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Research Article

Preliminary screening to assess the antimicrobial activities of extracts of evergreen woody species from South Korea against *Staphylococcus aureus*

Bo Kook Jang · Lai Won Chi · Ju Sung Cho · Cheol Hee Lee

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Abstract This study aimed to screen for plants with antimicrobial potential among the evergreen woody species of South Korea that are used for horticulture and landscaping and to provide basic information about plants with proven antimicrobial activity to underpin future research. The plant materials were extracted under various conditions, and the antimicrobial activities of the extracts were evaluated by agar diffusion assay. The screening tests demonstrated that the crude extracts of 43 species had inhibitory effects against *S. aureus*. The inhibitory activities of four species (*Elaeocarpus sylvestris*, *Camellia japonica*, *Cleyera japonica*, and *Quercus salicina*) were relatively higher than that of the synthetic antimicrobial agents methylparaben and phenoxyethanol. The highest inhibitory activity was observed with the leaf extracts (extracted with methanol for 30 minutes) of *E. sylvestris,* based on induction of the largest inhibition zone of 23.3 mm in size. In addition, solvent fractions of *E. sylvestris* were evaluated. The largest inhibitory zone of 23.1 mm was observed for the n-butanol fraction, which is likely to contain effective compounds that exhibit inhibitory activity against *S. aureus*. In contrast, n-hexane and residual aqueous fractions showed no antimicrobial activity. Overall, our findings confirm that evergreen woody plants native to South Korea have potential antimicrobial activity.

B. K. Jang · L. W. Chi · J. S. Cho · C. H. Lee (✉)
Division of Animal, Horticultural and Food Sciences, Chungbuk National University, Cheongju, 28644, Korea
e-mail: leech@chungbuk.ac.kr

B. K. Jang · L. W. Chi · J. S. Cho · C. H. Lee (✉)
Brain Korea 21 Center for Bio-Resource Development, Chungbuk National University, Cheongju, 28644, Korea

L. W. Chi
LOY Culture & Art College, Incheon, 21366, Korea

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Introduction

Disease has affected the survival of humans throughout their history, but it has been controlled by the development of medicines (Ríos and Recio 2005). Materials obtained from the natural environment have been the source for many medicines, and antibiotics and drugs have been derived directly or indirectly from endophytic microbes or plants (Newman et al. 2003). Although antibiotics obtained from many microorganisms have been developed and used, only a few plant materials with antimicrobial potential have been revealed, and these may possibly be used for antibiotic production (Newman and Cragg 2016).

The number of plant-derived antimicrobial products is increasing, but there are still many synthetic antimicrobials. Synthetic antimicrobials or antibiotics can effectively control microorganisms, but the development of antimicrobial resistance in microorganisms owing to the continued use of antibiotics has raised considerable issues. This has promoted research into plant-derived natural products (Chandra et al. 2017; Iwu et al. 1999; Lee and Shin 2010). The antimicrobial activity of plant-derived extracts has been demonstrated to be attributed to essential oils (Haloui et al. 2015), alkaloids (Altameme et al. 2015; Deng et al. 2011), terpenes (Moghrovyan et al. 2019), and flavonoids (Cushnie and Lamb 2005; Górniak et al. 2019). Microorganisms are less resistant to these plant-derived compounds than to synthetic compounds, and, thus, they can be used safely without causing side effects.

Staphylococcus aureus is a dangerous and versatile pathogen that can cause a multitude of diseases in addition to

food poisoning (Otto 2014). Most frequently, it causes skin infections and infections of the respiratory tract; it also causes diseases, such as pneumonia, toxic shock syndrome, scalded skin syndrome, and osteomyelitis. *S. aureus* has been controlled by the development of antibiotics, such as penicillin, but the emergence of its resistance to several antibiotics has created new problems (Lowy 1998; Lowy 2003). Typically, resistance to penicillin, methicillin, quinolone, and vancomycin is observed. The use of antibiotics against *S. aureus* is still effective, but the number of antibiotic-resistant *S. aureus* strains is predicted to increase. It is, thus, essential to find new drugs that can avoid microbial resistance.

Evergreen species native to South Korea have been planted in gardens, parks, and urban green spaces; along city streets; and for landscaping and windbreak forests since the late 1970s (Lee and Kim 2017). Such species have been planted for ornamental, horticultural, and landscaping purposes, and every year, many pruning by-products (mostly twigs and leaves) are produced. It is necessary to recycle these by-products to reduce processing costs and create a new added value. Some woody species have been used in traditional herbal medicines (Fenglin et al. 2004); extracts of *Elaeocarpus sylvestris* var. *ellipticus* are antimicrobial (Piao et al. 2009; Taguri et al. 2006) and extracts of *Quercus* species have shown antioxidant activity (Tuyen et al. 2016). In addition, the antimicrobial activity of extracts of several woody species have been reported in South Korea (Cho et al. 2018; Jang et al. 2018). Therefore, the present study was carried out to evaluate the potential for antimicrobial activity in 64 evergreen woody species native to South Korea. Our findings provide information on the antimicrobial activity of Korean evergreen woody plants and contribute to the development of plant-derived natural products.

