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Antibacterial Activity of *Curcuma longa* L. against Methicillin-resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has been emerging worldwide as one of the most important hospital and community pathogens. Therefore, new agents are needed to treat MRSA associated infections. The present study investigated the antimicrobial activity of ethyl acetate, methanol and water extracts of *Curcuma longa* L. (*C. longa*) against MRSA. The ethyl acetate extract of *C. longa* demonstrated a higher antibacterial activity than the methanol extract or water extract. Since the ethyl acetate extract was more active than the other extracts, the study examined whether the ethyl acetate extract could restore the antibacterial activity of β-lactams and alter the MRSA invasion of human mucosal fibroblasts (HMFs). In the checkerboard test, the ethyl acetate extract of *C. longa* markedly lowered the MICs of ampicillin and oxacillin against MRSA. In the bacterial invasion assay, MRSA intracellular invasion was significantly decreased in the presence of 0.125–2 mg/mL of *C. longa* extract compared with the control group. These results suggest that the ethyl acetate extract of *C. longa* may have antibacterial activity and the potential to restore the effectiveness of β-lactams against MRSA, and inhibit the MRSA invasion of HMFs. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: Curcuma longa L.; antibacterial activity; methicillin-resistant *Staphylococcus aureus.*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most important pathogens that causes suppuration, abscess formation, a variety of pyogenic infections and even fatal septicaemia in human beings. *S. aureus* was associated with 80% mortality in the preantibiotic era, and then was proved to be susceptible to the earliest antimicrobial substances such as penicillin (You *et al*., 1999). However, as antibiotic use increased, staphylococcal resistance rapidly developed (Bramley *et al*., 1989; You *et al*., 1999). Methicillin-resistant *Staphylococcus aureus* (MRSA), the resistance of which was due to penicillin-binding protein (PBP) 2′ production, was isolated in the early 1960s (Tsuchiya *et al*., 1996). MRSA is resistant to not only methicillin and other β-lactams but also to many other antibacterial agents such as macrolide (Stefani and Varaldo, 2003; Kim *et al*., 2004). Since MRSA exhibits multidrug resistance, it has been emerging worldwide as one of the most important hospital and community pathogens. Therefore, new agents are needed to treat MRSA associated infections. Some natural products are candidates for new antibiotic substances (Faizi *et al*., 2003; Gibbons *et al*., 2003).

Curcuma longa L. (*C. longa*), popularly known as turmeric, has long been used as a spice in Southeast Asia. It has also been used in Oriental folk medicines to treat infectious diseases (e.g. sinusitis, cough), cholecystitis and cholangitis and used as a therapy for hepatic disorders, rheumatism and anorexia (Kim, 1989). Previous works have shown that *C. longa* inhibited the growth of activity of some bacteria and fungi (Apisariyakul *et al.*, 1995; Negi *et al*., 1999; Singh *et al*., 2002; Chauhan *et al.*, 2003). However, little is known about the antimicrobial effects of *C. longa* on MRSA.

In the course of our screening project of the antibacterial activities of some natural products, it was recently found that extracts of *C. longa* had antibacterial activity against MRSA. In the present study, it was shown that *C. longa* had antimicrobial activity against MRSA and lowered the MICs of β-lactams. In addition, *C. longa* inhibited the invasion of MRSA to human mucosal fibroblasts (HMFs).

MATERIALS AND METHODS

Plant material and extraction. *C. longa* was obtained from the Korea Oriental Medical Herb Association (Seoul, South Korea). The identity was confirmed by Dr Bong-Seop Kil at the Department of Natural Science, Wonkwang University. Voucher specimen (number 7-00-12) has been deposited at the Herbarium of Department of Oral Biochemistry in Wonkwang University. Two 100 g portions of powder of dried

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Table 1. Bacterial strains used in the experiments

