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Antibacterial activity of bark of *Alnus pendula* against methicillin-resistant *Staphylococcus aureus*

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Abstract. – BACKGROUND AND OBJECTIVES, Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a rapidly growing health problem around the globe. Recently, there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms. In this study we evaluated the antibacterial activity of bark of *Alnus pendula* against MRSA.

MATERIALS AND METHODS, The MIC determination was done using the microdilution broth method and bacterial growth was determined by measuring optical density using spectrophotometer.

RESULTS, *Alnus pendula* bark EtOH extract and fractions (F-1, -2, -3 and -4) were investigated against MRSA. The most active fractions (F-3 and F-4) led to the isolation of oregonin (ORE) and hirsutanone (HIR). These compounds were active against MRSA strains with minimum inhibitory concentrations (MICs) ranging from 31.25 to 250 µg/ml MIC and 2 MIC of HIR completely inhibited the growth of MRSA.

CONCLUSIONS, The bark EtOH extract of *Alnus Pendula* has potent antibacterial activity against MRSA.

Key Words:

Antibacterial activity, MRSA, *Alnus pendula*, Oregonin, Hirsutanone.

Introduction

Staphylococcus (S.) aureus is an important human pathogen that can grow in inappropriately stored food causing a wide variety of diseases in humans either through toxin production or invasion[1]. This fatal pathogen is responsible for a variety of infections like skin and soft tissue infections, surgical and catheter infections, pneumonia, osteoarticular and bloodstream infections, and also for nosocomial infections[2,3]. The emergence of antibiotic resistant strains of *S. aureus* with infection outbreaks among hospitalized patients is a serious problem worldwide[4,5]. *Staphylococcus aureus* acquires methicillin resistance by the acquisition of the mecA gene that encodes a penicillin binding protein (PBP2a) with a low affinity for beta-lactams, usually carried on a larger piece of DNA called a staphylococcal cassette chromosome (SCCmec)[6]. Methicillin was introduced in Europe in 1959 and in the United States in 1961, and the initial cases of MRSA were reported in the United Kingdom in 1961, thereafter followed by reports in the world[7] that infections caused by MRSA are increasing in both hospital and community settings[8]. Consequently, there has been considerable interest in discovering and developing new anti-MRSA agents for potential therapeutic application[8]. Recently, there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms and many compounds of plant products have been shown to be specifically targeted against resistant pathogenic bacteria[9,10].

Alnus pendula belongs to Betulaceae and can be used as a water-erosion-control plant[11]. *Alnus*

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species grows well in Korea and the barks of these species have been used in folk oriental medicine as remedies for diarrhea, fever, alcoholism and hemorrhage[12]. *Alnus* species have various biological properties including antioxidative[13], anti-inflammatory[14] and anti-influenza[15]. Compounds isolated from *Alnus* plants include numerous diarylheptanoids along with triterpenoids and several biological activities of the diarylheptanoids, which are characteristic components of the *Alnus* species, were reported[12,13,16]. Diarylheptanoids have various biological properties including anticancer[17], anti-inflammatory[14], anti-influenza[15] and antioxidative avtivity[18]. In the present study, the bark EtOH extract of *Alnus pendula* showed strong antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA). The fractionation of the most active extracts F-3 and F-4 led to the isolation of oregonin and hirsutanone (Figure 1). These compounds were thereafter tested for their potential antimicrobial activity against two strains of *Staphylococcus aureus* and 16 other clinical isolates from the Wonkwang University Hospital. This study shows the isolation and antimicrobial activity of active compounds from the bark of *Alnus pendula* against MRSA.

Materials and Methods

Isolation of Compounds from Alnus pendula bark by EtOH Extraction

The dried and powdered bark (300 g) of *Alnus pendula* was extracted using 80% edible EtOH at room temperature for 3 days. After removing edible EtOH, the aqueous solution (46.73 g) and non-aqueous solution (28.65 g) was filtered. The filtrate (46.73 g) was concentrated and then applied to a column of Sephadex LH-20 (10-25 μm, GE Healthcare Bio-Science AB, Uppsala, Sweden) containing increasing proportion of MeOH (80→100%) giving 4 fractions (F-1, F-2, F-3 and F-4) after elution. Elution of F-3 containing an increasing proportion of MeOH (30→80%) afforded 3 fractions (F-3-1, F3-2, and F3-3). Repeated column chromatography of fraction F3-2 on the MCI-gel CHP 20P (75-150 μm, Mitsubishi Chemical, Tokyo, Japan) with a gradient solvent system of 30-100% MeOH led to compound 1 (13.26 g).