Materials and Methods

Plant collection

Leaves and twigs of 64 evergreen woody plants were collected in January 2013. The collection location for each sample is listed in Table 1. Forty-three species were collected from Wando, Jeollanam-do, South Korea and 21 species were collected from Jeju-si, Jeju-do.

Preparation of plant extracts

The samples were freeze dried (-70°C to -85°C, FD8512, Ilshin Biobase, Dongducheon, Korea) for 48 hours after washing in running tap water, except that part of each sample was retained for moisture content measurement as described below. The dry samples were finely ground (Hood mixer FM-681C, Hanil, Seoul, Korea) and stored in a deep freezer at -70°C (MDF-U73V, Sanyo Electric Co., Ltd., Osaka, Japan) as powder for use in our experiments. To screen the samples for antimicrobial activity, 1 g of dry leaf samples (powder) was ultrasonically extracted with methanol solvent for 30 minutes, and the inhibition zone was measured by agar diffusion assay.

Part of the fresh sample for each of the 64 species was used to measure moisture content. Fresh weight (W_1) was measured before oven drying at 60°C for 48 hours. After drying, the weight (W_2) was measured again, and the moisture content (%) was calculated as follows: (W_1 - W_2) / W_1. The content of soluble solids was also measured. The fresh weight of leaves and twigs was measured before freeze drying for 48 hours. A 5 ml sample of each 50-fold concentrated extract (methanol) was placed in an evaporating dish and dried at 60°C in an oven for 48 hours. After transferring it to a desiccator, the soluble solid content ($g \cdot g^{-1}$) of the extract was measured.

Microorganisms

The microorganisms used in the experiment were provided by the Korean Collection for Type Cultures (KCTC) (*Staphylococcus aureus* KCTC 1927). A culture medium in which Mueller-Hinton broth (275730, Difco Laboratories, Sparks, MD, USA) was mixed with 1.2% agar powder was prepared and cultured at 3 ~ 5-week intervals to maintain *S. aureus* activity. Two weeks before the experiment, 100 µl of *S. aureus* culture was activated in 10 ml broth at intervals of 3 ~ 5 days.

Extraction method

All powder samples were extracted with an ultrasonic cleaner (300 × 240 × 145 mm, 5510-DTH, Bransonic, Danbury, CT, USA) for 30 minutes, and then 1 g of the powder was added to a 200-ml glass bottle, and 30 ml methanol was added until the powder was immersed. The sample was extracted in the ultrasonic cleaner for 30 minutes. The extraction was repeated twice for each sample, and finally, two extracts were mixed and used. The resultant was filtered under reduced pressure with a vacuum pump using 55-mm quantitative filter paper. The filtered extract was quantified at 50-fold concentration (1 g / 50 ml) by adding a solvent (methanol). The extract was then concentrated under reduced

