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Comparative Analysis of Chemical Compositions and Antimicrobial Activities of Essential Oils from *Abies holophylla* and *Abies koreana*

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The chemical compositions and antibacterial and antifungal activities of essential oils extracted from *Abies holophylla* and *A. koreana* were investigated. GC-MS analysis revealed that 38 compounds comprised 95.88% of the *A. holophylla* essential oil, with the main components being bicyclo[2.2.1]heptan-2-ol (28.05%), δ3-carene (13.85%), α-pinene (11.68%), camphene (10.41%), dl-limonene (7.61%), β-myrcene (7.11%), *trans*-caryophyllene (5.36%), and α-bisabolol (3.67%). In the essential oil from *A. koreana*, 36 compounds comprised 98.67% of the oil, and the main compounds were bornyl ester (41.79%), camphene (15.31%), α-pinene (11.19%), dl-limonene (8.58%), fenchyl acetate (5.55%), and α-terpinene (2.29%). Both essential oils showed great potential of antibacterial activity against several bacteria tested, in the range of 2.2–8.8 μg per disc by the agar disc diffusion method, and minimal inhibitory concentration (MIC) values of 5.5–21.8 mg/ml by the microdilution method. Both oils showed very effective antifungal activities toward all pathogenic strains tested, including *Candida glabrata*, with MIC values in the range of 0.5–2.2 mg/ml. As a whole, *A. koreana* oil showed better antibacterial and antifungal properties than *A. holophylla* oil.

Keywords: Essential oil, antimicrobial activity, *Abies holophylla*, *Abies koreana*

Interest from the food and pharmaceutical industries in finding high-quality products from natural compounds exhibiting antimicrobial activity is growing rapidly [3, 8]. Essential oils are volatile oils from various parts of fragrant plants, such as the flower, flower bud, leaf, root, and stalk. Essential oils are considered to be important antimicrobial agents present in plants, and they may also have antioxidant and anti-inflammatory activities [2, 24]. Essential oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives such as alcohols, aldehydes, esters, ethers, ketones, and phenols, which have antitumor, antioxygen, anti-aging, anti-mutation, and sedative effects [15, 17, 18]. In addition, the high content of phenolic derivatives of essential oils contributes to their antimicrobial properties [4, 5]. Thus, essential oils from medicinal and other edible plants have been recognized as safe food-flavoring agents and aromatic disinfectants with antimicrobial and antioxidizing activities [7].

Abies is a genus that includes 51 species of evergreen conifers in the family *Pinaceae* [6]. *Abies holophylla* (Manchurian fir, also called needle fir), is a fir species native to mountainous regions of northern Korea, southern Ussuriland, and the provinces of Heilongjiang, Jilin, and Liaoning, China. *Abies koreana* Wilson (Korean fir) is a fir native to the higher mountains of South Korea, including Jeju Island. It grows at altitudes of 1,000–1,900 m in temperate rain forests with high rainfall and cool, humid summers and heavy winter snowfall. These species have been used in the treatment of colds, stomachache, indigestion, rheumatic diseases, and vascular and pulmonary diseases in folk medicine [25] and are reported to have antibacterial, antifungal, anti-inflammatory, and anti-ulcer activities [21, 23]. Various bioactive compounds such as lignans and triterpenoids have been isolated from several *Abies* species [13, 22]. Recently, the chemical composition of the essential oil prepared from the needles of *A. koreana* and its antibacterial activity against nine bacterial strains were reported [11]. However, there is no published report on the phytochemical composition and antimicrobial activity of the essential oil of *A. holophylla*. Therefore, we prepared the essential oils from the needles and twigs of *A. holophylla* as well as *A. koreana*, and compared their phytochemical compositions by gas chromatography–mass spectrometry (GC–MS) analysis. In addition, the antimicrobial properties of the two essential oils against 15 bacterial and 8 pathogenic fungal strains were also investigated.

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MATERIALS AND METHODS

Plant Material

The needles and twigs of *A. holophylla* and *A. koreana* were collected in November 2003 from Gwangneung, Gyeonggi Province, Korea. Voucher specimens (No. KNA20031154 for *A. holophylla* and No. KNA20031155 for *A. koreana*) have been deposited at the Korea National Arboretum, Gyeonggido, Korea.