Strain[a]	Class[b]	*mec*A gene[c]	β-lactamase activity[c]	Antibiotic resistance pattern[d]
S. aureus (ATCC 25923)	MSSA	–	–	–
S. aureus (WMC 1613)	MRSA	+	–	AM, OX, ME, GE
S. aureus (WMC 2512)	MRSA	+	+	AM, OX, ME, GE
S. aureus (WMC 3003)	MRSA	+	+	AM, OX, ME, E, GE
S. aureus (WMC 3104)	MRSA	+	+	AM, OX, ME, E, GE
S. aureus (WMC 4105)	MRSA	+	+	AM, OX, ME, E, GE
S. aureus (WMC 4201)	MRSA	+	+	AM, OX, ME, GE
S. aureus (WMC 4310)	MRSA	+	–	AM, OX, ME, E, GE
S. aureus (WMC 5002)	MRSA	+	+	AM, OX, ME, E, GE
S. aureus (WMC 5411)	MRSA	+	+	AM, OX, ME, GE
S. aureus (WMC 6209)	MRSA	+	+	AM, OX, ME, E, GE
S. aureus (WMC 7108)	MRSA	+	+	AM, OX, ME, E, GE
S. aureus (WMC 8106)	MRSA	+	+	AM, OX, ME, E, GE
S. aureus (WMC 8207)	MRSA	+	+	AM, OX, ME, E, GE

[a] *S. aureus*, *Staphylococcus aureus*; ATCC, American Type Culture Collection.
[b] MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*.
[c] +, positive; –, negative.
[d] AM, ampicillin; OX, oxacillin; ME, methicillin; E, erythromycin; C, chloramphenicol; VA, vancomycin; GE, gentamicin.

rhizomes of *C. longa* were soaked separately in 1000 mL of ethyl acetate and methanol for 72 h at room temperature. For water extraction, 100 g of powdered sample were boiled in 1000 mL of hot water for 60 min. The filtration of the extracted solution and evaporation under reduced pressure yielded ethyl acetate (8.16 g), methanol (8.26 g) and water (4.28 g) extracts.

Bacterial strains. The staphylococcal strains listed in Table 1 were 13 clinical isolates (MRSA) from Wonkwang University Hospital and the standard strain of *S. aureus* ATCC 25923, which is methicillin-sensitive *S. aureus* (MSSA). Antibiotic susceptibility was determined from the size of the inhibition zone, in accord with guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997), and the strains used were defined as MRSA based on the occurrence of the *mecA* gene and their resistance to oxacillin (Wallet *et al*., 1996). β-Lactamase activity was also determined using the DrySlide Beta Lactamase test (Difco Laboratories, Detroit, MI, USA) according to the manufacturer's specification. After culturing on Mueller-Hinton agar (Difco Laboratories), the bacteria were suspended in Mueller-Hinton broth (Difco Laboratories) and used for inoculation.

Detection of *mecA* gene. Detection of the *mecA* gene in the strains of MRSA was performed by PCR amplication. Total genomic DNA was obtained from *S. aureus* by the phenol chloroform extraction method as described earlier in the previous report (Tsen and Chen, 1992). Bacteria collected from 5 mL of the 18 h culture in Mueller-Hinton broth were used for DNA extraction after treatment with lysostaphin and RNase (Sigma, St Louis, MO, USA). The PCR assay was performed in a DNA thermal cycler, GeneAmp PCR system 9700 (PE Applied Biosystems, Mississauga, Ontario, Canada), by using a Gene Taq amplifying kit (Wako Pure Chemicals Industries, Ltd, Japan), according to the manufacturer's recommendations. Synthetic oligonucleotides used as primers were 5′-ATGAGATTAGGCATCGTTCC-3′ and 5′-TGGATGACAGTACCTGAGCC-3′ (Ryffel *et al*., 1990).

Disc diffusion method. For the first screening, the paper disc diffusion method was used to determine antibacterial activity, which is based on the method described previously (Ali *et al*., 2001). Sterile paper discs (6 mm; Toyo Roshi Kaihsa, Japan) were loaded with 50 μL of different amounts (0.25, 0.5 and 1 mg) of the extracts dissolved in dimethyl sulphoxide (DMSO) and were left to dry for 12 h at 37 °C in a sterile room. Bacterial suspensions were diluted to match the 0.5 MacFarland standard scale (approximately 1.5×10^8 CFU/mL) and they were further diluted to obtain a final inoculum. After Mueller-Hinton agar was poured into Petri dishes to give a solid plate and inoculated with 100 μL of suspension containing 1×10^8 CFU/mL of bacteria, the discs treated with extracts were applied to Petri dishes. Ampicillin and oxacillin were used as positive controls and paper discs treated with DMSO were used as a negative control. The plates were then incubated at 35 °C for 24 h in an incubator (Vision Co, Seoul, Korea). Inhibition zone diameters around each of the discs were measured and recorded at the end of the incubation time.