Table 1 List of plants used in this study

Sample number	Family	Species[a]	Korean name[b]	Collection region
(1)	Apocynaceae	*Nerium oleander* L.	Hyeop-juk-do	Jeju-si
(2)		**Trachelospermum asiaticum* var. *majus* Makino	Baek-hwa-deung	Wando
(3)		*Trachelospermum asiaticum* (Siebold & Zucc.) Nakai	Ma-sak-jul	Wando
(4)	Aquifoliaceae	*Ilex cornuta* Lindl. & Paxton	Ho-rang-ga-si-na-mu	Wando
(5)		**Ilex xwandoensis* C.F.Mill. & M.Kim	Wan-do-ho-rang-ga-si-na-mu	Wando
(6)		*Ilex crenata* Thunb.	Kkwang-kkwang-na-mu	Wando
(7)		*Ilex rotunda* Thunb.	Meon-na-mu	Jeju-si
(8)	Araliaceae	*Dendropanax morbiferus* H.Lév.	Hwang-chil-na-mu	Wando
(9)		*Fatsia japonica* (Thunb.) Decne. & Planch.	Pal-son-i	Wando
(10)		*Hedera rhombea* (Miq.) Siebold ex Bean	Song-ak	Wando
(11)	Caprifoliaceae	*Viburnum odoratissimum* Ker Gawl. ex Rümpler var. *awabuki* (K.Koch) Zabel	A-wae-na-mu	Wando
(12)	Cephalotaxaceae	**Cephalotaxus koreana* Nakai	Gae-bi-ja-na-mu	Wando
(13)	Cupressaceae	*Chamaecyparis obtusa* (Siebold & Zucc.) Endl.	Pyeon-baek	Wando
(14)		*Juniperus rigida* Siebold & Zucc.	No-gan-ju-na-mu	Wando
(15)	Daphniphyllaceae	*Daphniphyllum macropodum* Miq.	Gul-geo-ri-na-mu	Wando
(16)	Elaeagnaceae	*Elaeagnus glabra* Thunb.	Bo-ri-jang-na-mu	Jeju-si
(17)		*Elaeagnus umbellata* Thunb.	Bo-ri-su-na-mu	Wando
(18)	Elaeocarpaceae	*Elaeocarpus sylvestris* (Lour.) Poir.	Dam-pal-su	Jeju-si
(19)	Ericaceae	**Rhododendron brachycarpum* auct.	Man-byeong-cho	Wando
(20)		*Vaccinium bracteatum* Thunb.	Mo-sae-na-mu	Wando
(21)		*Vaccinium oxycoccos* L.	Neon-chul-wol-gyul	Jeju-si
(22)	Fagaceae	*Castanopsis sieboldii* (Makino) Hatus.	Gu-sil-jat-bam-na-mu	Wando
(23)		*Quercus acuta* Thunb.	Buk-ga-si-na-mu	Wando
(24)		*Quercus gilva* Blume	Gae-ga-si-na-mu	Jeju-si
(25)		*Quercus glauca* Thunb.	Jong-ga-si-na-mu	Wando
(26)		*Quercus myrsinifolia* Blume	Ga-si-na-mu	Wando
(27)		*Quercus salicina* Blume	Cham-ga-si-na-mu	Wando
(28)	Flacourtiaceae	**Xylosma japonica* A.Gray	San-yu-ja-na-mu	Jeju-si
(29)	Gramineae	**Phyllostachys pubescens* J.Houz.	Juk-sun-dae	Jeju-si
(30)		*Sasa quelpaertensis* Nakai	Je-ju-jo-rit-dae	Jeju-si
(31)	Hamamelidaceae	*Distylium racemosum* Siebold & Zucc.	Jo-rok-na-mu	Jeju-si
(32)	Illiciaceae	**Illicium anisatum* L.	But-sun-na-mu	Jeju-si
(33)	Lardizabalaceae	**Stauntonia hexaphylla* (Thunb.) Decne.	Meol-kkul	Wando
(34)	Lauraceae	**Actinodaphne lancifolia* (Blume) Meisn.	Yuk-bak-na-mu	Wando
(35)		**Cinnamomum loureirii* Nees	Yuk-gye-na-mu	Jeju-si
(36)		*Cinnamomum yabunikkei* H. Ohba	Saeng-dal-na-mu	Wando
(37)		*Cinnamomum camphora* (L.) J. Presl	Nok-na-mu	Jeju-si
(38)		*Laurus nobilis* L.	Wol-gye-su	Wando
(39)		*Litsea japonica* (Thunb.) Juss.	Kka-ma-gwi-jjok-na-mu	Wando
(40)		*Machilus japonica* Siebold & Zucc.	Sen-dal-na-mu	Wando
(41)		*Machilus thunbergii* Siebold & Zucc.	Hu-bak-na-mu	Wando
(42)		*Neolitsea aciculata* (Blume) Koidz.	Sae-deok-i	Jeju-si
(43)		*Neolitsea sericea* (Blume) Koidz.	Cham-sik-na-mu	Jeju-si
(44)	Loganiaceae	**Gardneria insularis* Nakai	Yeong-ju-chi-ja	Wando
(45)	Magnoliaceae	*Magnolia grandiflora* L.	Tae-san-mok	Jeju-si
(46)	Moraceae	**Ficus oxyphylla* Miq.	Mo-ram	Wando
(47)	Myricaceae	*Myrica rubra* (Lour.) Siebold & Zucc.	So-gwi-na-mu	Jeju-si
(48)	Myrsinaceae	*Ardisia crenata* Sims	Baek-ryang-geum	Jeju-si
(49)	Oleaceae	*Ligustrum lucidum* W.T.Aiton	Dang-gwang-na-mu	Wando
(50)		*Osmanthus fragrans* Lour.	Mok-seo	Wando
(51)		*Osmanthus fragrans* var. *aurantiacus* Makino	Geum-mok-seo	Wando
(52)	Pinaceae	*Pinus thunbergii* Parl.	Gom-sol	Wando
(53)	Rosaceae	*Eriobotrya japonica* (Thunb.) Lindl.	Bi-pa-na-mu	Wando

Table 1 List of plants used in this study (Continued)

