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Effect of *Saliva miltiorrhiza* Bunge on Antimicrobial Activity and Resistant Gene Regulation against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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This study was conducted in an effort to evaluate the antimicrobial activity and antibiotic-resistant gene regulation from *Saliva miltiorrhiza* Bunge on methicillin-resistant *Staphylococcus aureus* (MRSA). A variety of solvent fractions and methanol extracts of *S. miltiorrhiza* Bunge were tested in order to determine its antimicrobial activities against *S. aureus* and MRSA. As a result, the hexane fraction of *S. miltiorrhiza* Bunge evidenced the highest levels of antimicrobial activity against *S. aureus* and MRSA. The MICs of the hexane fraction against various MRSA specimens were 64<MICs≤128 μg/ml. The hexane fraction evidenced inhibitory effects superior to those of the chloroform fraction. The results showed inhibition zones of hexane (16 mm) and chloroform (14 mm) fractions against MRSA KCCM 40511 at 1,000 μg/disc. The hexane and chloroform fractions inhibited the expression of the resistant genes, *mecA*, *mecRI*, and *femA* in mRNA. Moreover, the results of Western blotting assays indicated that the hexane and chloroform fractions inhibited the expression of the resistant protein, PBP2a. These results reveal that the hexane and chloroform fractions of *S. miltiorrhiza* Bunge may prove to be a valuable choice for studies targeted toward the development of new antimicrobial agents.

Keywords: MRSA, *Salvia miltiorrhiza* Bunge, antimicrobial activity, resistant gene

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a principal cause of nosocomial infectious diseases, and has become a serious problem in hospitals. MRSA infections are quite difficult to cure, owing to the multidrug-resistance properties of MRSA, which is resistant to β-lactams as well as a host of other antibiotics. Due to the emergence of increasing drug resistance, most notably methicillin resistance in staphylococci, much attention has been focused on the search for new antimicrobial agents (Bramley *et al.*, 1989; Hiramatsu *et al.*, 1997).

Vancomycin (VCM) is the most effective antibiotic for MRSA infections, but clinical use often results in unexpected side effects and the development of infections with VCM-resistant enterococci (VRE) (Bailie and Neal, 1988; Berger-Bachi *et al.*, 1992). For these reasons, a substantial amount of attention has been focused on the exploration and utilization of plant extracts (phytochemicals) as an alternative to and/or in combination with traditional antibiotics for the treatment of MRSA infections (Iinuma *et al.*, 1994; Sato *et al.*, 1995; Schouten *et al.*, 2000).

During the course of our studies with medicinal plants, we utilized extracts from *S. miltiorrhiza* Bunge. The dried root of *S. miltiorrhiza* Bunge (Labiatae), referred to as "Danshen" in China, is one of the most popular traditional Chinese medicines. This plant has been employed for the treatment of a variety of diseases, including menstrual disorders, menorrhalgia, insomnia, blood circulation diseases, angina pectoris, and inflammation. The drug has proven particularly valuable in the treatment of coronary heart diseases (Tang and Eisenbrand, 1992). Different portions of the plant have been utilized for a variety of medicinal purposes. For example, the aqueous extracts of the fresh leaves, dried leaves, and dried roots and bark have been employed as an antidote for insecticide and ethyl alcohol poisoning; the dried root has also been utilized as an anti-inflammatory agent and antipyretic (Battaglia *et al.*, 1989; Saletu *et al.*, 1995). It has been recently reported that the extracts of *S. miltiorrhiza* leaves exert a protective effect on ethanol-induced hepatotoxicity, using hepatic lipid peroxidation, blood ethanol concentration, and alcohol dehydrogenase and aldehyde dehydrogenase activity as indicators (Yu *et al.*, 1998). *S. miltiorrhiza* has also been widely utilized for the treatment of vascular diseases, including hypertension (Wu *et al.*, 1998; Chen *et al.*, 2001a, 2001b; Hung *et al.*, 2001; Renet *et al.*, 2002; Ji *et al.*, 2003; Lay *et al.*, 2003).

The principal objective of this study was to determine the interesting effects of the *S. miltiorrhiza* Bunge extract and sub-fraction on *S. aureus* and MRSA, and whether resistant genes of the MRSA strain are affected by the extract and subfractions of *S. miltiorrhiza*.