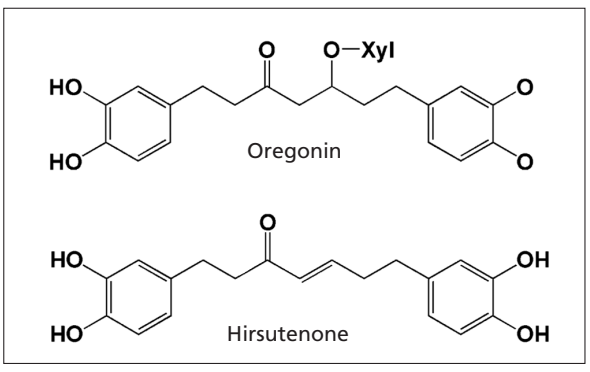

Figure 1. The chemical structure of oregonin (ORE) and hirsutenone (HIR).

The second fraction showing the highest activity (F-4) was applied on reversed-phase medium pressure liquid chromatography (MPLC) using Waters 650 system controller (Waters, Milford, MA, USA) equipped with a Gilson 110UV/VIS detector (Gilson Inc., Middleton, WI, USA) carried out on Daisogel (SP-120-40/60-ODS-B, Daiso Co. Ltd, Osaka, Japan) MPLC with MeOH (80-100%). The process afforded 4 fractions (F4-1, F4-2, F4-3 and F4-4). Repeated column chromatography of fraction F4-3 on the MCI-gel CHP 20P with a gradient solvent system of 80-100% MeOH yielded compound 1 (2.74 g) and compound 2 (0.23 g). Finally, the Fraction 4-4 was applied to a column filled with Sephadex LH-20 containing increasing proportion of MeOH (80→100%) afforded compound 2 (0.02 g).

The two compounds were thereafter structurally elucidated using Nulcear Magnetic Resonance spectroscopy (NMR).

Oregonin (compound 1), Brown amorphous powder, Negative FAB-MS m/z: 477 $[M-H]^-$, ^{1}H-NMR (600 MHz, DMSO-d_6+D_2O): δ 6.74-6.71 (4H in total, H-2',2''‘,5',5''), 6.53-6.50 (2H in total, m, H-6'',6'), 4.31 (1H, d, J =7.8 Hz, xyl-1), 4.14 (1H, m, H-5), 3.86 (1H, dd, J = 11.4, 6.1 Hz xyl-5e), 3.54 (1H, m, xyl-4), 2.83-2.52 (8H in total, H-1,2,4,7), 1.80-1.76 (2H in total, m, H-6), ^{13}C-NMR (125 MHz, DMSO-d_6+D_2O): 210.6 (C-3), 144.0-145.9 (C-4',4'',3',3''), 120.5-120.4 (C-6'',6'), 116.1-116.5 (C-2',2'',5',5''), 104.0 (xyl-1), 76.1 (C-5), 70.8 (xyl-4), 66.6 (xyl-5e), 38.3 (C-6), 48.2-29.7 (C-1,2,4,7), ^{1}H-NMR and ^{13}C-NMR data[16,19,20].

Hirsutenone (compound 2), Brown oil, Negative EI-MS m/z: 328 $[M]^+$, ^{1}H-NMR (Acetone-d6, 300 MHz): δ 6.92-6.85 (1H in total, m, H-5), 6.78-6.74 (4H in total, m, H-2',2'',5',5''), 6.57-6.53 (2H in total, m, H-6',6''), 6.11 (1H, d, J = 16.0 Hz, H-4), 2.84-2.45 (8H in total, m, H-1,2,6,7),^{13}C-NMR (125 MHz, DMSO-d_6+D_2O):

210.3 (C-3), 147.5 (C-5), 143.9-145.6 (C-4',4'',3',3''), 120.3-120.2 (C-6'',6'), 116.1-115.9 (C-2',2'',5',5''), 34 (C-6), 29.9-131.1 (C-1,2,4,7), ^{1}H-NMR and ^{13}C-NMR data[16,19,20].

