Combination of emodin with antibiotics against methicillin-resistant *Staphylococcus aureus* isolated from clinical specimens

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Abstract**—** *Emodin (3-methyl-1,6,8-trihydroxyanthraquinone), a natural anthraquinone compound, is an active compound derivative isolated from the rhizome of Rheum undulatum L, an herb widely used as a laxative in traditional Korean medicine. Emodin has been reported to have a variety of biological activities, such as anti-cancer, vasorelaxation, immunosuppressive, anti-inflammatory and wound healing properties. In this study, emodin was evaluated against 20 clinical isolates of MRSA, either alone or in combination with antibiotics. The emodin exhibited strong antibacterial activity against isolates MRSA with MICs/MBCs ranged between 64-256/64-512 μg/mL, for ampicillin 64-512/128-1024 μg/mL, and for oxacillin 8-64/16-64 μg/mL. The combination of emodin plus oxacillin or ampicillin was reduced by ≥4-fold against isolates MRSA tested, evidencing a synergistic effect as defined by a FICI of ≤ 0.5. Furthermore, a time-kill study evaluating the growth of the tested bacteria was completely attenuated after 2-6 h of treatment with the 1/2 MIC of emodin, regardless of whether it was administered alone or with oxacillin (1/2 MIC) or ampicillin (1/2 MIC). In conclusion, emodin exerted synergistic effects when administered with oxacillin or ampicillin and the antibacterial activity and resistant regulation of emodin against clinical isolates of MRSA might be useful in controlling MRSA infections.*

Keywords**— emodin, methicillin-resistant Staphylococcus aureus, minimum inhibitory concentrations, minimum bactericidal concentrations, time-kill curves, fractional inhibitory concentration.***

I. INTRODUCTION

Staphylococcus aureus is both a commensal bacterium and a human pathogen. Approximately 50% to 60% of individuals are intermittently or permanently colonized with *S. aureus* and, thus, there is relatively high potential for infections [1, 2]. Indeed, *S. aureus* is among the most prominent causes of bacterial infections in the United States and other industrialized countries. Simultaneously, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissue, pleuropulmonary, and devicerelated infections [3, 4]. Methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) are major causes of life-threatening infections including surgical site infections, bacteraemia, pneumonia and catheter-associated infections, leading to significant morbidity and mortality [5-7]. There is a very limited antimicrobial armamentarium to treat MRSA infections, of which vancomycin (a glycopeptide) and linezolid (an oxazolidinone antibiotic) are the major antibiotics [8, 9]. Antimicrobial drugs effective for treatment of patients infected with MRSA are limited. Thus, it is important and valuable to find compounds that potentiate antimicrobial activity of antibiotics. Plant medicines are used on a worldwide scale to prevent and treat infectious diseases [10, 11]. They are of great demand both in the developed as well as developing countries for the primary health care needs due to their wide biological and medicinal activities, higher safety margin and lesser costs [12, 13]. At the same time, because of the difficulty in developing chemical synthetic drugs and because of their side-effects, scientists are making more efforts to search for new drugs from plant resources to combat clinical multidrug-resistant microbial infections [13-15].

Emodin (3-methyl-1,6,8-trihydroxyanthraquinone), a natural anthraquinone compound, is an active compound derivative isolated from the rhizome of *Rheum undulatum L*, an herb widely used as a laxative in traditional Korean medicine [16, 17]. Emodin has been reported to have a variety of biological activities, such as anti-cancer, vasorelaxation, immunosuppressive, anti-inflammatory, antibacterial activity, and wound healing properties [18-22]. Emodin is shown to significantly inhibit biofilm formation in *P. aeruginosa*, induces proteolysis of a known AHL-binding protein, and can be used as a potential QS inhibitor for the control of biofilm formation and growth [23]. Emodin from *Polygonum cuspidatum* exhibits strong

antibacterial activity against *Haemophilus parasuis in vitro*. The antibacterial mechanism of emodin to *H. parasuis* attributed to producing alterations on the physical structure and increasing cell membrane permeability [24].

In this study, the antimicrobial activities of emodin against methicillin-resistant *Staphylococcus aureus* isolated in a clinic were assessed using broth microdilution method and the checkerboard and time-kill methods for synergistic effect of the combination with antibiotics.

II. MATERIALS AND METHODS

2.1 Preparation of bacterial strains

20 isolates of methicillin-resistant *Staphylococcus aureus* isolated from the Wonkwang University Hospital, as well as standard strains of methicillin-sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin-resistant *S. aureus* (MRSA) ATCC 33591 were used. Antibiotic susceptibility was determined in testing the inhibition zones (inoculums 0.5 McFarland suspension, 1.5×10^8 CFU/ml) and MIC/MBC (inoculums 5×10^5 CFU/ml) for strains, measured as described in the National Committee for Clinical Laboratory Standards (NCCLS, 1999). Briefly, the growth of bacteria was examined at 37°C in 0.95 mL of BHI broth containing various concentrations of emodin. These tubes were inoculated with 5×10^5 colony-forming units (CFU)/mL of an overnight culture grown in BHI broth, and incubated at 37°C. After 24 h of incubation, the optical density (OD) was measured spectrophotometrically at 550 nm. Three replicates were measured for each concentration of tested drugs. To rapidly identifying the methicillin-resistance, presence of *mecA* gene in MRSA isolates was detected using PCR method as the following [25].