Sample number	Family	Species[a]	Korean name[b]	Collection region
(54)		*Rhaphiolepis indica* var. *umbellata* (Thunb.) H.Ohashi	Da-jeong-keum-na-mu	Wando
(55)	Rutaceae	**Citrus xjunos* Siebold ex Yu. Tanaka	Yu-ja-na-mu	Wando
(56)	Schisandraceae	*Kadsura japonica* (L.) Dunal	Nam-o-mi-ja	Wando
(57)	Taxaceae	*Torreya nucifera* (L.) Siebold & Zucc.	Bi-ja-na-mu	Wando
(58)	Taxodiaceae	*Cryptomeria japonica* (Thunb. ex L.f.) D.Don	Sam-na-mu	Wando
(59)	Theaceae	*Camellia japonica* L.	Dong-baek-na-mu	Wando
(60)		*Cleyera japonica* Thunb.	Bi-jju-gi-na-mu	Jeju-si
(61)		*Eurya emarginata* (Thunb.) Makino	U-muk-sa-seu-re-pi	Jeju-si
(62)		*Eurya japonica* Thunb.	Sa-seu-re-pi-na-mu	Wando
(63)		*Ternstroemia gymnanthera* (Wight & Arn.) Sprague	Hu-pi-hyang-na-mu	Wando
(64)	Thymelaeaceae	*Daphne odora* Thunb.	Seo-hyang	Jeju-si

*Indicates unresolved name or synonym (TPL, 2013).
[a]Indicates accepted name, refer to The Plant List (TPL), 2013.
[b]Refers to Korea National Arboretum, 2017.

pressure again and dissolved in dimethyl sulfoxide to adjust the final concentration to 50 mg·ml^{-1}. The extract was stored in a -70°C deep freezer until use in the experiment. In all experiments, the concentration of the crude extract was diluted to 50 mg·ml^{-1}. The concentration of the extract to be used in the agar diffusion assay was diluted to 2.00 mg/disc.

Agar diffusion assay

The agar diffusion assay was performed following the method of Shin (2010) with minor modifications. Preparation of the agar diffusion plate used in the experiment was as follows: 1) The broth medium with 0.7% soft agar was sterilized and stored at 60°C in a constant temperature water bath. 2) The optical density of the activated *S. aureus* culture was adjusted to 1.0 using a visible spectrophotometer. 3) The adjusted *S. aureus* culture was added to the sterilized broth medium at 1% (v/v) ratio and stirred before pipetting 12 ml per petri dish.

On the surface of the agar diffusion plate, an 8-mm paper disk injected with the extract (2 mg / disc (40 μl)) was attached. The paper-disk-attached medium was cooled for 1 hour at 4°C and then transferred to a growth chamber at 37°C and incubated for 24 hours. After incubation, the inhibition zone, including the diameter of the paper disc, was measured. As controls, phenoxyethanol (0H2108, Junsei Chemical Co., Ltd., Tokyo, Japan) and methylparaben (8K5015, Junsei Chemical Co.) at concentrations of 0.40, 1.00, 2.00, and 4.00 mg / disc were used. The same method was used for all experiments.

Antimicrobial activities according to the extraction conditions of four species

Four species (*E. sylvestris*, *Camellia japonica*, *Cleyera japonica*, and *Quercus salicina*), the extracts of which induced large inhibition zones, were selected from the 64 species screened (Table 2). The antimicrobial activity of the extracted parts (leaf and twig), extraction solvents (distilled water, 80% ethanol, and methanol), and extraction time (15, 30, and 45 minutes) were compared.

Antimicrobial activities according to solvent fractions of *E. sylvestris*

Solvent fractionation of the final *E. sylvestris* extract was carried out to determine inhibitory activity. Fractions were obtained using a ratio of powdered sample:distilled water: n-hexane (v:v:v) at 1:9:10. The n-hexane aqueous layer was sequentially partitioned into chloroform, ethyl acetate, and n-butanol (Fig. 1). Each solvent fraction was concentrated under reduced pressure and freeze dried to obtain a powdered sample, which was dissolved in distilled water and used in the antimicrobial experiment in the same manner as the agar diffusion method.

Statistical analysis

For the agar diffusion assay, treatments included three replicates. In the case of the extraction conditions of the four species and solvent fractions of *E. sylvestris*, six replicates were performed twice. SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA) was used to calculate

the mean ± standard error for each treatment, and a factorial analysis was performed using Duncan's multiple range test with a significance level of $p < 0.05$.

Results

Screening for antimicrobial activity of 64 evergreen woody species

The antimicrobial activity against *S. aureus* was observed in crude extracts of 43 of the 64 evergreen woody species used in this study (Table 2). Forty-three leaf extracts exhibited inhibition zones of 8.05 ~ 23.30 mm. The *E. sylvestris* leaf extract had the largest inhibition zone (23.30 mm), followed by *Cleyera japonica* (18.95 mm), *Q. salicina* (18.65 mm), and *Camellia japonica* (16.70 mm).