Bacterial Strains and Growth Conditions

Among the 16 *S. aureus* strains used in this study, 14 clinical isolates (MRSA) were obtained from 14 different patients at Wonkwang University Hospital (Iksan, South Korea). The other 2 strains were *Staphylococcus aureus* ATCC 33591 (methicillin-resistant strain) and *Staphylococcus aureus* ATCC 25923 (methicillin-susceptible strain). ATCC 25923 (American Type Culture Collection, Manassas, VA, USA) and ATCC 33591 were commercially purchased. Before use, all bacteria were stored in 30% glycerol and frozen at –70°C. The bacteria were cultured in Mueller-Hinton broth (MHB) and Mueller-Hinton agar (MHA) (Difco Laboratories, Baltimore, MD, USA). The bacteria were suspended in MHB and then incubated at 37°C for 24 h. MSSA and MRSA strains were selected as test microorganisms because for 2 decades the therapeutics options have been very limited. In the case of MRSA, it is resistant not only to β-lactams but to other types of antibiotics as well.

Determination of the MecA Gene

Detection of the mecA gene in the MRSA strains was performed by PCR (Polymerase chain reaction) amplification. Prior to the DNA extraction, frozen bacteria were subcultured twice on Muller Hinton agar plates (MHA plates). For rapid extraction, one to five bacterial colonies were suspended in 300 µl of cell lysis buffer and heated at 100°C for 20 minutes. After centrifugation at 12,000 RPM for 10 minutes, 2 µl of the supernatant was used for the DNA extraction. PCR reactions were performed using a MRSA Primer Mix Kit (Genotek Co, Daejeon). The PCR amplification consisted of 30 cycles (94°C, 60 sec; 55°C, 60 sec; 72°C, 60 sec). The PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide. We have previously reported the sequences of these primers[21].

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth dilution method[21]. Briefly, a preparation of the microorganisms inocula was done on 24 h broth cultures and the suspensions were adjusted to a 0.5 McFarland standard turbidity (approximately 10^8 CFU/ml). Final inoculums were adjusted to the 10^4 CFU/ml. The MHB was supplemented with serial ampicillin and *Alnus pendula* bark EtOH extract and fractions (F-1, F-2, F-3 and F-4), ORE and HIR concentrations. The MIC was defined as the lowest concentration in which there is no visible growth after 24 h of incubation at 37°C.

Measurement of Bacterial Growth

The test strains were grown in MHB at 37°C. Overnight cultures were diluted with MHB and the bacteria [approximately 1×10^5 CFU (colony forming unit)] were grown at 37°C in 1 ml of the fresh MHB, to which was added 2 MIC, MIC and 1/2 MIC of HIR. The bacterial growth was determined by measuring optical density after 24 h (up to 20 h) at 600 nm using spectrophotometer (OD 600_{nm}). The inhibition of bacterial growth was indicated by the decreases in $OD600_{nm}$. $OD600_{nm}$ of 1.5 corresponds to approximately 10^{12} CFU/ml of medium and $OD600_{nm}$ of 0 to 0.35 corresponds to no growth. The numbers of viable cells were determined on an antibiotic-free MHA plate after 24 h incubation. Colony counts were performed on plates, yielding 30-300 colonies. The bacterial growth inhibition experiments were performed at least thrice for confirmation of the result; the data presented are mean ± standard deviation[22].

Statistical Analysis

All the experiments were performed in triplicates. The MIC data for each microorganism were analyzed using one-way analysis of variance (ANOVA) and differences among group means were analyzed using the Dunnett's multiple comparisons test. p value < 0.05 was considered as significant[23].

Results

Antimicrobial Susceptibility

The aim of present study was to evaluate the antibacterial effects of *Alnus pendula* bark EtOH extracts against the MSSA and MRSA strains. *Alnus pendula* EtOH extracts as shown in Table I has a MIC ranging from 125 to 250 µg/ml. The *Alnus pendula* EtOH bark extract were then fractioned into fractions (F-1, -2, -3 and -4). The extracts along with ampicillin were used for MIC determination using the microdilution broth method. The results were recorded as MIC in

Table I. The minimum inhibitory concentrations (MIC)s of *Alnus pendula* EtOH extract (AJM) and ampicillin (AMP) against *S. aureus strains*.

Strain	MIC (μg/ml)	
	AJM	AMP
S. aureus ATCC 25923 (MSSA)	125	0.12
S. aureus ATCC 33591 (MRSA)	250	125
Clinical isolates		
DPS 1 (MRSA)[a]	250	125
DPS 2 (MRSA)	250	125

[a]DPS: indicates staphylococcal strains from the Department of Plastic Surgery, Wonkwang University Hospital.