2.2 Minimum inhibitory concentrations/minimum bactericidal concentrations assay

The antimicrobial activities of emodin against clinical isolates MRSA 20 and reference strains were determined *via* the broth dilution method [26, 27]. The minimum inhibitory concentrations (MICs) were recorded as the lowest concentration of test samples resulting in the complete inhibition of visible growth. For clinical strains, MIC_{50}s and MIC_{90}s, defined as MICs at which, 50 and 90%, respectively of the isolates were inhibited, were determined. The minimum bactericidal concentrations (MBCs) were determined based on the lowest concentration of the extracts required to kill 99.9% of bacteria from the initial inoculum as determined by plating on agar.

2.3 Checkerboard dilution test

The synergistic combinations were investigated in the preliminary checkerboard method performed using the MRSA, MSSA, and one clinical isolate strains via MIC determination [26, 27]. The fractional inhibitory concentration index (FICI) and fractional bactericidal concentration index (FBCI) are the sum of the FICs and FBCs of each of the drugs, which were defined as the MIC and MBC of each drug when used in combination divided by the MIC and MBC of each drug when used alone. The FIC and FBC index was calculated as follows: FIC = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone) and FBC = (MBC of drug A in combination/MBC of drug A alone) + (MBC of drug B in combination/MBC of drug B alone). FIC indices (FICI) and FBCI were interpreted as follows: ≤ 0.5, synergy; >0.5-≤1.0, additive; >1.0-≤2.0, indifference; and >2.0, antagonism.

2.4 Time-kill curves

The bactericidal activities of the drugs evaluated in this study were also evaluated using time-kill curves constructed using the isolated and reference strains. Cultures with an initial cell density of $5\text{-}8\times10^6$ CFU/ml were exposed to the MIC of emodin alone, or emodin (1/2 MIC) plus oxacillin (1/2 MIC) or emodin (1/2 MIC) plus ampicillin (1/2 MIC). Viable counts were conducted at 0, 0.5, 1, 2, 3, 4, 5, 6, 12, and 24 h by plating aliquots of the samples on agar and subsequent incubation for 24 hours at 37°C. All experiments were repeated several times and colony counts were conducted in duplicate, after which the means were determined.

III. RESULTS AND DISCUSSION

The results of the antibacterial activity showed that the emodin exhibited inhibitory activities against isolates MRSA and reference stains, MRSA ATCC33591 and MSSA ATCC25923. In Table 1, the emodin displayed varying degrees of activity against clinical isolated MRSA 1-20 with MIC in the range of 64-256 μg/mL and MBC in the range of 64-256 μg/mL. The MICs/MBCs for ampicillin were determined to be either 64/128 or 1024/2048 μg/mL; for oxacillin, either 8/16 or 64/64 μg/mL against MRSA 1-20 isolates. The range of MIC_{50} and MIC_{90} were 16-64 μg/mL and 64-256 μg/mL against MRSA 1-20 isolates, respectively. Various anthraquinones constitute an important class of phytochemicals which possess diverse

biological activities against MRSA [21, 28, 29]. A chemical structure-activity relationship study revealed that two hydroxyl units at the C-1 and C-2 positions of anthraquinone play important roles in antibiofilm and anti-hemolytic activities [28]. Emodin exhibits strong antibacterial activity against *H. parasuis in vitro* [24]. Emodin exhibits antimicrobial activity against a broad range of gram-positive, including *S. aureus*, and Mycobacterium tuberculosis as well as other microorganisms [21, 24, 30].

TABLE 1
ANTIBACTERIAL ACTIVITY OF EMODIN AND ANTIBIOTICS IN ISOLATED MRSA AND SOME OF REFERENCE BACTERIA

Samples	emodin (μg/mL)			Ampicillin	Oxacillin
	$MIC_{50<}$	$MIC_{90<}$	(1) MIC/MBC	MIC/MBC (μg/mL)	
MSSA ATCC 25923 [1]	64	256	256/512	8/16	0.25/1
MRSA ATCC 33591 [2]	32	128	128/256	1024/2048	8/16
MRSA 1[3]	8	64	64/128	512/1024	16/32
MRSA 2	16	64	64/128	128/256	16/32
MRSA 3	32	64	128/256	512/2048	8/16
MRSA 4	64	128	256/256	256/512	16/64
MRSA 5	32	128	128/256	128/256	16/32
MRSA 6	16	64	64/128	256/512	8/32
MRSA 7	16	64	64/256	128/512	16/32
MRSA 8	32	128	128/256	256/512	8/32
MRSA 9	32	256	256/512	256/512	32/64
MRSA 10	64	128	256/256	64/128	8/16
MRSA 11	32	128	128/256	128/256	16/64
MRSA 12	16	64	64/64	256/512	32/64
MRSA 13	16	64	64/128	64/128	64/64
MRSA 14	16	64	64/128	128/512	16/32
MRSA 15	32	128	128/256	64/128	16/32
MRSA 16	32	128	128/256	128/256	16/32
MRSA 17	64	256	256/256	128/256	8/16
MRSA 18	64	256	256/512	64/128	16/32
MRSA 19	16	64	64/128	128/256	16/64
MRSA 20	32	128	128/256	128/512	16/32

[1]MSSA (ATCC 25923): reference strain Methicillin-sensitive *Staphylococcus aureus.*
[2]MRSA (ATCC 33591): reference strain Methicillin-resistant *Staphylococcus aureus.*
[3]MRSA (1-20): Methicillin-resistant *Staphylococcus aureus* isolated a clinic.