Soluble solid and moisture contents of the 64 evergreen woody species are shown in Table 3. The moisture content ranged from 41.58 to 79.33% for leaf samples and 32.80 to 65.68% for twig samples. Soluble solid content ranged from 50 to 400 $mg \cdot g^{-1}$ for leaf samples and 100 to 340

Table 2 Antimicrobial activities of extracts obtained from the leaves of 64 species against *Staphylococcus aureus*

Sample number	Concentration (mg/disc)	Inhibition zone (mm)	Sample number	Inhibition zone (mm)
Methylparaben[a]	0.40	-[b]	(29)	-
	1.00	-	(30)	10.10 ± 2.40 f
	2.00	11.20 ± 2.90 ef	(31)	15.45 ± 1.35 c
	4.00	13.80 ± 2.30 d	(32)	10.35 ± 0.45 f
Phenoxyethanol[a]	0.40	-	(33)	8.55 ± 0.35 h
	1.00	-	(34)	10.35 ± 0.30 f
	2.00	8.50 ± 0.70 f	(35)	9.95 ± 0.05 g
	4.00	9.80 ± 1.80 g	(36)	-
(1)	2.00	-	(37)	10.00 ± 0.00 f
(2)	2.00	-	(38)	9.40 ± 0.10 g
(3)	2.00	-	(39)	-
(4)	2.00	-	(40)	9.20 ± 1.00 g
(5)	2.00	-	(41)	-
(6)	2.00	-	(42)	-
(7)	2.00	-	(43)	-
(8)	2.00	15.75 ± 0.85 c	(44)	-
(9)	2.00	-	(45)	14.85 ± 0.95 cd
(10)	2.00	-	(46)	8.75 ± 0.15 gh
(11)	2.00	12.45 ± 0.35 e	(47)	11.70 ± 0.40 ef
(12)	2.00	-	(48)	9.10 ± 0.20 g
(13)	2.00	11.50 ± 0.40 ef	(49)	10.45 ± 0.35 f
(14)	2.00	10.40 ± 0.20 f	(50)	-
(15)	2.00	8.35 ± 0.15 h	(51)	-
(16)	2.00	-	(52)	12.50 ± 0.50 e
(17)	2.00	-	(53)	12.50 ± 0.00 e
(18)	2.00	23.30 ± 0.80 a	(54)	11.30 ± 0.00 ef
(19)	2.00	12.65 ± 0.85 e	(55)	8.05 ± 0.05 h
(20)	2.00	12.15 ± 0.05 e	(56)	10.15 ± 0.15 f
(21)	2.00	11.90 ± 0.10 ef	(57)	9.00 ± 0.10 g
(22)	2.00	13.80 ± 1.60 d	(58)	12.65 ± 0.55 e
(23)	2.00	12.40 ± 0.60 e	(59)	16.70 ± 1.30 bc
(24)	2.00	9.35 ± 0.15 g	(60)	18.95 ± 0.15 b
(25)	2.00	11.00 ± 0.50 f	(61)	10.40 ± 0.40 f
(26)	2.00	9.50 ± 0.50 g	(62)	12.30 ± 0.02 e
(27)	2.00	18.65 ± 2.30 b	(63)	16.00 ± 2.10 bc
(28)	2.00	8.95 ± 0.15 gh	(64)	12.35 ± 0.45 e

Mean ± S.E. (n = 10). Different lowercase letters indicate a significant difference at $p < 0.05$ based on Duncan's multiple range test.
[a]Positive control.
[b]Not detected.