Table II. Among these fractions, F-3 and F-4 present the best antibacterial effects for the all strains of *Staphylococcus aureus* (Table II). This is an encouraging result in regards to the ability of MRSA to be resistant to most antibiotics. The two fractions (F-3, -4) subsequently led to the isolation of oregonin (ORE) and hirsutanone (HIR) illustrated in Figure 1. The ORE and HIR compounds were later tested against different strains of *S. aureus* as shown in Table III. From the fraction (F-3 and -4), ORE and HIR appear to be the sole isolated compounds showing antibacterial activity with MICs ranging from 31.25 to 125 μg/ml.

Effects of Hirsutanone (HIR) on Bacterial Growth

HIR greatly decreased the growth rate of ATCC 33591 (MRSA), ATCC 25923 (MSSA) and 1 strain of clinical isolates (DPS-1). Figure 2 shows that 1/2 MIC had weak effect on the growth rate but that the simultaneous addition of MIC and 2 MIC of HIR completely inhibited the growth of ATCC 25923 (MSSA), ATCC 33591 (MRSA) and DPS-1. HIR decreased the growth rate in all strains of MRSA examined. This clearly demonstrates that MRSA can be completely inhibited at MIC and 2 MIC by HIR.

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem in human medicine that may have catastrophic effects. Therefore, there is an urgent need to develop new drugs. Recently, there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms[9,10]. Here, we tested antibacterial activity of *Alnus Pendula* against MSSA, MRSA and 14 of clinical bacteria. The bark of *Alnus Pendula* has never been tested for antimicrobial activity against MRSA. Its EtOH extracts as shown in Table I has a MIC ranging from 125 to 250 μg/ml. The *Alnus Pendula* EtOH bark extract were then fractioned into fractions (F-1, F-2, F-3 and -4). Among these fractions, F-3 and F-4 present the best antibacterial effects for the all strains of MRSA. This is an encouraging result in regards to the ability of MRSA to be resistant to most antibiotics. The two fractions (F-3, -4) subsequently led to the isolation of ORE and HIR illustrated in Figure 1. The ORE and HIR compounds were later tested against different strains of MRSA as shown in Table III. From the fraction (F-3 and -4), ORE and HIR appear to be the sole isolated compounds showing antibacterial activity with MICs ranging from 31.25 to 125 μg/ml. This shows HIR as a more potent antimicrobial than

Table II. Antimicrobial activity of *Alnus pendula* EtOH extract and fractions -1 (F-1), fractions -2 (F-2), fractions -3 (F-3), fractions -4 (F-4) and ampicillin (AMP) against *S. aureus strains*.

Strain	MIC (μg/ml)				
	F-1	F-2	F-3	F-4	AMP
S. aureus ATCC 25923 (MSSA)	2000	500	250	125	0.12
S. aureus ATCC 33591 (MRSA)	2000	500	250	125	125
Clinical isolates					
DPS 1 (MRSA)[a]	> 2000	500	250	250	125
DPS 2 (MRSA)	> 2000	500	250	250	125

[a]DPS: indicates staphylococcal strains from the Department of Plastic Surgery, Wonkwang University Hospital.

Table III. Antimicrobial activity of oregonin (ORE), hirsutanone (HIR) isolated from *Alnus pendula* EtOH extract and ampicillin (AMP) against *S. aureus strain*.

Strain	mecA gene	MIC (μg/ml)		
		ORE	HIR	AMP
S.aureus ATCC 25923 (MSSA)	–	250	62.5	0.12
S.aureus ATCC 33591 (MRSA)	+	250	62.5	125
Clinical isolates				
DPS 1 (MRSA)[a]	+	250	125	125
DPS 2 (MRSA)	+	250	62.5	125
DPS 3 (MRSA)	+	125	62.5	250
DPS 4 (MRSA)	+	125	62.5	125
DPS 5 (MRSA)	+	125	125	62.5
DPS 6 (MRSA)	+	62.5	62.5	62.5
DPS 7 (MRSA)	+	62.5	62.5	31.25
DPS 8 (MRSA)	+	62.5	31.25	31.25
DPS 9 (MRSA)	+	62.5	31.25	31.25
DPS 10 (MRSA)	+	62.5	31.25	31.25
DPS 11 (MRSA)	+	62.5	31.25	31.25
DPS 12 (MRSA)	+	125	125	31.25
DPS 13 (MRSA)	+	62.5	62.5	31.25
DPS 14 (MRSA)	+	62.5	62.5	31.25

[a]DPS: indicates staphylococcal strains from the Department of Plastic Surgery, Wonkwang University Hospital.