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Materials and Methods

Extract preparation

S. miltiorrhiza Bunge was purchased from the Yakryeong (Drug) Market (Korea). The dried plant material (2.4 kg) was extracted three times with 80% methanol under reflux for 5 h, and the supernatant was filtered using 10 μm cartridge paper. The filtered extract was then evaporated with a rotary evaporator (Eyela, Japan) under reduced pressure, yielding a viscous methanol extract. The concentrated extract was freeze-dried. This process generally produced 950.6 g of brown powder. After freeze-drying, the powder extract was fractionated with hexane, chloroform, ethylacetate, butanol, and water. A voucher specimen was deposited at the Food Science and Technology Department, Keimyung University, Daegu, Korea.

Microbial strains

The bacterial strains utilized in this study were standard *Staphpylococcus aureus* (ATCC 25923) and methicillin-resistant *Staphylococcus aureus* (KCCM 11812, KCCM 40510, KCCM 40511, and KCCM 40512) strains, obtained from the Korean Culture Center of Microorganisms. All strains were grown aerobically in Tryptic Soy Broth (Difco, USA) for 24 h at 37°C.

Determination of minimum inhibitory concentration

A broth microdilution broth susceptibility assay was conducted, in accordance with NCCLS (1999) guidelines, for the MIC determinations. All tests were performed in Tryptic Soy Broth and the strains were cultured overnight at 37°C. Bacterial suspensions were diluted to match the 0.5 Mac Farland standards (approximately 1.5×10^8 CFU/ml). The extracted compounds were dissolved in dimethyl sulfoxide (DMSO) and 2-fold serial dilutions were prepared, then added to tryptic soy agar plates [1% (v/v); final concentration range of 100-1,000 μg/ml]. The plates were incubated under normal atmospheric conditions for 24 h at 37°C. Bacterial growth was indicated by the presence of a white pellet on the bottom of the tube. The MIC value was defined as the lowest concentration of extracts at which no visible growth could be observed.

Disc diffusion method

The agar disc diffusion method was utilized to evaluate the antimicrobial activities of the *S. miltiorrhiza* Bunge. In brief, a suspension of the tested microorganism (0.1 ml of 10^8 cells/ml) was spread onto solid media plates. Sterilized paper discs (8 mm in diameter) were impregnated with 50 μl of the extract and fractions, then placed on the incubated plates. These plates, after 2 h of maintenance at 4°C, were incubated for 24 h at 37°C. The diameters of the inhibition zones were measured in millimeters. All tests were conducted in duplicate.

Scanning electron microscopy

In order to assess the changes in surface MRSA (KCCM 40511), we visualized the *S. miltiorrhiza* Bunge samples under scanning electron microscopy. The specimens were fixed in 2% phosphate buffered glutaraldehyde, rinsed in 0.1 M phosphate buffer, and post-fixed in 1% buffered osmium tetroxide. The fixed specimens were then dehydrated via a graded series of ethanol. The dried specimens were coated with platinum with a Hitachi E-1010 Ion sputterer (Hitachi Co., Japan) and observed via scanning electron microscopy (Hitachi S-450, Japan) at an accelerating voltage of 20 kV.

Transmission electron microscopy

The bacteria were fixed in glutaraldehyde followed by osmium tetroxide. Thin sections of specimens were post-stained in methanolic uranyl acetate and lead citrate, then observed via transmission electron microscopy (Hitachi H-7600, Japan).

