the essential oils of *A. koreana* grown in Mt. Halla were limonene, bornyl acetate, α-pinene, and camphene. These data were very similar to those reported for the needles of *A. koreana,* originated from Mt Halla in Jeju [Baran *et al.*, 2007], the major components being bornyl acetate, camphene, α-pinene, and limonene. On the other hand, the main constituents of the essential oils of *A. koreana* grown in Mt. Dukyu were borneol, α-pinene, β-pine, and bornyl acetate, and camphene and limonene were contained in trace amounts [Jeong *et al.*, 2007]. These changes might have arisen from the environmental differences, including climatic, seasonal, geographical, and genetic differences, among the mountainous regions (Mt. Dukyu, Mt. Chiri, and Mt. Halla) [Perry *et al.*, 1999].

Antimicrobial activities of the essential oils. The antimicrobial activities of the essential oils from *A. koreana, C. japonica*, and *T. nucifera* were evaluated by the disk diffusion method (Table. 2), and the MICs were determined against the two bacteria (*E. coli* and *S.*

epidermidis) and one yeast (*C. albicans*) strains (Table. 3). The results showed that the essential oil of *A. koreana* has stronger and broader spectrum of antimicrobial activities than those of *C. japonica* and *T. nucifera.* The yeast *C. albicans,* with the strongest inhibition zone (34 mm), was the most susceptible strain tested against the oil of *A. koreana*. The oil of *A. koreana* was effective against all strains tested in the study, at a range of 2.05-3.81 mg/mL, whereas the essential oil of *C. japonica* showed activities against *S. epidermidis* and *C. albicans*. All tested oils inhibited the growth of *S. epidermidis*. The *A. koreana* oil had the lowest MIC value of 3.81 mg/mL. It is of interest to note that the antifungal effect on the growth inhibition of *C. albicans* was observed in all essential oils except from that of *T. nucifera,* suggesting that the composition of each essential oil may vary in its effectiveness against *C. albicans*. Interestingly, these results indicate that the essential oil from *A. koreana* has both antibacterial and antifungal effects. *A. koreana* oil exhibited stronger activity than did *C. japonica* and *T. nucifera*.

The differences in the antimicrobial activity could be due to the differences in the chemical compositions of the oils. The content of monoterpene hydrocarbons (especially α-pinene) in *A. koreana* was higher than those of *C. japonica* and *T. nucifera*. In addition, α-pinene and β-pinene have been reported to have significant antibacterial activities [Couladis *et al*., 2003; Chalchat *et al*., 2000]. It is possible that these essential oils identified from the coniferous trees can be used antibacterial or antifungal agents in foods or in other products.

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