ORE and thus the most active compound by its complete inhibition of the growth of all the test microorganisms. HIR greatly decreased the growth rate of ATCC 33591 (MRSA), ATCC 25923 (MSSA) and 1 strain of clinical isolates (DPS-1). Figure 2 shows that 1/2 MIC had weak effect on the growth rate but that the simultaneous addition of MIC and 2 MIC of HIR completely inhibited the growth of ATCC 25923 (MSSA), ATCC 33591 (MRSA) and DPS-1. HIR decreased the growth rate in all strains of MRSA examined. This clearly demonstrates that MRSA can be completely inhibited at MIC and 2MC by HIR. Furthermore, biologically active diarylheptanoids such as ORE and HIR are isolated from Alnus spices[13,24] and *Pinus flexilis*[25]. It has been shown that ORE and HIR have antioxidant[13], anti-tumor[26], anti-inflammatory activities[27]. This is the first study describing the antibacterial activity of *Alnus pendula* bark against MRSA. The results of the present investigation demonstrated that the EtOH extract of *Alnus pendula* and its component, HIR and ORE exert potent inhibitory effects on MRSA. This compound also evidenced a significant inhibitory effect against MRSA. These results indicate that HIR from *Alnus pendula* may be a good candidate as an inhibitor of MRSA. While the antimicrobial mechanism action is not yet well understood, HIR appears to be a very good natural product that can be used against multi drug resistant bacteria in the likeness MRSA awaiting further testing. Furthermore, we plan to conduct additional researches into the mechanisms underlying the inhibitory properties and to study for *in vivo* activity, toxicity, and bioavailability of this compound and EtOH extract of *Alnus pendula* bark.

Conclusions

Alnus Pendula bark EtOH extract has potent antibacterial activity against MRSA. Because *Alnus Pendula* has been used as a traditional medicine in Korea, the EtOH extract of *Alnus Pendula* bark as well as its active components could have great potential for the MSSA and MRSA. HIR and ORE markedly lowered the MICs of ampicillin against the MSSA and MRSA. Namely, the results of this study are promising and may enhance the use of natural products instead of antibiotics.

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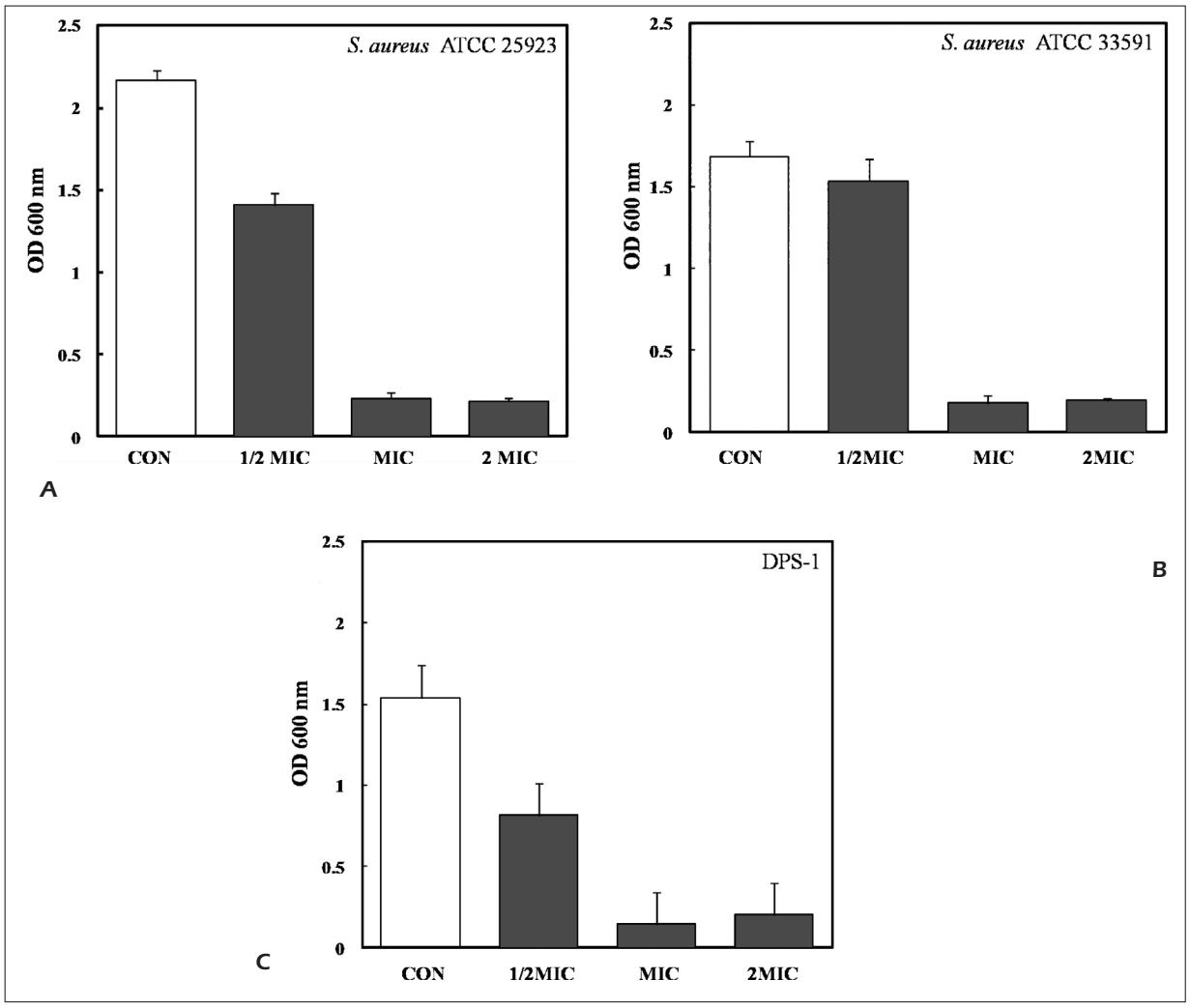