RNA isolation and RT-PCR analysis

Total RNA was isolated from the strains with Trizol reagent (Molecular Research Center, Inc.) in accordance with the manufacturer's specifications. RNA concentrations were estimated via spectrophotometry at 260 nm. 1-5 μg of total RNA plus 0.5 μg of oligo dT was denatured at 70°C for 10 min, then preincubated for 2 min at 42°C after the addition of 10 mM dithioothreitol (DTT), 2.5 mM each of dNTPs, and reaction buffer. Two hundreds units of Superscript II reverse transcriptase were added and incubated for 50 min at 42°C. The reaction was then heated to 70°C for 15 min, and any remaining cRNA was removed via the addition of 2 units RNase H at 37°C for 20 min. Ten percents of the RT products were added to a PCR reaction which included PCR buffer (pH 8.4, 20 mM Tris, 50 mM KCl), 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 2 mM primers, and 5 units Taq DNA polymerase. Twenty-seven PCR cycles were then conducted as follows: denaturation at 95°C, extension at 72°C. Primer sequences were as follows: *mecA* (554 bp, PCR product, annealing temperature: 51.9°C) F; 5'-ATGAGATTAGGC ATCGTTCC-3', R; 5'-TGGATGACAGTACCTGAGCC-3'; *mecI* (268 bp, PCR product, annealing temperature: 49.5°C) F; 5'-CTGCAGAATGGGAAGTTATG-3', R; 5'-ACAAGTGA ATTGAAACCGCC-3'; *mecR1* (235 bp, PCR product, annealing temperature: 53.9°C) F; 5'-AAGCACCGTTACTAT CTGCACA-3', R: 5'-GAGTAAATTTTGGTCGAATGCC-3'; *femA* (372 bp, PCR product, annealing temperature: 52.6°C) F; 5'-CAT GATGGCGAGATTACAGGCC-3', R; 5'-CGCTA AAGGTACTAACACACGG-3'; *nucA* (270 bp, PCR product, annealing temperature: 56.0°C) F; 5'-GCGATTGATGGTGAT ACGGTT-3', R; 5'-AGCCAAGCCTTGACGAACTAAAGC-3'; GAPDH (514 bp, PCR product, annealing temperature: 51.0°C) F; 5'-TGAGAACGGGAAGCTTGTCA-3', R; 5'-GG AAGGCCATGCCAGTGA-3'.

Western blot analysis

MRSA were treated with various concentrations of *S. miltiorrhiza* Bunge extracts and fractions. The bacterial lysates were prepared in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 2 mM ethylenediaminetetraacetic acid (EDTA), 150 mM NaCl, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 mM NaF, 1 mM Na_3PO_4, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT), 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1% NP-40. The cells were then disrupted and extracted for 30 min at 4°C. Following 15 min of centrifugation at 18,928×g, the supernatant was obtained as the cell lysate. Protein concentrations were measured via Bio-Rad protein assay. Aliquots of cellular proteins (30 μg/lane) were then electrophoresed on 10%

SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to Immobilon-P-membranes (Millipore, USA). The membranes were probed with anti-PBP2a (Abcam, UK) and the same membranes were stripped and reprobed with anti-mouse IgG (Cell Signaling, USA). Loading differences were normalized with polyclonal anti-actin antibodies.

Results

Minimal inhibitory concentration of S. miltiorrhiza Bunge extracts and fractions

In order to determine the antimicrobial activity of *S. miltiorrhiza* Bunge against MRSA, we measured the MIC of samples via a medium dilution method. The results of the MRSA tests are shown in Table 1. As a result, the MICs of *S. miltiorrhiza* Bunge methanol extract against various MRSA samples were 1<MICs≤4 mg/ml. The sub-fractions evidenced profound antimicrobial activity against *S. aureus* and MRSA. In particular, the hexane and chloroform fractions evidenced the highest levels of antimicrobial activity against various MRSA, with the exception of the ethyl acetate, butanol, and water fractions. The MICs of the hexane fraction against various MRSA samples were 64<MICs≤128 μg/ml.

Antimicrobial activity of S. miltiorrhiza Bunge extract and fractions

Table 2 shows the antimicrobial activity of *S. miltiorrhiza* Bunge extract and sub-fractions and penicillin as β-lactam antibiotics against *S. aureus* and MRSA. The antimicrobial effects of *S. miltiorrhiza* Bunge were observed at concentrations that corresponded to the MICs (Fig. 1). The hexane (16 mm) and chloroform (14 mm) fractions evidenced the largest inhibition zone against MRSA KCCM 40511 at a concentration of 1,000 μg/disc. The other sub-fractions (ethylacetate, butanol and aqueous fractions) manifested less activity, or none at all.

Table 1. Minimum inhibition concentration of *S. miltiorrhiza* Bunge extract and fractions against *S. aureus* and MRSA

Microorganism		MIC[a]							
		Methanol	Hexane	Chloroform	Ethylacetate	Butanol	Water	DMSO[c]	Penicillin
S. aureus	ATCC 25923	1<MIC≤2	32<MIC≤64	32<MIC≤64	N.A.[b]	N.A.	N.A.	N.A.	4<MIC≤8
MRSA	KCCM 11812	1<MIC≤2	64<MIC≤128	64<MIC≤128	N.A.	N.A.	N.A.	N.A.	16<MIC≤32
	KCCM 40510	1<MIC≤2	64<MIC≤128	64<MIC≤128	N.A.	N.A.	N.A.	N.A.	32<MIC≤64
	KCCM 40511	2<MIC≤4	64<MIC≤128	64<MIC≤128	N.A.	N.A.	N.A.	N.A.	32<MIC≤64
	KCCM 40512	1<MIC≤2	64<MIC≤128	64<MIC≤128	N.A.	N.A.	N.A.	N.A.	32<MIC≤64