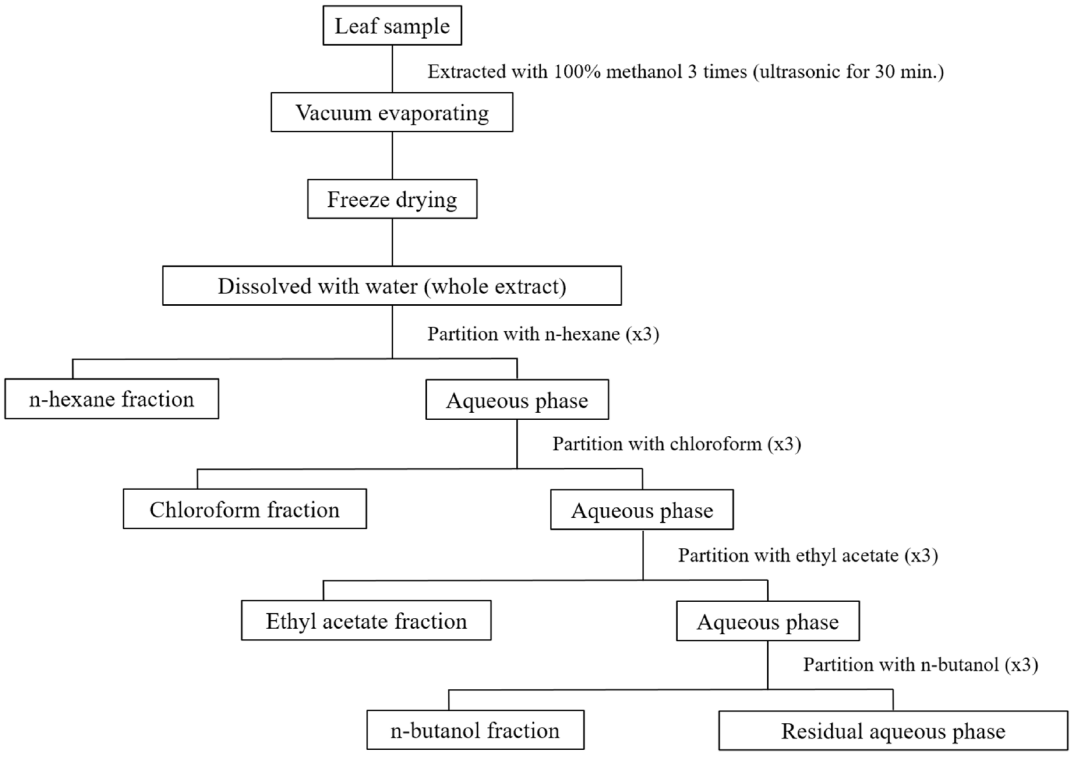

Fig. 1 Schematic diagram of the solvent fractionation of the MeOH extract using n-hexane, chloroform, ethyl acetate, and n-butanol

$mg \cdot g^{-1}$ for twig samples.

Antimicrobial activities according to extraction conditions of four species

The extracts of the four plants exhibited different levels of inhibitory activity against *S. aureus* depending on the extraction conditions used (Table 4). The leaf extracts of *E. sylvestris* and *Cleyera japonica* were found to have a stronger inhibitory effect on *S. aureus* than the twigs. However, for *Camellia japonica*, the twig extract was more effective. Leaf extracts of *E. sylvestris* exhibited strong inhibitory effects regardless of the solvent used: methanol (23.3 mm, 30 min), 80% ethanol (21.8 mm, 15 min), and distilled water (21.4 mm, 45 min). In the extracts of *Cleyera japonica*, the inhibition zone against *S. aureus* was observed in methanol (21.3 mm, 30 min) and 80% ethanol (20.6 mm, 15 min), but no inhibition zone was formed in distilled water. *Camellia japonica* twigs showed the highest inhibitory activity with 80% ethanol for 45 minutes (18.5 mm). Antimicrobial activity against *S. aureus* was observed in both *Q. salicina* leaf (19.7 mm) and twig (19.3 mm) extracts. In these plants, strong inhibition zones were observed at 45 min extraction with 80% ethanol regardless of the extraction part.

Antimicrobial activities according to solvent fractions of *E. sylvestris*

The inhibition zone of each solvent fraction was measured (Table 5). The n-butanol fraction showed the largest inhibition zone at 23.1 mm (Fig. 2), whereas no inhibition zone was observed for the residual aqueous or n-hexane fractions. Inhibition zones of 13.5 and 11.4 mm were observed for the chloroform and ethyl acetate fractions, respectively. Therefore, substances that exhibit antimicrobial activity against *S. aureus* are most likely to be found in the n-butanol fraction.

Discussion

We screened evergreen woody plants in South Korea for antimicrobial activity. The plant groups used in the study were more effective than the synthetic antimicrobial agents used as controls, and the results suggested that they have potential for new plant-derived natural products. The leaf extracts of four species strongly inhibited *S. aureus*, the causative agent of skin problems. However, antimicrobial activity differed in the different parts (leaves and twigs), and the soluble solid content examined was also different. In previous reports, it was demonstrated that there were differences in the compounds of essential oils in the

Table 3 Soluble solid and moisture contents of extracts obtained from leaves and twigs of 64 species

Sample number	Soluble solids content ($mg \cdot g^{-1}$)		Moisture content (%)		Sample number	Soluble solids content ($mg \cdot g^{-1}$)		Moisture content (%)	
	Leaf	Twig	Leaf	Twig		Leaf	Twig	Leaf	Twig
(1)	160	140	51.54	56.89	(33)	290	150	63.09	59.62
(2)	150	130	58.16	46.72	(34)	160	180	71.54	52.55
(3)	100	270	56.05	32.80	(35)	120	270	57.97	40.74
(4)	190	240	46.63	47.75	(36)	270	320	47.39	50.28
(5)	240	180	65.49	40.72	(37)	200	260	53.15	51.77
(6)	280	340	57.77	55.80	(38)	160	150	58.31	49.43
(7)	180	220	64.98	51.81	(39)	320	210	57.00	57.13
(8)	270	250	71.19	59.83	(40)	260	310	55.21	52.72
(9)	260	300	60.31	60.28	(41)	150	220	50.24	52.49
(10)	230	270	54.77	59.14	(42)	200	340	51.20	53.49
(11)	210	180	65.86	65.42	(43)	210	220	55.08	54.90
(12)	330	210	69.21	64.67	(44)	400	250	49.81	51.49
(13)	270	150	50.17	33.72	(45)	220	300	63.79	50.76
(14)	270	180	52.52	46.83	(46)	50	270	48.99	46.20
(15)	150	320	67.40	62.34	(47)	200	140	65.86	52.38
(16)	260	340	65.66	56.90	(48)	360	240	51.75	65.68
(17)	140	200	60.27	54.32	(49)	240	180	46.64	48.72
(18)	350	140	63.91	58.39	(50)	270	340	79.33	41.64
(19)	240	240	54.44	48.09	(51)	270	220	48.03	45.21
(20)	370	220	63.86	52.63	(52)	190	250	58.21	52.13
(21)	210	220	57.76	53.86	(53)	220	180	57.08	57.69
(22)	190	330	55.00	51.74	(54)	270	210	59.59	53.82
(23)	220	270	49.10	51.20	(55)	220	150	49.72	47.64
(24)	180	150	52.25	55.08	(56)	290	180	74.47	64.52
(25)	330	100	44.59	40.53	(57)	320	270	60.87	56.24
(26)	120	190	44.14	42.63	(58)	340	320	67.17	52.37
(27)	190	240	54.83	43.10	(59)	200	260	54.16	42.60
(28)	200	250	49.76	43.97	(60)	140	150	59.73	48.94
(29)	230	300	55.84	53.87	(61)	240	200	48.71	51.26
(30)	150	270	51.37	54.87	(62)	220	210	61.35	43.97
(31)	260	180	41.58	52.25	(63)	220	310	59.94	53.87
(32)	280	210	66.94	59.32	(64)	330	220	75.46	53.30