Figure 2. Diversity of inhibition of growth by hirsutanone in S.aureus ATCC 25923 ***(A)***, *S. aureus* ATCC 33591 ***(B)*** and DPS-1 ***(C)***. Bacteria were cultured at 37°C in MHB to which was added 1/2MIC, MIC and 2MIC of hirsutanone (HIR). The bacterial growth was monitored with a spectrophotometer every hour for 24 hr. The control experiment was performed by using culture medium without hirsutanone. The results represent means of triplicate determinations undertaken on two separate occasions.

References

1) Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev1997; 10: 505-520.

2) Baltch AL, Ritz WJ, Bopp LH, Michelse PB, Smith RP. Antimicrobial activities of daptomycin, vancomycin, and oxacillin in human monocytes and of daptomycin in combination with gentamicin and/or rifampin in human monocytes and in broth against *Staphylococcus aureus*. Antimicrob Agents Chemother 2007; 51: 1559-1562.

3) Denis O, Deplano A, Nonhoff C, Hallin M, De RR, Vanhoof R, De MR, Struelens MJ. *In vitro* Activities of Ceftobiprole, Tigecycline, Daptomycin, and 19 Other Antimicrobials against Methicillin-Resistant *Staphylococcus aureus* Strains from a National Survey of Belgian Hospitals. Antimicrob Agents Chemother 2006; 50: 2680-2685.

4) Geha DJ, Uhl JR, Gustaferro CA, Persing DH. Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. J Clin Microbiol 1994; 32:1768-1772.

5) Mastoraki A, Kriaras I, Douka E, Mastoraki S, Stravopodis G, Geroulanos S. Methicillin-resistant *Staphylococcus aureus* preventing strategy in cardiac surgery. Interact. Cardiovasc Thorac Surg 2008; 7: 452-456.

6) Hartman BJ, Tomasz A. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 1986; 29: 85-92.

7) Rice LB. Antimicrobial resistance in gram-positive bacteria. Am J Infect Control 2006; 34: 11-19.

8) Chopra I. Antibiotic resistance in *Staphylococcus aureus*: concerns, causes and cures. Expert Rev Anti Infect Ther 2003; 1: 45-55.

9) Aqil F, Khan MS, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. J Basic Microbiol 2005; 45: 106-114.

10) Choi JG, Kang OH, Obiang-Obounou B, Lee YS, Chae HS, Oh YC, Sohn DH, Park H, Choi HG, Kim SG, Shin DW, Kwon DY. Antibacterial activity of Ecklonia cava against methilillin-resistant *Staphylococcus aureus* and Salmonella spp. Foodborne Pathog Dis 2010; 7: 435-441.