Determination of minimum inhibitory concentrations (MICs). MICs were determined by the agar dilution method, which is based on the method described previously (Chang *et al*., 1995). The MICs of ampicillin and oxacillin were also determined. A final inoculum of 1×10^4 CFU/mL was spotted with a multipoint inoculator (Denley Instruments, Sussex, UK) onto agar plates. The plates were then incubated at 35 °C for 24 h in the incubator (Vision Co, Seoul, Korea). The MIC was defined as the lowest concentration of extracts at which no visible growth was observed. The minimum concentration of extracts that inhibited 50% and 90% of

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the isolates tested was defined as MIC_{50} and MIC_{90}, respectively.

Checkerboard dilution test. The antibacterial effects of a combination of the ethyl acetate extract of *C. longa*, which exhibited the highest antimicrobial activity, and β-lactams were assessed by the checkerboard test as previously described (Chang *et al*., 1995). The antimicrobial combinations assayed included *C. longa* extract plus ampicillin and *C. longa* extract plus oxacillin. Serial dilutions of two different antimicrobial agents were mixed in cation-supplemented Mueller-Hinton broth. Inocula were prepared from colonies grown on Mueller-Hinton agar after overnight culture. The final bacterial concentration after inoculation was 5×10^5 CFU/mL. After 24 h of incubation at 35 °C, the MIC was determined to be the minimal concentration at which there was no visible growth.

Bacterial invasion assay. Bacterial invasion into cells and tissues is one of the important pathogenic mechanisms in oral infection (Schuster and Burnett, 1981). To investigate the inhibitory effect of an ethyl acetate extract of *C. longa*, which exhibited the highest antimicrobial activity on bacterial invasion to cultured monolayers of HMFs, previously reported methods were used with a slight modification (Jung *et al*., 2001). HMFs were obtained from the patients undergoing oral surgery. The HMFs were grown routinely in monolayers in α-minimum essential medium (α-MEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin G and 100 µg/mL streptomycin sulphate. Before use, the cells were seeded at 5×10^4 cells/well in 24-well tissue culture plates (Costar, Cambridge, MA, USA) for binding and invasion assays and grown into confluent monolayers for 1 day at 37 °C in a 5% CO_2 incubator (Vision Co, Seoul, Korea).

The number of bacteria attached to HMFs was quantified by determining the number of recovered bacterial colonies after co-culture. Approximately 16 h prior to the experiments, the HMFs were washed three times with invasion medium (growth medium without antibiotics) and held in this medium. Just before beginning the experiment, the medium was removed and the HMFs were washed once with invasion medium followed by a further addition of 1 mL of fresh invasion medium. Appropriate wells of HMFs were inoculated with 1 mL of invasion medium containing 0, 0.125, 0.25, 0.5 and 1 mg/mL of *C. longa* extract and 1×10^5 CFU/mL/well of bacteria for the specified times at 37 °C in air with 5% CO_2. Subsequently, the medium was removed from the infected monolayers, before washing three times with sterile Ca^{2+}- and Mg^{2+}-free PBS to remove non-adherent bacteria. The HMFs were treated with 0.25% trypsin in Hanks' balanced salt solution (Gibco BRL) and further lysed with 0.025% Triton X-100 (Sigma Chemical Co., St Louis, MO, USA) in sterile distilled water. Cell lysates were serially diluted and plated in triplicate on blood agar plates; the plates were then incubated overnight at 37 °C and the CFU were counted. At this time, colonies of *S. aureus* were identified by Gram stain, catalase and coagulase tests.