Combination antibiotic therapy has been studied to promote the effective use of antibiotics in increasing *in vivo* activity of antibiotics, in preventing the spread of drug-resistant strains, and in minimizing toxicity [26, 31, 32]. The combination of oxacillin and emodin showed in a reduction in the MICs/MBCs for all bacteria, with the MICs/MBCs of 8/16 or 64/128 μg/mL for oxacillin becoming 2-8/4-16 μg/mL and reduced by ≥4-fold in most of *S. aureus* tested, evidencing a synergistic effect as defined by a FICI of ≤ 0.5 except clinic MRSA 8, 11, and 15 at MIC and clinic MRSA 1, 4, 10, 15, and 17 at MBC (Table 2). In combination with emodin, the MICs/MBCs for ampicillin were reduced by ≥4-fold in most of *S. aureus* tested, evidencing a synergistic effect as defined by a FICI of ≤ 0.5 except clinic MRSA 7, 9, and 19 at MIC and clinic MRSA 4, 7, 10, 12, and 19 at MBC by FICI of > 0.625 (Table 3). The some plant derived compounds can improve the in vitro activity of some cell-wall inhibiting antibiotics by directly attacking the same target site, that is, peptidoglycan [21, 33].

TABLE 2
SYNERGISTIC EFFECTS OF EMODIN WITH OXACILLIN IN ISOLATED MRSA AND SOME OF REFERENCE BACTERIA

Samples	Agent	MIC/MBC (μg/mL)		FIC/FBC	FICI/FBCI[2]	Outcome
		Alone	Combination[1]			
MSSA ATCC 25923[3]	Emodin	256/512	128/256	0.5/0.5	0.75/0.75	**Additive/ Additive**
	Oxacillin	0.25/1	0.0625/0.25	0.25/0.25		
MRSA ATCC 33591[4]	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	8/16	2/4	0.25/0.25		
MRSA 1[5]	Emodin	64/128	16/32	0.25/0.25	0.5/0.75	**Synergistic/ Additive**
	Oxacillin	16/32	4/16	0.25/0.5		
MRSA 2	Emodin	64/128	16/32	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	16/32	4/8	0.25/0.25		
MRSA 3	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	8/16	2/4	0.25/0.25		
MRSA 4	Emodin	256/256	64/128	0.25/0.5	0.5/0.75	**Synergistic/ Additive**
	Oxacillin	16/64	4/16	0.25/0.25		
MRSA 5	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	16/32	4/8	0.25/0.25		
MRSA 6	Emodin	64/128	16/32	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	8/32	2/4	0.25/0.25		
MRSA 7	Emodin	64/256	16/32	0.25/0.125	0.5/0.375	**Synergistic/ Synergistic**
	Oxacillin	16/32	4/8	0.25/0.25		
MRSA 8	Emodin	128/256	64/64	0.5/0.25	0.75/0.5	**Additive/ Synergistic**
	Oxacillin	8/32	2/8	0.25/0.25		
MRSA 9	Emodin	256/512	64/128	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	32/64	8/16	0.25/0.25		
MRSA 10	Emodin	256/256	64/128	0.25/0.5	0.5/0.75	**Synergistic/ Additive**
	Oxacillin	8/16	2/4	0.25/0.25		
MRSA 11	Emodin	128/256	64/64	0.5/0.25	0.75/0.375	**Additive/ Synergistic**
	Oxacillin	16/64	4/8	0.25/0.125		
MRSA 12	Emodin	64/64	8/16	0.125/0.25	0.375/0.5	**Synergistic/ Synergistic**
	Oxacillin	32/64	8/16	0.25/0.25		
MRSA 13	Emodin	64/128	16/32	0.25/0.25	0.375/0.5	**Synergistic/ Synergistic**
	Oxacillin	64/64	8/16	0.125/0.25		
MRSA 14	Emodin	64/128	8/32	0.125/0.25	0.375/0.5	**Synergistic/ Synergistic**
	Oxacillin	16/32	4/8	0.25/0.25		
MRSA 15	Emodin	128/256	32/64	0.25/0.25	0.75/0.75	**Additive/ Additive**
	Oxacillin	16/32	8/16	0.5/0.5		
MRSA 16	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	16/32	4/8	0.25/0.25		
MRSA 17	Emodin	256/256	64/128	0.25/0.5	0.5/1.0	**Synergistic/ Additive**
	Oxacillin	8/16	2/8	0.25/0.5		
MRSA 18	Emodin	256/512	64/128	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	16/32	4/8	0.25/0.25		
MRSA 19	Emodin	64/128	16/32	0.25/0.25	0.5/0.375	**Synergistic/ Synergistic**
	Oxacillin	16/64	4/8	0.25/0.125		
MRSA 20	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Oxacillin	16/32	4/8	0.25/0.25		

[1]The MIC and MBC of emodin with oxacillin
[2] the FIC/FBC index
[3]MSSA (ATCC 25923): reference strain Methicillin-sensitive *Staphylococcus aureus.*
[4]MRSA (ATCC 33591): reference strain Methicillin-resistant *Staphylococcus aureus.*
[5]MRSA (1-20): Methicillin-resistant *Staphylococcus aureus* isolated a clinic.