different extracted parts (Hafsé et al. 2013; Haloui et al. 2015). The results of the present study indicate that the leaf extracts exhibited stronger antimicrobial activity than twig extracts, suggesting that they contain compounds with high antimicrobial activity.

The active ingredient of the plant-derived extract shows different effects depending on the extraction conditions, even from the same plant. Depending on the solvent used, extractions can be used to extract water-soluble or water-insoluble compounds. In the present study, the crude extracts of four species extracted with organic solvents exhibited higher overall antimicrobial activity than those extracted with distilled water. Previous reports on *Achillea millefolium* subsp. (Candan et al. 2003), *Hypericum capitatum* and *H. scabrum* (Sokmen et al. 1999) demonstrated that water-insoluble extracts contain more antimicrobial compounds than water-soluble extracts. These results suggest that compounds that exhibit inhibitory activity against *S. aureus* may be water-insoluble rather than water-soluble.

Non-polar extraction with organic solvents is more effective for extracting compounds from plants, including non-polar compounds (Webster et al. 2008). The crude extract of *E. sylvestris*, which showed the strongest antimicrobial activity, contained a mixture of various chemical compounds (i.e., 1,2,3,4,6-penta-*O*-galloyl-β-d-glucose, coniferyl alcohol, umbelliferone, scopoletin, β-sitosterol, and daucosterol). To isolate the active component, fractionation according to solvent polarity was performed. Different solvent fractions exhibited different levels of antibiotic activity, and the strongest inhibitory activity against *S. aureus* was observed for the n-butanol fraction.

According to a recent study, the leaves of *E. sylvestris* contain gallotannin precursors 1,2,3,4,6-penta-*O*-galloyl-β-d-glucose, which show high antioxidant potential (Piao et

Table 4 Antimicrobial activities of extracts obtained from leaves and twigs of four species against *Staphylococcus aureus* at varying concentrations according to extraction conditions, compared with those of chemical controls