Phytochemical screening. Phytochemical tests of extracts were performed as previously described (Houghton and Raman, 1998; Woo, 2001). Mayer's reagent was used for alkaloids, ferric chloride reagent for phenolics, Molish test for glycosides, Biuret reagent for proteins, Mg-HCl reagent for flavonoids, Liebermann-Burchard reagent for steroids, silver nitrate reagent for organic acids.

Statistical analysis. All experiments were carried out in triplicate. Data were analysed using the statistical package for social sciences (SPSS). Differences between the means of the experimental and control groups were evaluated by the Student's *t*-test.

RESULTS

Table 2 shows the antimicrobial activity of *C. longa* extracts determined by the disc diffusion method. The MICs for the ethyl acetate extract, methanol extract and water extract of *C. longa* against 13 strains of MRSA and 1 standard strain of MSSA were also determined (Table 3). The determination of the inhibition zones by the disc diffusion method revealed antimicrobial activity of the *C. longa* against MRSA as well as the standard MSSA, and these results were confirmed by the data expressed as MIC in the agar dilution method. The ethyl acetate extract of *C. longa* demonstrated a higher inhibitory activity (MIC: 2 mg/mL) than the methanol extract (MIC: 8 mg/mL) and the water extract (MIC: 64 mg/mL) against the standard MSSA. The MICs of ampicillin and oxacillin against standard MSSA were 0.125 and 0.031×10^{-3} mg/mL, respectively. The MICs against MRSA were broadly similar to the those against MSSA. The MIC_{50} of the ethyl acetate extract, methanol extract and water extract were 2 mg/mL, 4 mg/mL and 64 mg/mL, respectively. The ethyl acetate extract of *C. longa* demonstrated a higher antibacterial activity than the other extracts. The MIC_{90} and MIC range of ethyl acetate extract were 4 mg/mL and 1–4 mg/mL, respectively.

Following the determination of MIC values for MRSA or the standard MSSA, the study examined whether the ethyl acetate extract of *C. longa*, which exhibited highest antimicrobial activity, may lower the MICs of β-lactams by the checkerboard dilution method. The results of the checkerboard dilution test against MRSA and the standard MSSA are shown in Table 4. The ethyl acetate extract of *C. longa* markedly lowered the MICs of ampicillin and oxacillin against MRSA and a standard MSSA (Table 4).

To determine whether the ethyl acetate extract of *C. longa*, which exhibited the highest antimicrobial activity, inhibited the MRSA invasion of HMFs, the cells were treated with various concentrations of the ethyl acetate extract of *C. longa*, and bacterial invasion was assayed. The effect of various concentrations of *C. longa* extract on MRSA invasion of HMFs is presented in Fig. 1. The MRSA intracellular invasion was significantly decreased in the presence of 0.125–1 mg/mL of the ethyl acetate extract of *C. longa* extract compared with the control group. The effect of *C. longa* extract on MRSA invasion appeared bo be dose dependent. These findings suggest that the ethyl acetate extract of *C. longa* may inhibit the MRSA invasion of HMFs.

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Table 2. Antimicrobial activity (inhibition zones diameter in mm) of *Curcuma longa* L. extracts against 13 MRSA and 1 standard MSSA

	Zone of inhibition (mm)										
	EA[a] (mg)			M (mg)			W (mg)			Ampicillin[b]	Oxacillin[c]
Strain	0.25	0.5	1	0.25	0.5	1	0.25	0.5	1	(10×10^{-3} mg)	(1×10^{-3} mg)
S. aureus (ATCC 25923)	ND[d]	11	14	ND	ND	12	ND	ND	7	34	24
S. aureus (WMC 1613)	12	14	10	ND	12	15	ND	ND	9	15	ND
S. aureus (WMC 2512)	ND	10	13	ND	ND	10	ND	ND	8	11	ND
S. aureus (WMC 3003)	ND	13	15	ND	ND	10	ND	ND	7	11	ND
S. aureus (WMC 3104)	ND	13	16	ND	ND	13	ND	ND	7	11	ND
S. aureus (WMC 4105)	ND	11	14	ND	ND	11	ND	ND	8	11	ND
S. aureus (WMC 4201)	ND	11	15	ND	10	12	ND	ND	ND	12	9
S. aureus (WMC 4310)	9	12	15	ND	9	14	ND	ND	7	17	ND
S. aureus (WMC 5002)	10	12	14	ND	10	12	ND	ND	8	11	ND
S. aureus (WMC 5411)	ND	10	13	ND	ND	12	ND	ND	8	11	ND
S. aureus (WMC 6209)	12	14	17	ND	11	15	ND	ND	7	10	ND
S. aureus (WMC 7108)	11	12	14	ND	ND	10	ND	ND	7	10	ND
S. aureus (WMC 8106)	ND	12	15	ND	10	14	ND	ND	9	10	ND
S. aureus (WMC 8207)	ND	12	15	ND	ND	12	ND	ND	7	8	ND