TABLE 3
SYNERGISTIC EFFECTS OF EMODIN WITH AMPICILLIN IN ISOLATED MRSA AND SOME OF REFERENCE BACTERIA

Samples	Agent	MIC/MBC (µg/mL)		FIC/FBC	FICI/FBCI[2]	Outcome
		Alone	Combination[1]			
MSSA ATCC 25923[3]	Emodin	256/512	64/128	0.25/0.25	0.75/0.75	**Additive/ Additive**
	Ampicillin	8/16	4/8	0.5/0.5		
MRSA ATCC 33591[4]	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	1024/2048	256/512	0.25/0.25		
MRSA 1[5]	Emodin	64/128	16/32	0.25/0.25	0.375/0.375	**Synergistic/ Synergistic**
	Ampicillin	512/1024	64/128	0.125/0.125		
MRSA 2	Emodin	64/128	16/32	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	128/256	32/64	0.25/0.25		
MRSA 3	Emodin	128/256	32/64	0.25/0.25	0.375/0.3125	**Synergistic/ Synergistic**
	Ampicillin	512/2048	64/128	0.125/0.0625		
MRSA 4	Emodin	256/256	64/128	0.25/0.5	0.5/0.75	**Synergistic/ Additive**
	Ampicillin	256/512	64/128	0.25/0.25		
MRSA 5	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	128/256	32/64	0.25/0.25		
MRSA 6	Emodin	64/128	16/32	0.25/0.25	0.5/0.375	**Synergistic/ Synergistic**
	Ampicillin	256/512	64/64	0.25/0.125		
MRSA 7	Emodin	64/256	32/128	0.5/0.5	1.0/0.75	**Additive/ Additive**
	Ampicillin	128/512	64/128	0.5/0.25		
MRSA 8	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	256/512	64/128	0.25/0.25		
MRSA 9	Emodin	256/512	64/64	0.25/0.125	0.75/0.375	**Additive/ Synergistic**
	Ampicillin	256/512	128/128	0.5/0.25		
MRSA 10	Emodin	256/256	64/128	0.25/0.5	0.5/0.75	**Synergistic/ Additive**
	Ampicillin	64/128	16/32	0.25/0.25		
MRSA 11	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	128/256	32/64	0.25/0.25		
MRSA 12	Emodin	64/64	16/32	0.25/0.5	0.375/0.625	**Synergistic/ Additive**
	Ampicillin	256/512	32/64	0.125/0.125		
MRSA 13	Emodin	64/128	16/16	0.25/0.125	0.5/0.375	**Synergistic/ Synergistic**
	Ampicillin	64/128	16/32	0.25/0.25		
MRSA 14	Emodin	64/128	16/16	0.25/0.125	0.5/0.25	**Synergistic/ Synergistic**
	Ampicillin	128/512	32/64	0.25/0.125		
MRSA 15	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	64/128	16/32	0.25/0.25		
MRSA 16	Emodin	128/256	32/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	128/256	32/64	0.25/0.25		
MRSA 17	Emodin	256/256	64/64	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	128/256	32/64	0.25/0.25		
MRSA 18	Emodin	256/512	64/128	0.25/0.25	0.5/0.5	**Synergistic/ Synergistic**
	Ampicillin	64/128	16/32	0.25/0.25		
MRSA 19	Emodin	64/128	32/64	0.5/0.5	0.75/0.75	**Additive/ Additive0**
	Ampicillin	128/256	32/64	0.25/0.25		
MRSA 20	Emodin	128/256	32/64	0.25/0.25	0.375/0.375	**Synergistic/ Synergistic**
	Ampicillin	128/512	16/64	0.125/0.125		

[1]The MIC and MBC of emodin with ampicillin
[2] the FIC/FBC index
[3]MSSA (ATCC 25923): reference strain Methicillin-sensitive *Staphylococcus aureus*
[4]MRSA (ATCC 33591): reference strain Methicillin-resistant *Staphylococcus aureus*
[5]MRSA (1-20): Methicillin-resistant *Staphylococcus aureus* isolated a clinic

The efficacy of emodin administered with oxacillin or ampicillin on standard (MSSA and MRSA) and clinical isolates of MRSA (MRSA 1-20) was confirmed by time-kill curve experiment (Fig. 1-4). Cultures of each strain of bacteria with a cell density of 5-8×10^6 CFU/mL were exposed to the MIC of emodin alone or/and emodin (1/2 MIC) with oxacillin (1/2 MIC) or/and ampicillin (1/2 MIC). Interestingly, the combination of the emodin plus oxacillin or/and ampicillin exhibited a steady reduction of 5-8 × 10^6 CFU/mL to 10^3 CFU/mL within 6 h and did not recover within 24 h, as compared to that observed with emodin (MIC) alone. A powerful bactericidal effect was exerted when a combination of drugs was utilized. Although emodin has no influence on genes related to cell wall synthesis and lysis as well as β-lactamase activity and drug accumulation, emodin reduces membrane fluidity and disrupted membrane integrity [21]. This perturbation of the cell membrane coupled with the action of β-lactams on the transpeptidation of the cell membrane could lead to the enhanced antimicrobial effect [32, 33].