[a]MIC, minimum inhibitory concentration; values given as mg/ml for the methanol extract and as μg/ml for various fractions and penicillin
[b]N.A.: not active
[c]DMSO: 2% of DMSO was used as an control solvent

Table 2. Results of the antimicrobial tests of the investigated *S. miltiorrhiza* Bunge extract and fractions in disc diffusion assay

Extract	Concn.	*S. aureus* ATCC 25923	MRSA KCCM 11812	MRSA KCCM 40510	MRSA KCCM 40511	MRSA KCCM 40512
Methanol	2.5 mg/disc	18[a]	13	10	N.D.[b]	10
	5 mg/disc	20	18	12	11	12
	10 mg/disc	25	20	16	13	14
Hexane	250 μg/disc	13	10	10	N.D.	10
	500 μg/disc	15	14	11	12	13
	1000 μg/disc	19	16	15	16	17
Chloroform	250 μg/disc	11	10	10	N.D.	9
	500 μg/disc	14	12	11	11	12
	1000 μg/disc	18	14	13	14	16
Ethylacetate	1000 μg/disc	N.D.	N.D.	N.D.	N.D.	N.D.
Butanol	1000 μg/disc	N.D.	N.D.	N.D.	N.D.	N.D.
Water	1000 μg/disc	N.D.	N.D.	N.D.	N.D.	N.D.
Penicillin	62.5 μg/disc	18	14	11	13	14
	125 μg/disc	23	19	16	16	17
	250 μg/disc	32	28	24	23	24

[a]Inhibition zones including the diameter of the paper disc (8 mm)
[b]N.D.: Not detected

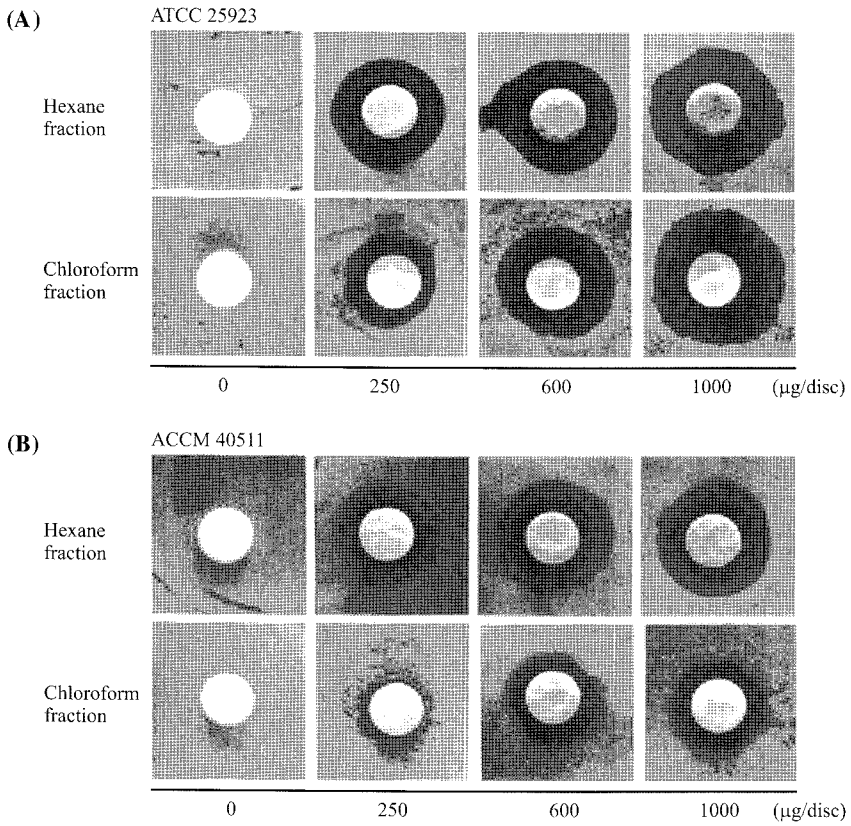

Fig. 1. Antimicrobial effect of *S. miltiorrhiza* Bunge via disc diffusion assay. *S. aureus* (A) and MRSA KCCM 40511 (B) treated with the indicated concentrations of the hexane and chloroform fractions.