Isolation of the Essential Oil

Each sample (1 kg) of plants was immersed in 9 l of distilled water and submitted to steam distillation using a manufactured apparatus with a condenser (Hanil Labtech, Korea). Distillation continued for 2 h at 100°C, and the volatile compounds containing the water-soluble fraction were allowed to settle for 20 min. The essential oil layer was separated and purified by microfiltration. The temperature of the water used for cooling the condenser was 4°C.

GC Analysis

GC analysis was performed using a Hewlett-Packard (HP, Santa Clara, U.S.A.) model 6890 gas chromatograph equipped with an apolar capillary DB-5MS 1127 column (30 m×0.32 mm i.d., 0.25 μm film thickness; J&W Scientific, Ringoes, U.S.A.) and a split-splitless injection port (split mode). The oven temperature was programmed from 50 to 250°C at 2°C/min and held at this temperature for 30 min. Injector and FID (flame ionization detector) temperatures were 250°C. The carrier gas was helium at a flow rate of 1.5 ml/min. The GC–MS was performed using an HP model 5973 mass spectrometer operating in the electrospray ionization mode at 70 eV, combined with the GC described above. Most constituents were identified by GC by comparison of their GC retention indices to those in the literature or to those of standards available in our laboratories. Further identification was made by comparison of their mass spectra to those stored in the Wiley 7N Library or with mass spectra from the literature [1, 10].

Microbial Strains

The essential oils were tested for antibacterial activities against *Acinetobacter calcoaceticus* ATCC 19606, *Citrobacter freundii* ATCC 6750, *Enterobacter aerogenes* ATCC 13048, *E. cloacae* ATCC 13047, *Escherichia coli* ATCC 10536, *E. coli* ATCC 25922, *E. coli* ATCC 33312, *Klebsiella pneumoniae* ATCC 10031, *K. oxytoca* ATCC 10031, *Pseudomonas aeruginosa* NCTC 10490, *Serratia marcescens* ATCC 25419, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538P, and *S. aureus* 503. The essential oils were also tested for antifungal activities against *Candida albicans* B02630, *C. krusei* ATCC 6258, *C. glabrata* YFCC 062, *C. tropicalis* ATCC 13803, *C. pseudotropicalis* KCCM 11658, *C. parapsilosis* ATCC 34136, *Cryptococcus neoformans* B.42419, and *Aspergillus fumigatus* B.19119,

Antibacterial Assay

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oils [16]. Briefly, a 100 μl suspension containing 10^5 colony-forming units (CFU)/ml of bacteria was spread on Mueller Hinton (MH) agar. The discs (6 mm in diameter) were impregnated with 10 μl of essential oil diluted with 5% dimethylsulfoxide (DMSO) under aseptic conditions and placed on the inoculated agar. Negative controls were prepared using the same solvent that was spread on the agar plates. Chloramphenicol (Catalog No. 857440; Aldrich, St. Louise, U.S.A.) served as a positive reference standard to determine the sensitivity of each bacterial strain tested [9]. The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zone of growth inhibition against the test organisms.

Minimal Inhibitory Concentration (MIC) Evaluation Using a Microwell Dilution Assay

MIC values were evaluated for the bacterial strains and unicellular fungi using a broth microdilution method [18]. For bacterial strains, all tests were performed in MH broth. Overnight broth cultures of each strain were prepared, and the final concentration in each well was adjusted to 1×10^4 CFU/ml. The essential oils were dissolved in nine volumes of DMSO, and then a 2-fold serial doubling dilution of the oil was prepared using MH broth in a 96-well microtiter plate. The oil was tested against all bacterial cultures, standards, and controls. The plate was covered with a sterile plate sealer, the wells were then mixed on a plate shaker for 20 sec, and the plates were incubated at 37°C for 18 h. The MIC is defined as the lowest concentration of the sample at which the microorganism does not demonstrate visible growth. Microbial growth was indicated by turbidity using a microplate reader (Model 680; Bio-Rad, Hercules, U.S.A.). For fungal strains, all tests were performed in a similar fashion to that used for bacterial strains, except the broth used was modified RPMI 1640 (Gibco BRL, Carlsbad, U.S.A.) to have 0.165 M MOPS buffer (pH 7.0) and L-glutamine instead of $NaHCO_3$, and the inoculum size for each well was adjusted to 5×10^3 CFU/ml. Fungal growth was indicated by turbidity using a microplate reader for unicellular fungi or by the naked eye for filamentous fungi. Chloramphenicol and ketoconazole (Catalog No. K0045; TCI Co., Tokyo, Japan) served as positive references standard to determine the sensitivity of each bacterial and fungal strain, respectively.