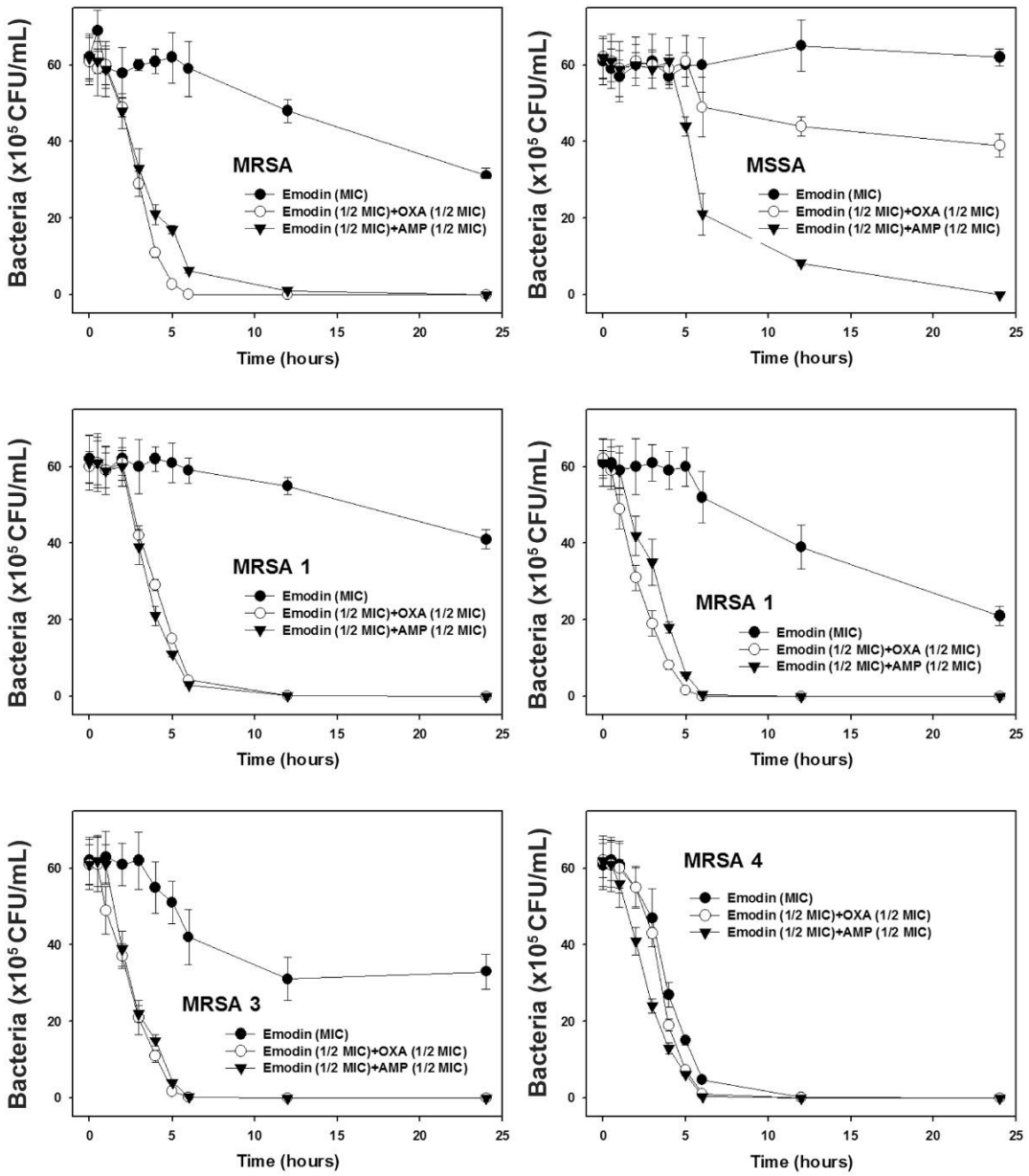

FIG. 1. TIME-KILL CURVES OF MIC OF EMODIN ALONE AND 1/2 MIC OF EMODIN WITH 1/2 MIC OF OXACILLIN OR AMPICILLIN AGAINST ISOLATES MRSA (1-4) AND METHICILLIN-SENSITIVE *S. AUREUS* (MSSA) ATCC 25923 AND METHICILLIN-RESISTANT *S. AUREUS* (MRSA) ATCC 33591 STRAINS. BACTERIA WERE INCUBATED WITH EMODIN ALONE (●) AND WITH AMPICILLIN (○) OR OXACILLIN (▼) OVER TIME. CFU, COLONY-FORMING UNITS.