11) Suga T, Iwata N, Asakawa Y. Chemical Constituents of the Male Flower of *Alnus pendula* (Betulaceae). Bull Chem Soc Jpn 1972; 45: 2058-2060.

12) Lee SJ. Korea Fold Medicine, Seoul National University Publishing Center Press 1996; p. 40.

13) Kuroyanagi M, Shimomae M, Nagashima Y, Muto N, Okuda T, Kawahara N, Nakane T, Sano T. New diarylheptanoids from Alnus japonica and their antioxidative activity. Chem Pharm Bull 2005; 53: 1519-1523.

14) Kang G, Kong PJ, Yuh SV, Chun W, Kim SS. Curcumin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression by inhibiting activator protein 1 and nuclear factor kappa b bindings in BV2 microglial cells. J Pharmacol Sci 2004; 94: 325-328.

15) Tung NH, Kwon HJ, Kim JH, Ra JC, Ding Y, Kim JA, Kim YH. Anti-influenza diarylheptanoids from the bark of Alnus japonica. Bioorg Med Chem Lett 2010; 20: 1000-1003.

16) Jeong DW, Kim JS, Cho SM, Lee YA, Kim KH, Kim SW, Lee MW. Diarylheptanoids from the stem barks of Alnus hirsuta var. sibirica. Kor J Pharmacog 2000; 31: 28-33.

17) Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 2003; 23: 363-398.

18) Muthenna P, Suryanarayana P, Gunda SK, Petrash JM, Reddy GB. Inhibition of aldose reductase by dietary antioxidant curcumin: mechanism of inhibition, specificity and significance. FEBS Lett 2009; 583: 3637-3642.

19) Kim HJ, Kim KH, Yeom SH, Kim MK, Shim JG, Lim HW, Lee MW. New Diarylheptanoids from the barks of Alnus japonica Steudel. Chin Chem Lett 2005; 16:1337-1340.

20) Lee MW, Park MS, Jeong DW, Kim KH, Kim HH, Toh SH. Diarylheptanoids from the leaves of Alnus hirsuta Turcz. Arch Pharm Res 2000; 23: 50-53.

21) Lee YS, Han SH, Lee SH, Kim YG, Park CB, Kang OH, Keum JH, Kim SB, Mun SH, Shin DW, Kwon DY. Synergistic effect of tetrandrine and ethidium bromide against methicillin-resistant *Staphylococcus aureus* (MRSA). J Toxicol Sci 2011; 36: 645-651.

22) Qiu J, Zhang X, Luo M, Li H, Dong J, Wang J, Leng B, Wang X, Feng H, Ren W, Deng X. Subinhibitory concentrations of perilla oil affect the expression of secreted virulence factor genes in *Staphylococcus aureus*. PLoS One 2011; 6: 16160.

23) Choi JG, Kang OH, Lee YS, Oh YC, Chae HS, Obiang-Obounou B, Park SC, Shin DW, Hwang BY, Kwon DY. Antimicrobial activity of the constituents of Smallanthus sonchifolius leaves against methicillin-resistant *Staphylococcus aureus*. Eur Rev Med Pharmacol Sci 2010; 14: 1005-1009.

24) Lee SL, Huang WJ, Lin WW, Lee SS, Chen CH. Preparation and anti-inflammatory activities of diarylheptanoid and diarylheptylamine analogs. Bioorg Med Chem 2005; 13: 6175-6181.

25) Lee KK, Bahler BD, Hofmann GA, Mattern MR, Johnson RK, Kingston DG. Isolation and structure elucidation of new PKCα inhibitors from Pinus flexilis. J Nat Prod 1998; 61: 1407-1409.

26) Park D, Kim HJ, Jung SY, Yook CS, Jin C, Lee YS. A new diarylheptanoid glycoside from the stem bark of Alnus hirsuta and protective effects of diarylheptanoid derivatives in human HepG2 cells. Chem Pharm Bull 2010; 58: 238-241.

27) Lee CS, Ko HH, Seo SJ, Choi YW, Lee MW, Myung SC, Bang H. Diarylheptanoid hirsutenone prevents tumor necrosis factor-alpha-stimulated production of inflammatory mediators in human keratinocytes through NF-kappaB inhibition. Int Immunopharmacol 2009; 9: 1097-1104.