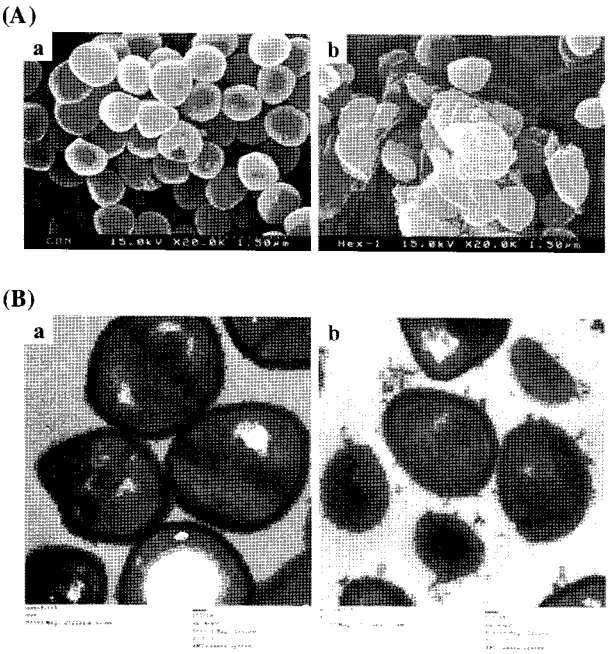

Fig. 2. Antimicrobial effects of *S. miltiorrhiza* Bunge hexane fraction on methicillin resistant *S. aureus* KCCM 40510 [×30,000 magnification; (A) SEM, (B) TEM]. White (a) control, White (b) treated with hexane fraction (25 μg/ml), Black (a) control, Black (b) treated with hexane fraction (25 μg/ml).

Morphology changes against MRSA treated with S. miltiorrhiza Bunge fraction

To observe the morphological changes of MRSA after treatment with the hexane fraction, SEM and TEM were used to visualize MRSA KCCM 40511 at a concentration of 25 μg/ml. The cell walls of MRSA were destroyed as the result of an inhibition of PBP2a synthesis. We were able to visualize the damaged bacteria, which evidenced shrinkage (Fig. 2).

Detection and inhibition of resistant gene against MRSA

Fig. 3 shows the result of resistant expression for MRSA using RT-PCR (Reverse transcriptase-polymerase chain reaction). We were unable to confirm the presence of the *mecA*, *mecI*, *mecR1*, and *femA* genes in the Standard ATCC 25923, as well as MRSA (KCCM 11812, 40510, 40512), bbut we clearly detected them in KCCM 40511. As can be observed in Figs. 3(A) and 3(B), the expression of the *mecA* gene was inhibited in a dose-dependent manner, and *mecR1* was also dose-dependently inhibited by the hexane and chloroform fractions. Also, *femA,* which is known to be related to cell wall composition, was demonstrated to have an inhibitory effect on expression. On the other hand, the *nucA* (nuclease A) gene is expressed not only in *S. aureus,* but also in MRSA. This *nucA* gene was employed in our comparison of resistant gene expression patterns. The *S. aureus* micrococcal *nucA* is a small (~18 kDa), stable, extracellular enzyme, which is secreted as a target protein