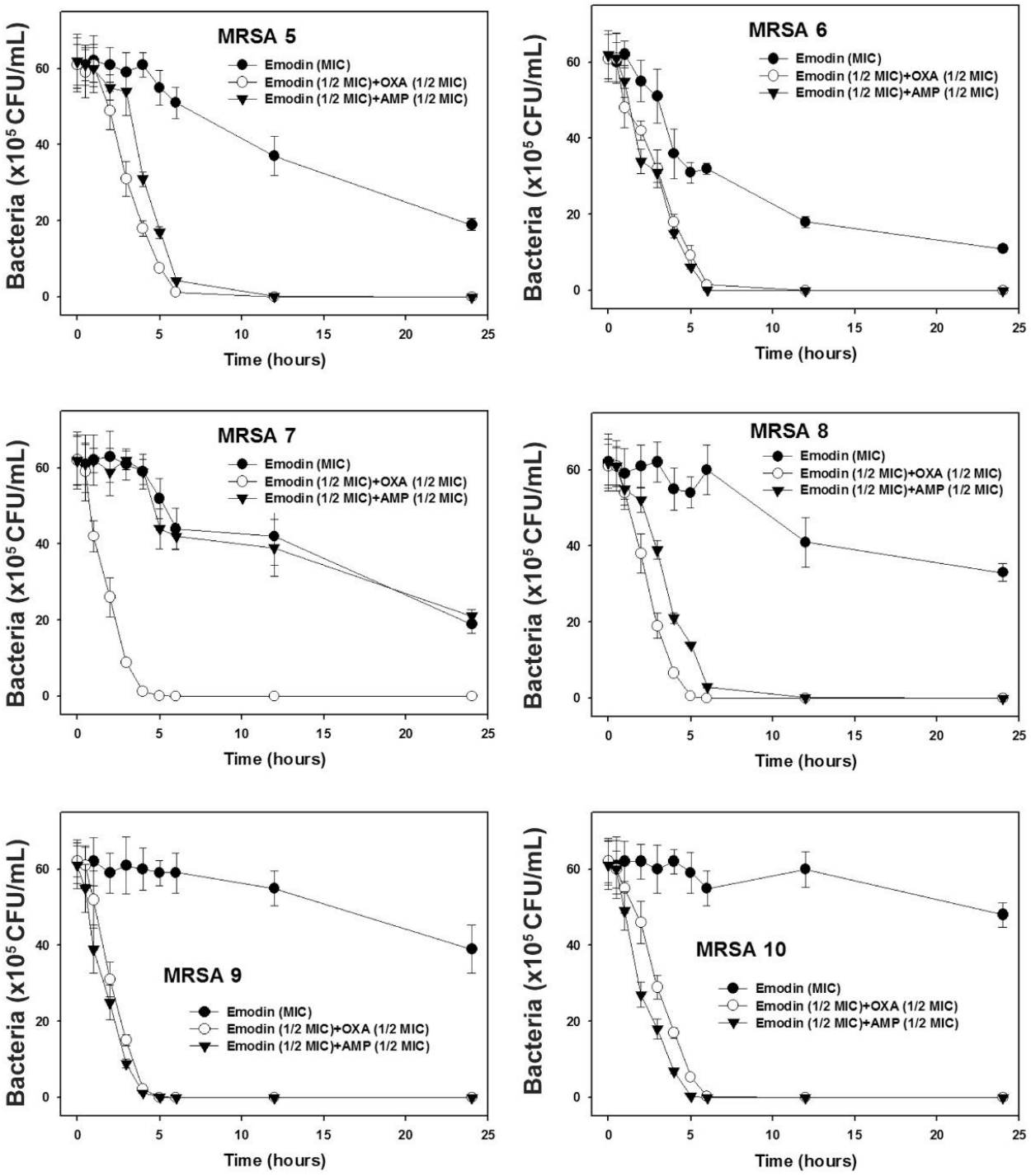

FIG. 2. TIME-KILL CURVES OF MIC OF EMODIN ALONE AND 1/2 MIC OF EMODIN WITH 1/2 MIC OF OXACILLIN OR AMPICILLIN AGAINST ISOLATES MRSA (5-10). BACTERIA WERE INCUBATED WITH EMODIN ALONE (●) AND WITH AMPICILLIN (○) OR OXACILLIN (▼) OVER TIME. CFU, COLONY-FORMING UNITS.

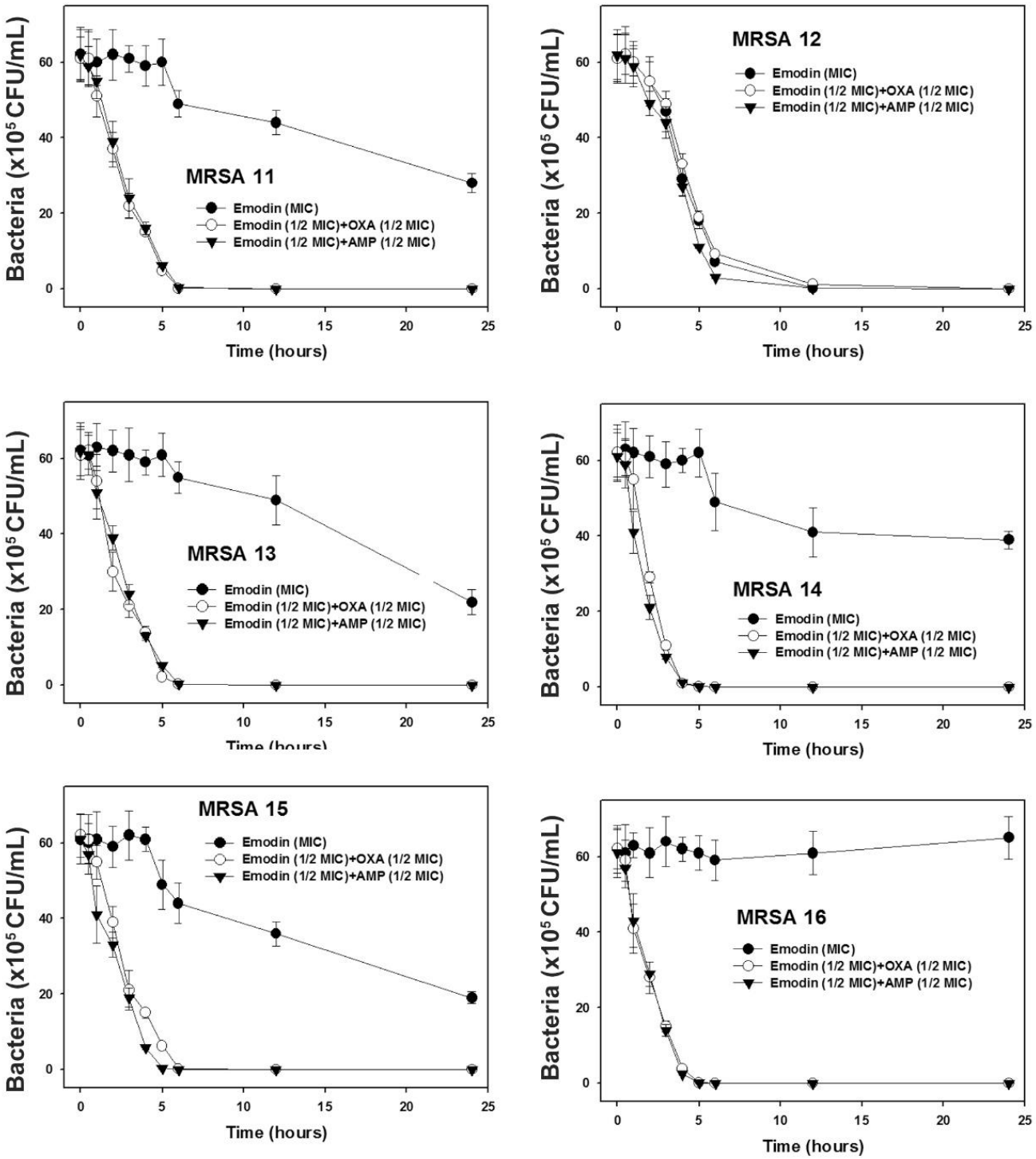

FIG. 3. TIME-KILL CURVES OF MIC OF EMODIN ALONE AND 1/2 MIC OF EMODIN WITH 1/2 MIC OF OXACILLIN OR AMPICILLIN AGAINST ISOLATES MRSA (11-16). BACTERIA WERE INCUBATED WITH EMODIN ALONE (●) AND WITH AMPICILLIN (○) OR WITH OXACILLIN (▼) OVER TIME. CFU, COLONY-FORMING UNITS.