RESULTS AND DISCUSSION

The essential oil was prepared from the needles and twigs of plants by distillation for 2 h at 100°C. As a result, the hydrodistillation of *A. holophylla* and *A. koreana* yielded 0.8% and 0.6% (*v/w*) essential oils, respectively. In the previous report, the yield from the needles of *A. koreana* by the same distillation process for 3 h was 0.9% (*v/w*) [11]. Our lower yield may have been caused by the plant material used or the time of distillation.

The GC–MS analysis revealed that 38 compounds comprised 99.55% of the *A. holophylla* essential oil and the peak areas of each component were relatively calculated in percent area (Table 1). The main components were bicyclo[2.2.1]heptan-2-ol (28.05%), δ3-carene (13.85%), α-pinene (11.68%), camphene (10.41%), dl-limonene (7.61%), β-myrcene (7.11%), *trans*-caryophyllene (5.36%), and α-bisabolol (3.67%).

In the essential oil from *A. koreana*, 36 compounds comprised 98.67% of the oil (Table 1). The main components of the oil were bornyl ester (41.79%), camphene (15.31%),

Table 1. Chemical composition of the essential oils from *Abies koreana* and *Abies holophylla*.

Retention time (min)	Component[a]	Peak area (%)	
		Abies holophylla	*Abies koreana*
6.51	Bicyclo[2.2.1]hepta-2-ene	-	1.80
7.86	Tricyclene	0.87	1.56
8.37	α-Pinene	11.68	11.19
8.83	Camphene	10.41	15.31
10.03	2-β-Pinene	1.57	1.93
10.03	Sabinene	1.50	-
10.83	β-Myrcene	7.11	0.50
11.75	δ3-Carene	13.85	0.55
11.98	α-Terpinene	-	2.29
12.10	1-Methyl-2-iso-propylbenzene	-	0.25
12.11	Cymene	0.19	-
12.69	dl-Limonene	7.61	8.58
14.20	γ-Terpinene	0.08	-
15.84	α-Terpinolene	1.05	0.19
18.69	Exo-methyl-camphenilol	0.13	-
19.87	Borneol L	0.26	0.30
24.41	Thymyl methyl ether	0.22	0.38
26.14	Linalyl acetate	-	0.31
27.62	α-Fenchyl acetate	-	5.55
28.11	Bicyclo[2.2.1]heptan-2-ol	28.05	
28.16	Bornyl ester	-	41.79
32.40	α-Cubebene	-	0.13
32.67	Nerol	0.16	0.08
33.85	Geranyl acetate	0.84	0.11
34.28	β-Bourbonene	0.16	0.89
35.34	Junipene	0.21	-
35.41	(−)-Isoledene	-	1.24
36.25	*trans*-Caryophyllene	5.36	0.51
38.19	α-Caryophyllene	1.23	-
38.22	β-Selinene	-	0.24
39.81	Germacrene D	0.50	0.14
40.82	Alloocimene	0.45	-
41.10	α-Muurolene	0.14	0.22
41.74	α-Amorphene	0.21	0.39
41.83	β-Bisabolene	1.00	0.42
42.45	γ-Cadinene	0.28	-
44.65	Nerolodol isomer	0.08	0.40
45.10	Caryophyllene oxide	0.31	0.15
48.44	t-Cadinol	-	0.12
49.50	α-Cadinol	-	0.10
51.23	α-Bisabolol	3.67	0.84
69.94	Abieta-7,13-diene	0.17	
Total		99.33[b]	98.46[b]

[a]Compounds listed in order of their elution on the DB-5MS 1127 column.
[b]Compounds with peak area less than 1% are omitted.
-, Not detected.