[a] EA, ethyl acetate extract; M, methanol extract; W, water extract.
[b] Ampicillin resistance ≤ 28 mm.
[c] Oxacillin resistance ≤ 10 mm.
[d] ND, no detected activity at this concentration.

Table 3. MICs of *Curcuma longa* L. extracts, ampicillin and oxacillin against 13 MRSA and 1 standard MSSA

	MIC (mg/mL)					
		C. longa				
Strain	Class	EA[a]	M	W	Ampicillin[b]	Oxacillin[c]
S. aureus (ATCC 25923)	MSSA	2	8	64	0.125×10^{-3}	0.031×10^{-3}
S. aureus (WMC 1613)	MRSA	1	2	16	4×10^{-3}	4×10^{-3}
S. aureus (WMC 2512)	MRSA	4	4	16	64×10^{-3}	4×10^{-3}
S. aureus (WMC 3003)	MRSA	4	4	64	64×10^{-3}	4×10^{-3}
S. aureus (WMC 3104)	MRSA	2	4	64	64×10^{-3}	4×10^{-3}
S. aureus (WMC 4105)	MRSA	4	4	64	32×10^{-3}	4×10^{-3}
S. aureus (WMC 4201)	MRSA	2	4	64	32×10^{-3}	8×10^{-3}
S. aureus (WMC 4310)	MRSA	1	4	64	4×10^{-3}	4×10^{-3}
S. aureus (WMC 5002)	MRSA	4	4	64	32×10^{-3}	4×10^{-3}
S. aureus (WMC 5411)	MRSA	4	4	32	64×10^{-3}	16×10^{-3}
S. aureus (WMC 6209)	MRSA	1	4	64	64×10^{-3}	8×10^{-3}
S. aureus (WMC 7108)	MRSA	2	8	64	32×10^{-3}	8×10^{-3}
S. aureus (WMC 8106)	MRSA	4	4	64	64×10^{-3}	16×10^{-3}
S. aureus (WMC 8207)	MRSA	4	4	64	64×10^{-3}	16×10^{-3}

[a] EA, ethyl acetate extract; M, methanol extract; W, water extract.
[b] Ampicillin MICs of ≥ 0.25×10^{-3} mg/mL should be considered resistant.
[c] Oxacillin MICs of ≥ 4×10^{-3} mg/mL should be considered resistant.

Preliminary phytochemical tests for the ethyl acetate, methanol and water extracts are shown in Table 5. The ethyl acetate and methanol extracts gave positive tests for phenolics, flavonoids, glycosides and steroids. The water extract gave a positive test for proteins.

DISCUSSION

The present study investigated the antimicrobial activity of *C. longa* extracts against clinical isolates of MRSA and the effect of *C. longa* extract on the invasion by MRSA of HMFs. The results indicate that the *C. longa* extract showed antimicrobial activity against all tested strains of MRSA and a standard MSSA (Table 2). The fact that the extracts of *C. longa* inhibited the growth of *S. aureus* provides some scientific rationale for the fact that the local inhabitants used the extracts as antimicrobial agents. Although all the fractions of *C. longa* produced some inhibitory activities against MRSA and standard MSSA, the ethyl acetate extract exerted more inhibitory activity than the methanol and water extracts. These results suggest that ethyl acetate would be a better solvent than methanol or water in an attempt to isolate the antibacterial principles. In previous studies, curcumin, demethoxycurcumin, bisdemethoxycurcumin, oleoresin and essential oils were isolated from *C. longa* (Apisariyakul *et al.*, 1995; Song *et al.*, 2001). The essential oil (5.8%), obtained by steam