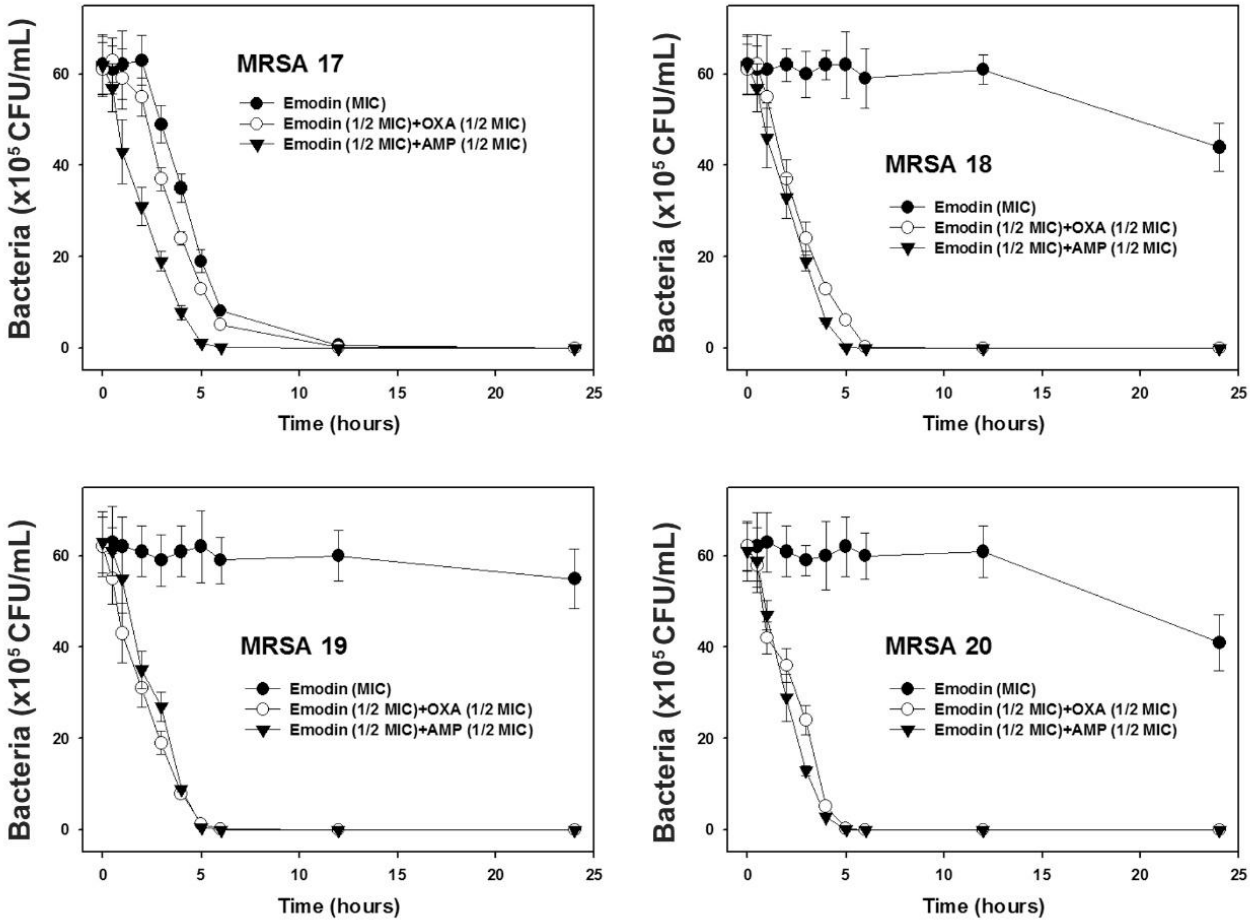

FIG. 4. TIME-KILL CURVES OF MIC OF EMODIN ALONE AND 1/2 MIC OF EMODIN WITH 1/2 MIC OF OXACILLIN OR AMPICILLIN AGAINST ISOLATES MRSA (17-20). BACTERIA WERE INCUBATED WITH EMODIN ALONE (●) AND WITH AMPICILLIN (○) OR WITH OXACILLIN (▼) OVER TIME. CFU, COLONY-FORMING UNITS.

In conclusion, our results of the antibacterial activity showed that emodin exhibited strong inhibitory activities against isolates MRSA. The combination effects of emodin with antibiotics were synergistic effect by FIC/FBC index <0.5 against most of tested clinic isolated MRSA. Emodin is expected to be recognized as natural sources for the development of new functional drugs against multi-resistant *S. aureus*, MRSA.

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by a Korea Research Foundation Grant funded by the Korean Government (KRF-20110023479).

AUTHORS' CONTRIBUTION

Jeong-Dan Cha and Sung-Mi Choi have substantial contributions to conception and design and drafting and revising it. Su-Mi Cha and Eun-Jin Jang have substantial contributions to acquisition and analysis of data.