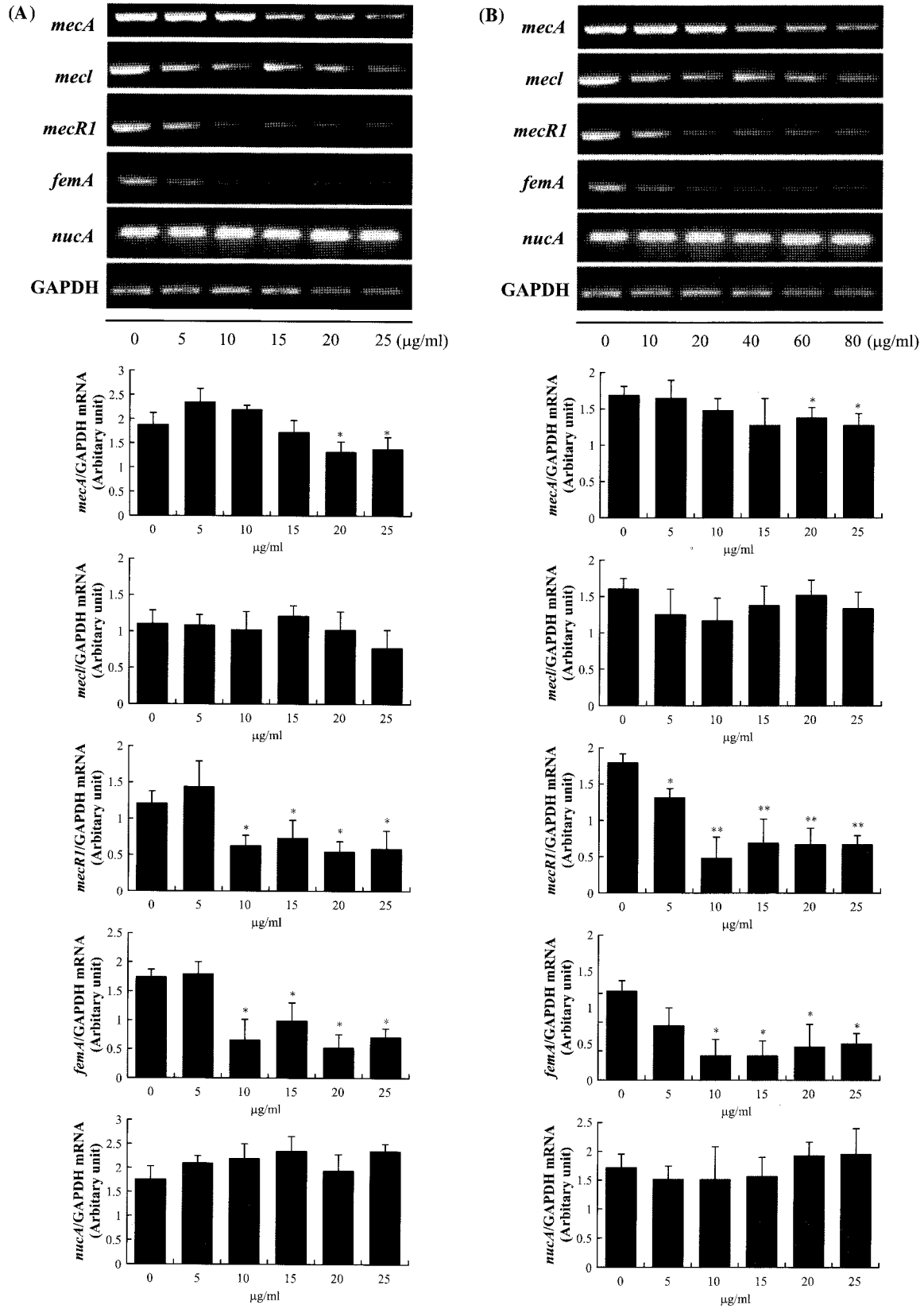

Fig. 3. mRNA expression of the amplified fragments *mecA, mecI, mecR1, femA,* and *nucA* in MRSA KCCM 40511 strains. MRSA KCCM 40511 treated with the indicated concentrations of the hexane (A) and chloroform (B) fractions. mRNA expression levels were described in terms of intensity using an image analyzer. GAPDH was employed as an internal control. Each bar represents the Mean±S.D. *$P<0.05$ vs control. **$P<0.001$ vs control.

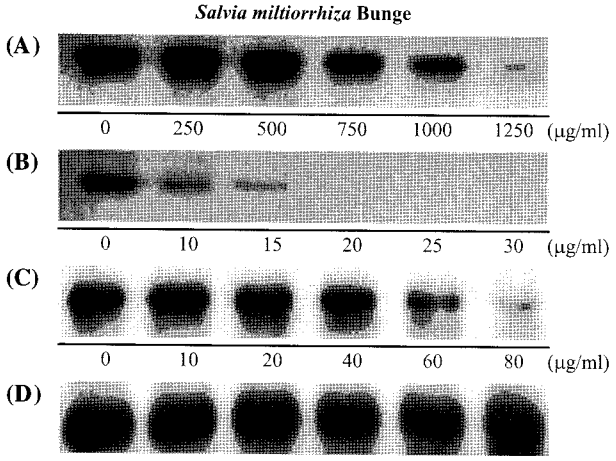

Fig. 4. Effect of *S. miltiorrhiza* Bunge on the expression of PBP2a protein against MRSA strains. The MRSA KCCM 40511 treated with the indicated concentration of methanol extract, as well as the hexane and chloroform fractions. The samples in each of the lanes are as follows: (A), methanol extract; (B), hexane fraction; (C), chloroform fraction; (D), anti-actin. Anti-actin was used as an internal control.

in *S. aureus* (Dutton *et al.*, 2000; Myscofski *et al.*, 2001). As a result, the hexane and chloroform fractions did not attenuate the expression of *nucA*. This result was caused by the *S. miltiorrhiza* Bunge fraction-induced reduction in methicillin resistance coupled with the inhibition of *mecA* gene expression. Moreover, the results of Western blotting assays indicated that the hexane and chloroform fractions inhibited the expression level of the resistant protein, PBP2a (Fig. 4).