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Table 4. MICs for β-lactams used in combination with the ethyl acetate extract of *Curcuma longa* L. against 13 MRSA and 1 standard MSSA

	MIC (mg/mL) of ampicillin				MIC (mg/mL) of oxacillin			
	C. longa (mg/mL)				*C. longa* (mg/mL)			
Strain	0	0.25	0.5	1	0	0.25	0.5	1
S. aureus (ATCC 25923)	0.125×10^{-3}	0.031×10^{-3}	0.016×10^{-3}	0.008×10^{-3}	0.031×10^{-3}	0.031×10^{-3}	0.016×10^{-3}	0.008×10^{-3}
S. aureus (WMC 1613)	4×10^{-3}	2×10^{-3}	1×10^{-3}	0	4×10^{-3}	2×10^{-3}	1×10^{-3}	0
S. aureus (WMC 2512)	64×10^{-3}	64×10^{-3}	32×10^{-3}	32×10^{-3}	4×10^{-3}	4×10^{-3}	2×10^{-3}	2×10^{-3}
S. aureus (WMC 3003)	64×10^{-3}	32×10^{-3}	32×10^{-3}	16×10^{-3}	4×10^{-3}	2×10^{-3}	2×10^{-3}	1×10^{-3}
S. aureus (WMC 3104)	64×10^{-3}	32×10^{-3}	32×10^{-3}	16×10^{-3}	4×10^{-3}	2×10^{-3}	2×10^{-3}	1×10^{-3}
S. aureus (WMC 4105)	32×10^{-3}	16×10^{-3}	8×10^{-3}	8×10^{-3}	4×10^{-3}	2×10^{-3}	1×10^{-3}	1×10^{-3}
S. aureus (WMC 4201)	32×10^{-3}	16×10^{-3}	16×10^{-3}	8×10^{-3}	8×10^{-3}	4×10^{-3}	4×10^{-3}	4×10^{-3}
S. aureus (WMC 4310)	4×10^{-3}	2×10^{-3}	1×10^{-3}	0	4×10^{-3}	2×10^{-3}	1×10^{-3}	0
S. aureus (WMC 5002)	32×10^{-3}	16×10^{-3}	16×10^{-3}	16×10^{-3}	4×10^{-3}	2×10^{-3}	2×10^{-3}	2×10^{-3}
S. aureus (WMC 5411)	64×10^{-3}	64×10^{-3}	16×10^{-3}	16×10^{-3}	16×10^{-3}	16×10^{-3}	8×10^{-3}	4×10^{-3}
S. aureus (WMC 6209)	64×10^{-3}	32×10^{-3}	4×10^{-3}	0	8×10^{-3}	4×10^{-3}	2×10^{-3}	0
S. aureus (WMC 7108)	32×10^{-3}	16×10^{-3}	16×10^{-3}	8×10^{-3}	8×10^{-3}	4×10^{-3}	4×10^{-3}	2×10^{-3}
S. aureus (WMC 8106)	64×10^{-3}	64×10^{-3}	32×10^{-3}	32×10^{-3}	16×10^{-3}	8×10^{-3}	8×10^{-3}	8×10^{-3}
S. aureus (WMC 8207)	64×10^{-3}	32×10^{-3}	16×10^{-3}	4×10^{-3}	16×10^{-3}	8×10^{-3}	4×10^{-3}	4×10^{-3}

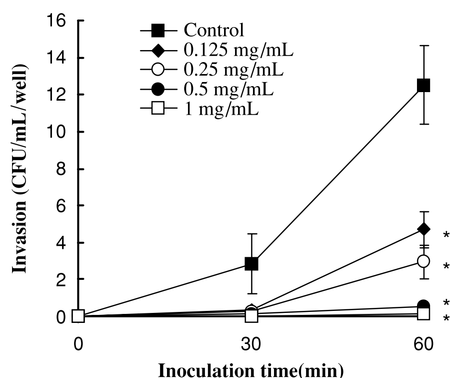

Figure 1. Effect of ethyl acetate extract of *Curcuma longa* L. on MRSA invasion of HMFs. Cells were infected with *Staphylococcus aureus* (WMC 8207) in the different sub-MIC concentrations of *Curcuma longa* L. extract for several time periods, followed by measurement of CFU recovered from cell monolayers. Each point represents mean ± standard errors. * $p < 0.05$ compared with control group.