Part		Concentration (mg/disc)	Inhibition zone (mm)			
			Sample (18)	Sample (59)	Sample (60)	Sample (27)
Methylparaben[a]		0.40	-[b]	-	-	-
		1.00	-	-	-	-
		2.00	11.0 ± 0.41 gh	11.0 ± 0.41 i	11.0 ± 0.41 g	11.0 ± 0.41 hi
		4.00	13.7 ± 0.26 fg	13.7 ± 0.26 f-h	13.7 ± 0.26 e	13.7 ± 0.26 de
Phenoxyethanol[a]		0.40	-	-	-	-
		1.00	-	-	-	-
		2.00	8.7 ± 0.22 i	8.7 ± 0.22 j	8.7 ± 0.22 i	8.7 ± 0.22 j
		4.00	9.9 ± 0.10 hi	9.9 ± 0.10 i	9.9 ± 0.10 gh	9.9 ± 0.10 i
	Solvent	Extraction time (min)				
Leaf[c]	MeOH	15	19.5 ± 0.62 b-d	13.4 ± 2.05 f-h	17.5 ± 0.20 c	17.6 ± 0.03 b
		30	23.3 ± 0.79 a	13.4 ± 0.56 f-h	21.3 ± 0.64 a	16.6 ± 0.20 bc
		45	18.6 ± 0.55 c-e	13.8 ± 0.27 f-h	17.1 ± 0.41 c	17.3 ± 0.58 b
	80% EtOH	15	21.8 ± 0.55 ab	12.6 ± 1.80 h	20.6 ± 0.74 ab	16.1 ± 0.50 c
		30	21.2 ± 0.55 a-c	13.8 ± 0.58 f-h	19.8 ± 0.54 b	17.2 ± 0.28 bc
		45	18.9 ± 0.20 c-e	16.9 ± 0.19 bc	21.3 ± 0.46 a	19.7 ± 0.17 a
	DW	15	18.0 ± 0.46 de	14.5 ± 2.14 ef	-	12.7 ± 0.75 ef
		30	19.6 ± 0.64 b-d	13.9 ± 0.09 fg	-	10.8 ± 0.54 hi
		45	21.4 ± 1.03 a-c	13.0 ± 0.49 gh	-	12.1 ± 0.44 fg
Twig[c]	MeOH	15	11.5 ± 0.50 gh	16.2 ± 0.09 b-d	10.3 ± 0.25 gh	11.5 ± 0.33 gh
		30	14.3 ± 0.50 f	15.6 ± 0.56 de	12.1 ± 0.25 f	10.8 ± 0.33 hi
		45	18.8 ± 0.18 c-e	16.0 ± 0.31 cd	15.2 ± 0.64 d	14.3 ± 0.19 d
	80% EtOH	15	16.4 ± 0.14 ef	17.2 ± 0.22 b	9.4 ± 0.23 hi	14.6 ± 0.32 d
		30	14.5 ± 0.14 f	18.5 ± 0.22 a	8.7 ± 0.10 i	18.6 ± 0.10 a
		45	18.7 ± 0.29 c-e	17.4 ± 0.40 b	9.3 ± 0.29 hi	19.3 ± 0.18 a
	DW	15	-	10.2 ± 0.28 i	-	16.1 ± 0.50 c
		30	-	-	-	16.8 ± 0.25 bc
		45	-	-	-	13.8 ± 0.23 de
Part (A)			***	***	***	*
Solvent (B)			NS	***	**	***
Time (C)			*	***	**	***
A × B			NS	***	***	***
A × C			***	NS	***	**
B × C			**	***	***	***
A × B × C			NS	**	***	***

Mean ± S.E. (n = 10). Different lowercase letters indicate a significant difference at $p < 0.05$ based on Duncan's multiple range test. NS, *, **, ***; nonsignificant or significant at $p < 0.05$, 0.01, or 0.001, respectively.
[a]Positive control.
[b]Not detected.
[c]Concentration of 2.00 mg/disc was used for all plant extracts

al. 2009). Taguri et al (2006) reported that *E. sylvestris* extracts inhibited bacterial growth. Furthermore, 70% ethanol extract of *E. sylvestris* inhibited cytomegalovirus and varicella-zoster virus (Bae et al. 2017; To et al. 2014). It is considered that the leaves of *E. sylvestris* may contain a variety of inhibitory compounds, especially the n-butanol fraction, which may contain compounds that exhibit potent antimicrobial activity against *S. aureus*. This inhibitory activity was consistent across the results of two screening experiments using crude extracts.

In conclusion, 43 of the 64 evergreen woody species in this study showed potential antimicrobial activity against *S. aureus*. In particular, the strongest antimicrobial activity was observed for the n-butanol fraction of *E. sylvestris* leaf crude extract. It is expected that this fraction can be developed as a plant-derived natural product in the future. Our study also indicates that extracts from Korean evergreen woody plants have antimicrobial activity against *S. aureus*.

Table 5 Antimicrobial activities of solvent fractions obtained from leaves of Elaeocarpus sylvestris (18) against *Staphylococcus aureus*

Control/fraction	Concentration (mg/disc)	Inhibition zone (mm)
Methylparaben[a]	0.40	-[b]
	1.00	10.0 ± 0.12 e
	2.00	13.9 ± 0.07 c
	4.00	15.0 ± 0.12 b
Phenoxyethanol[a]	0.40	-
	1.00	-
	2.00	-
	4.00	11.9 ± 0.52 d
Whole extract	2.00	23.3 ± 0.79 a
n-hexane fraction	2.00	-
Chloroform fraction	2.00	13.5 ± 0.23 c
Ethyl acetate fraction	2.00	11.4 ± 0.13 d
n-butanol fraction	2.00	23.1 ± 0.25 a
Residual aqueous fraction	2.00	-

Mean ± S.E. (n = 10). Different lowercase letters indicate a significant difference at $p < 0.05$ based on Duncan's multiple range test.
[a]Positive control.
[b]Not detected.

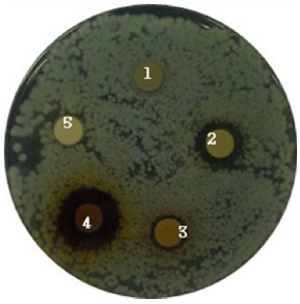

Fig. 2 Antimicrobial activities of different solvent fractions of *Elaeocarpus sylvestris* (18) MeOH extract against *Staphylococcus aureus* represented by inhibition zones. 1, n-hexane; 2, chloroform; 3, ethyl acetate; 4, n-butanol; 5, residual aqueous

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