α-pinene (11.19%), dl-limonene (8.58%), fenchyl acetate (5.55%), and α-terpinene (2.29%). However, according to Jeong *et al.* [11], the main components of the essential oil from the needles of *A. koreana* were borneol (27.9%), α-pinene (23.2%), β-pinene (5.8%), terpinene-4-ol (3.8%), bornyl acetate (3.4%), and α-terpineol (3.1%). This may reflect not only the difference in plant material used and distillation time, but also the influence of geographic circumstance and climate on the content of the essential oil from the plant. In fact, there have been many reports that

Table 2. Antimicrobial activities of the essential oils from *Abies koreana* and *Abies holophylla*

Microorganisms	Zone of inhibition (mm)[a]						
	Abies holophylla			*Abies koreana*			CAM[b]
	8.8 μg	4.4 μg	2.2 μg	8.8 μg	4.4 μg	2.2 μg	30 μg
Escherichia coli 1507 E	-	-	-	11.0	-	-	25.0
Escherichia coli ATCC 33312	13.0	10.0	-	14.0	12.0	10.0	28.0
Klebsiella oxytoca ATCC 10031	15.0	13.5	12.0	19.0	15.0	12.0	34.0
Bacillus subtilis ATCC 6633	11.0	9.5	-	11.0	9.5	-	30.0
Staphylococcus aureus ATCC 6538P	-	-	-	10.0	-	-	25.0
Staphylococcus aureus ATCC 25923	10.0	-	-	10.0	-	-	24.0
Staphylococcus aureus 503	11.0	-	-	11.0	-	-	25.0

[a]Diameter of zone of inhibition (mm) including disc diameter 6 mm.
[b]Chloramphenicol as the positive control
-, Complete lack of activity.

essential oil composition is variable between sites, species, and ages, even in individual plants [19].

The *in vitro* antimicrobial activities of essential oils against various microorganisms and their potential activities were assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters, and MIC values. According to the results presented in Tables 2 and 3, the essential oil of *A. koreana* showed potential antimicrobial activity against all bacteria tested. In particular, the antimicrobial assay using the agar disc diffusion method showed that 2.2 μg of *A. koreana* oil most effectively inhibited the growth of *E. coli* ATCC 33312 (10.0 mm) and *K. oxytoca* ATCC 10031 (12.0 mm). The essential oils from *A. holophylla* showed the strongest antimicrobial activity against *K. oxytoca* ATCC 10031 (12.0 mm) at 2.2 μg per disc and also against many other microorganisms in the range of 4.4–8.8 μg per disc, except for *S. aureus* ATCC 6538P and *E. coli* 1507E (Table 2).

The MIC value of *A. holophylla* for *K. pneumoniae* ATCC 10031 was 10.9 mg/ml, but other strains resulted in MIC values higher than 21.8 mg/ml (Table 3). The MIC values of *A. koreana* for *K. pneumoniae* ATCC 10031, *B. subtilis* ATCC 6633, and *E. coli* ATCC 33312 were 5.5, 10.9, and 10.9 mg/ml, respectively, and other strains resulted in MIC values higher than 21.8 mg/ml. In the previous report, *A. koreana* essential oil exhibited broad-spectrum antibacterial activity against the nine organisms tested, including methicillin-resistant *S. aureus* [11]. Similarly, our data clearly show that the essential oils from *A. holophylla* and *A. koreana* can effectively inhibit the growth of several microorganisms, which was more effective on Gram-negative, including *E. coli* and *K. pneumoniae* strains, prior to Gram-positive bacteria. However, the MIC values (5.5–21.8 mg/ml) of the essential oils were comparatively higher than those of other essential oils. For example, essential oil from the Tibetan medicinal herb *Dracocephalum heterophyllum*

Table 3. Minimal inhibitory concentrations of the essential oils from *Abies koreana* and *Abies holophylla* toward bacteria.

Microorganisms	MIC		
	Abies holophylla[a]	*Abies koreana*[a]	CAM[b]
Acinetobacter calcoaceticus ATCC 19606	>21.8	>21.8	16.0
Citrobacter freundii ATCC 6750	>21.8	>21.8	16.0
Enterobacter aerogenes ATCC 13048	21.8	21.8	8.0
Enterobacter cloacae ATCC 13047	21.8	21.8	8.0
Escherichia coli ATCC 10536	21.8	21.8	2.0
Escherichia coli ATCC 25922	21.8	21.8	2.0
Escherichia coli ATCC 33312	21.8	10.9	2.0
Klebsiella pneumoniae ATCC 10031	10.9	5.5	1.0
Pseudomonas aeruginosa NCTC 10490	>21.8	>21.8	16.0
Serratia marcescens ATCC 25419	>21.8	>21.8	4.0
Bacillus subtilis ATCC 6633	21.8	10.9	2.0
Staphylococcus aureus ATCC 25923	21.8	21.8	8.0
Staphylococcus aureus ATCC 6538P	>21.8	>21.8	8.0

[a] Values given as mg/ml
[b]Chloramphenicol as the positive control. Values given as μg/ml

Table 4. Minimal inhibitory concentrations of the essential oils from *Abies koreana* and *Abies holophylla* toward fungi.