Discussion

Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility, and approximately 80% of the rural population of Korea depends on herbal medicine as a primary health care modality. Over the years, the World Health Organization has advocated that countries should interact with traditional medicine with a view toward the identification and exploitation of different aspects of traditional medicine protocols, specifically those that effect safe and effective remedies for ailments of both microbial and non-microbial origin (WHO, 1978).

Danshen is an annual sage plant, known in the West as *S. miltiorrhiza* Bunge. The plant grows indigenously in Korea, China, Japan, and Mongolia. In China, it is located primarily in hilly regions of the west, southwest, and southeast. *S. miltiorrhiza* is among the most popular of the medicinal herbs used in China. It has been utilized as a treatment for stroke since 1970 (Wu *et al.*, 2005), and is also used for the treatment of angina and heart attacks, as an antihypertensive and a sedative (Ji *et al.*, 2000). *S. miltiorrhiza* harbors several pharmacologically active compounds, most notably the diterpenoids known as tanshinones. A related plant, *S. columbariae*, from California, USA also harbors tanshinones, especially cryptotanshinone (Adams *et al.*, 2005).

S. miltiorrhiza has other effects relevant to stroke, including anti-inflammatory, free radical-scavenging, antioxidant, and mitochondrial protection effects. Tanshinone I from *S. miltiorrhiza* has been shown to inhibit arachidonic acid metabolism and the production of interleukin-12, and also evidences anti-inflammatory effects (Kang *et al.*, 2000; Kim *et al.*, 2002). Neutrophil activation is inhibited by an unspecified tanshinone (Li and Tang, 1991) which is also known to function as an anti-inflammatory agent. *S. miltiorrhiza* antioxidant activity is also expressed as increases in superoxide dismutase, catalase, glutathione peroxidase, and glutathione transferase activities (Ji *et al.*, 2003; Sun *et al.*, 2005). Free radical scavenging and mitochondrial protective activities have also been associated with *S. miltiorrhiza* (Cao *et al.*, 1996; Wang *et al.*, 2003; Zhou *et al.*, 2003).

S. miltiorrhiza preparations contain a host of pharmacologically active compounds. Among all of the active compounds harbored by the plant, diterpenoids and salvianolic acid derivatives are the best studied thus far. The principal diterpenoids of *S. miltiorrhiza* are tanshinones and isotanshinone. Miltirone, salviol, and other diterpenoids have also been identified in the plant. Miltirone has sedative activity and is a benzodiazepine receptor agonist (Chang *et al.*, 1991). Purified tanshinone IIA and IIB are neuroprotective in cerebral ischemia and reperfusion (Lam *et al.*, 2003). Tanshinone I, cryptotanshinone, and tanshinone V exhibit protective effects against myocardial ischemia and reperfusion (Ji *et al.*, 2000). Tanshinones also function as anti-inflammatory agents. Tanshione I, dihydrotanshinone, and cryptotanshinone inhibit the production of interleukin-12 and interferon-gamma (Kang *et al.*, 2000). Tanshinone I inhibits arachidonic acid metabolism by phospholipase A_2 (Kim *et al.*, 2002).