Table 5. Phytochemical analysis of *Curcuma longa* L. extracts

Plant constituent	EA[a]	M	W
Alkaloids	–[b]	–	–
Phenolics	+ + +	+ +	–
Flavonoids	+ + +	+ +	–
Glycosides	+ +	+ + +	–
Proteins	–	–	+ + +
Steroids	+ +	+ + +	–
Organic acids	–	–	–

[a] EA, ethyl acetate extract; M, methanol extract; W, water extract.
[b] + + + strong; + + medium; + poor presence; – absence.

distillation of the rhizomes, has the following constituents; α-phellandrene 1%, sabinene 0.6%, cineol 1%, borneol 0.5%, zingiberene 25% and sesquiterpenes 53% (Eigner and Scholz, 1999). In this investigation, it was found that the ethyl acetate extract of *C. longa* contains a lot of flavonoids. Since several reports have shown that some compounds belonging to flavonoids have antibacterial activity (Sato *et al.*, 2000), it is considered that the flavonoids in *C. longa* may, in part, be related to the antibacterial effects in the present study. However, further studies are needed to elucidate the antimicrobial principles in *C. longa.*

Several mechanisms by which microorganisms can overcome the toxicity of antimicrobial agents are known. These include the production of drug insensitive enzymes, modification of the targets for drugs and the extrusion of drugs from bacterial cells by a multidrug resistance (MDR) pump. It seems that the genes responsible for MDR are present mainly in the *mec* region of the MRSA chromosome and several other genes such as *fem*, *llm* and *sigB* are also involved (Shiota *et al.*, 1999). In the present study, the MICs of *C. longa* extract against MRSA were not higher than the standard MSSA. These data show that the tested stains of MRSA in this experiment may not have resistance against *C. longa* extract, although many MRSA exhibit the multidrug resistance (Shiota *et al.*, 1999). Since recent reports showed that some natural products may lower the MIC of β-lactams (Shiota *et al.*, 1999; Liu *et al.*, 2000), the study examined whether the ethyl acetate extract of *C. longa* (which exhibited highest antimicrobial activity) could lower the MICs of β-lactams by the checkerboard dilution method. As expected, the *C. longa* extract markedly lowered the MICs of ampicillin and oxacillin against MRSA. For all the strains there were 2- to 16-fold reductions in the MICs. To our knowledge, this is the first report that the *C. longa* extract lowered the MICs of β-lactam antibiotics. However, it is not yet clear how the *C. longa* extract enhanced the antibacterial activity of β-lactam antibiotics against MRSA. Recent reports showed that some medical plants have MDR pump inhibitors, which may lower the MIC of antimicrobial agents (Stermitz *et al.*, 2000). Further studies are needed to elucidate whether the *C. longa* extract may have the MDR pump inhibitors.

Since bacterial invasion into cells and tissues is the one of the important pathogenic mechanisms in oral infection (Schuster and Burnett, 1981), the study examined whether the ethyl acetate extract of *C. longa* could affect the intracellular invasion by MRSA of HMFs. Surprisingly, *C. longa* extract inhibited the MRSA invasion of HMFs. In the invasion mechanism

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of *Staphylococcus aureus*, Staphylococcal protein A (SPA) may have an important role (Jung *et al*., 2001). Additional experiments are required to determine the possibility of inhibition of SPA role by *C. longa* extract.

In conclusion, the results reported here showed that the ethyl acetate extract of *C. longa* possesses antimicrobial activity, lowers the MICs of β-lactam antibiotics against MRSA and inhibits the MRSA invasion of HMFs.

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