Microorganisms	MIC[a]		
	Abies holophylla	*Abies koreana*	Ketoconazole
Candida albicans B02630	>2.2	>2.2	0.05
Candida krusei ATCC 6258	>2.2	2.2	0.40
Candida glabrata YFCC 062	2.2	0.5	0.40
Candida tropicalis ATCC 13803	>2.2	>2.2	0.10
Candida pseudotropicalis KCCM 11658	>2.2	1.1	0.05
Candida parapsilosis ATCC 34136	>2.2	1.1	0.05
Cryptococcus neoformans B 42419	0.5	1.1	0.05
Aspergillus fumigatus B 19119	>2.2	1.1	0.80

[a]Values given as mg/ml.

Benth is reported to have MIC values of 0.039–0.156 mg/ml against various Gram-positive and Gram-negative bacteria [26]. This difference can be attributed to the bacterial strains and assay conditions, especially media used for cultivating bacteria, as well as the composition of the essential oils used in each particular study. We adopted the same condition for antimicrobial assay as that in the previous report by Hong *et al.* [9], and our data showed similar but stronger antimicrobial activity than those reported in spite of different oil compositions.

Among the major components, long-chain alcohols, such as terpinen-4-ol, α-terpineol, and borneol, are believed to be the antibacterial constituents of essential oils [4, 20], and α-pinene is also thought to be active in the inhibition of microbial growth [12]. The essential oils prepared in this study did not contain terpinen-4-ol; however, relatively higher contents of α-pinene, a monoterpene, were detected in the oils from both *A. holophylla* (11.68%) and *A. koreana* (11.19%), which may be responsible for the antibacterial activity.

The fungiostatic activities of the essential oils were tested on various fungal strains that commonly cause foot rot and other diseases (Table 4). The essential oil from *A. koreana* was the most effective for *C. glabrata* YFCC 062 with a MIC value of 0.5 mg/ml, and was also effective for *C. neoformans* B42419, *C. pseudotropicalis* KCCM 11658, *C. parapsilosis* ATCC 34136, and *A. fumigatus* B19119 with MIC values of 1.1 mg/ml. *A. holophylla* oil was the most effective for *C. neoformans* B42419 (MIC, 0.5 mg/ml) and showed MIC values above 2.2 mg/ml for other fungal strains tested. In the case of the essential oil from *D. heterophyllum* Benth, MIC values toward yeast and fungal strains were in the range of 0.156 mg/ml and 0.313–2.5 mg/ml, respectively [26]. Although there were many differences in experimental condition, the fungiostatic activity of essential oils from *A. koreana* and *A. holophylla* seemed to be as strong as that from *D. heterophyllum* Benth.

According to Knobloch *et al.* [14], essential oils can cause damage to biological membranes; in particular, phenolic alcohols or aldehydes interfere with membrane-associated enzyme proteins. Additionally, it was suggested that essential oils may disrupt the permeability of cell membranes and inhibit respiration, and its efficiency depends on the rate of monoterpene penetration through the fungal cell wall and cell membrane structure [4]. Although the major components of essential oils that endow the profound antifungal effect need to be further identified, our data clearly demonstrate that the oils prepared from *A. holophylla* and *A. koreana* have great potential antifungal activity against most fungi tested.

In conclusion, the chemical composition of the essential oils prepared from the needles and twigs of *A. holophylla* as well as *A. koreana* was analyzed by GC–MS. Comparison of our data with those of *A. koreana* oil prepared from the needles described in the previous report suggested that the plant part, such as needle and twig, had great influence on the oil composition and antimicrobial activity [11]. Additionally, we demonstrated that both oils had very effective antifungal activities toward most pathogenic strains tested, with MICs values as low as one-tenth of MICs for antibacterial activity, indicating that they may be practically useful as safe and natural antifungal agents.

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