Our results indicate that the polar fractions (ethylacetate, butanol, and water) evidenced no antimicrobial activity, but the methanol extract and the non-polar hexane and chloroform fractions exhibited profound activity against *S. aureus*. Park *et al.* (2001) previously evaluated the antimicrobial activity of an extract of *Rubus coreanus*. The growth inhibition zone was determined as 16.6 mm at a concentration of 10 mg/disc. Nostro *et al.* (2001) determined the MICs of *Helichrysum italicum* extract against MRSA. The values ranged from 125 to 500 μg/ml. The methanol extract of *S. miltiorrhiza* Bunge evidenced antimicrobial activity at a lower density than was observed with *Rubus coreanus*. In particular, the hexane fraction of *S. miltiorrhiza* Bunge evidenced the most profound antimicrobial activity against not only *S. aureus,* but also MRSA, that was seen in other sub-fractions. Its nonpolar extract contains tanshinones, which may protect the myocardium against ischemia-induced damage. The crude extract of *S. miltiorrhiza*, in the form of phenolic compounds, has been shown to be effective in protecting liver microsomes, hepatocytes, and erythrocytes against oxidative damage (Liu *et al.*, 2001; Li *et al.*, 2002). Therefore, several active components, including tanshinones, D(+)3,4-dihydroxyphenol lactic acid, protocatechuic aldehyde, salvianolic acid (A, B, C, D, E, F) and rosmarinic acid (Wang *et al.*, 1991; Li, 1997) have been isolated and identified thus far.

According to a previous report, the principal constituents of the root of *S. miltiorrhiza* Bunge are abietane-type diterpene pigments, the so-called tanshinones, all of which possess *ortho-* or *para*-naphthoquinone chromophores (Ryu *et al.*, 1997).

These are unique components found in the *Salvia* genus, and have been associated with a variety of biological activities (Wang *et al.*, 1989; Chang *et al.*, 1990; Sato *et al.*, 1992). In this fashion, the most profound activities of the hexane and chloroform fractions could also be attributed to the presence of several types of polyphenols belonging to different classes, including tanshinones in the hexane and chloroform fractions. These results indicate that the hexane and chloroform fractions of *S. miltiorrhiza* Bunge may represent a valuable choice for studies intended to detect the development of new antimicrobial activities.

However, the mechanism underlying methicillin resistance appears to be quite complex, and has yet to be thoroughly elucidated; however, it is believed to involve the overproduction of β-lactamase (Sabath, 1982), and the expression of *mecA,* which generates penicillin-binding protein (PBP) 2a, which has a low affinity for β-lactam antibiotics, as well as a change in PBP type (Hiramatsu *et al.*, 2001; Bertrand *et al.*, 2005). In a previous study, factors other than the expression of the *mecA* gene were detected. These factors appear to control the induction of PBP 2a production in *S. aureus* E 67-0, and are collectively termed as *mecR*. *mecR* has been shown to be present in some variants of *Staphylococcus aureus*. It suppresses the synthesis of PBP 2a present in a certain genetic background, and mediates influences on the expression of methicillin resistance. This *mecR* has been identified as two different genes, *mecI* and *mecR1,* both of which are located within the upstream region of the *mecA* gene. These genes were previously cloned and sequenced by Zhang *et al.* (2001), and it was also explained that *mecI* encodes for the MecI suppression protein. However, *mecR1* encodes for the inducing protein, MecR1, which is required for the induction of PBP 2a production. Upon contact with β-lactam, which is equivalent to an MRSA inducing factor, *mecR1* is activated, and its signal binds to the promoter region of *mecA* and is transduced to *mecI,* thereby suppressing transcription. In this manner, suppression is cleared. In addition, Berger-Bachi *et al.* (1989) reported that the *femA* gene also performs a crucial role in this process. The action mechanism of the *femA* gene remains to be fully elucidated at present; nevertheless, it is believed that it plays not part in PBP 2a synthesis. Rather, it appears to be involved in the synthesis of cell wall components of *S. aureus*, and also mediates drug sensitivity, and thus in involved in methicillin resistance.

As a result, the hexane and chloroform fractions inhibited the expression of the resistant genes *mecA, mecR1,* and *femA* in a dose-dependent manner. Moreover, the results of Western blotting assays indicated that the hexane fractions inhibited the expression levels of the resistant protein, PBP2a, at a density of 10 μg/ml. As far as could be ascertained in our literature survey, the active compounds implicated herein have proven effective in other studies. However, the majority of the reports published thus far have addressed the antimicrobial activity of these compounds only, and did not address the capacity of the extract and fractions to regulate resistance properties.

In conclusion, we determined herein that the hexane and chloroform fractions of *S. miltiorrhiza* Bunge evidenced profound antimicrobial activity, and inhibited resistant gene expression against *S. aureus* and MRSA. Therefore, with more detailed research into the identification, action mechanisms, and toxicity of active compounds, the hexane and chloroform fractions from *S. miltiorrhiza* Bunge are expected to be recognized as natural sources for the development of new functional foods, and will also comprise a component of the fight against MRSA.

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