REFERENCES

[1] B. Pourakbari, G. Rezaizadeh, S. Mahmoudi, and S. Mamishi "Epidemiology of nosocomial infections in pediatric patients in an Iranian referral hospital," *J Prev Med Hyg*, vol. 53, no. 4, pp. 204-206, 2012.

[2] S. Krishna, and L. S. Miller "Host-pathogen interactions between the skin and *Staphylococcus aureus*," *Curr Opin Microbiol*, vol. 15, no. 1, pp. 28-35, 2012.

[3] B. E. Cleven, M. Palka-Santini, J, Gielen, S. Meembor, M. Kronke, and O. Krut "Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray," *J Clin Microbiol*, vol. 44, no. 7, pp. 2389-2397, 2006.

[4] A. Zecconi, and F. Scali "*Staphylococcus aureus* virulence factors in evasion from innate immune defenses in human and animal diseases," *Immunol Lett*, vol. 150, no. 1-2, pp.12-22, 2013.

[5] S. Stefani, and P. E. Varaldo "Epidemiology of methicillin resistant staphylococci in Europe," *Clin Microbiol Infect*, vol. 9, no. 12, pp. 1179-1186, 2003.

[6] E. J. Choo, and H. F. Chambers "Treatment of Methicillin-Resistant Staphylococcus aureus Bacteremia," *Infect Chemother*, vol. 48, no. 4, pp. 267-273, 2016.

[7] S. M. Purrello, J. Garau, E. Giamarellos, T. Mazzei, F. Pea, A. Soriano, and S. Stefani "Methicillin-resistant Staphylococcus aureus infections: A review of the currently available treatment options," *J Glob Antimicrob Resist*, vol. 7, pp.178-186, 2016.

[8] J. Yue, B. R. Dong, M. Yang, X. Chen, T. Wu, and G. J. Liu "Linezolid versus vancomycin for skin and soft tissue infections," *Cochrane Database Syst Rev*, vol. 7, no. 1, pp.CD008056, 2016.

[9] C. Eckmann, D. Nathwani, W. Lawson, S. Corman, C. Solem, J. Stephens, C. Macahilig, J. Li, C. Charbonneau, N. Baillon-Plot, and S. Haider "Comparison of vancomycin and linezolid in patients with periopheral vascular disease and/or diabetes in an observational European study of complicated skin and soft-tissue infections due to methicillin resistant Staphylococcus aureus," *Clin Microbiol Infect*, vol. 21 no. Suppl 2, pp. S33-9, 2015.

[10] P. Dahiya, and S. Purkayastha "Phytochemica screening and antimicrobial activity of some medicinal plant multi-drug resistant bacteria from clinical isolates," *Indian J Pharm Sci*, vol. 74, no. 5, pp. 443-450, 2012.

[11] X. Su, A. B. Howell, and D. H. D'Souza "Antibacterial effects of plant-derived extracts on methicillin-resistant *Staphylococcus aureus," Foodborne Pathog Dis*, vol. 9, no. 6, pp. 573-578, 2012.

[12] F. Aqil, M. S. Khan, M. Osais, and I. Ahmad "Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin-resistant *Staphylococcus aureus*," *J Basic Microbiol*, vol. 45, no. 2, pp. 106-114, 2005.

[13] J. N. Eloff "Which extractant should be used for the screening and isolation of antimicrobial components from plants?," *J Ethnopharmacol*, vol. 60, no. 1, pp. 1-8, 1998.

[14] D. E. Djeussi, J. A. K. Noumedem, B. T. Ngadjui, and V. Kuete "Antibacterial and antibiaotic-modulation activity of six Cameroonian medicinal plants against Gran-negative multi-drug resistant phenotypes," *Bmc Compem Altern*, vol. 4, no. 16, pp.124, 2016.

[15] J. Rios, and M. Recio "Medicinal plants and antimicrobial activity," *J Ethnopharmacol*, vol. 100, no. 1-2, pp. 80-84, 2005.

[16] S. Z. Choi, S. O. Lee, K. U. Jang, S. H. Chung, S. H. Park, H. C. Kang, E. Y. Yang, H. J. Cho, and K. R. Lee "Antidiabetic stilbene and anthraquinone derivatives from *Rheum undulatum," Arch Pharm Res*, vol. 28, no. 9, pp. 1027-1030, 2005.

[17] Y. Li, H. Liu, X. Ji, and J. Li "Optimized separation of pharmacologically active anthraquinones in rhubarb by capillary electrochromatography," *Electrophoresis*, vol. 21, no. 15, pp. 3109-3115, 2000.

[18] J. Lu, Y. Xu, X, Wei, Z. Zhao, J. Xue, and P. Liu "Emodin Inhibits the Epithelial to Mesenchymal Transition of Epithelial Ovarian Cancer Cells," *Biomed Res Int*, vol. 2016, pp. 6253280, 2016.

[19] S. Y. Park, M. L. Jin, M. J. Ko, G. Park, and Y. W. Choi "Anti-neuroinflammatory Effect of Emodin in LPS-Stimulated Microglia: Involvement of AMPK/Nrf2 Activation," *Neurochem Res*, vol. 41, no. 11, pp. 2981-2992, 2016.

[20] C. L. Zhang, L. N. Cong, R. Wang, Y. Wang, K. T. Ma, L. Zhao, J. Q. Si, and L. Li "Emodin-induced increase in expression of β1 subunit of BKCa channel mediates relaxation of cerebral basilar artery in spontaneously hypertensive rats," *Sheng Li Xue Bao*, vol. 66, no. 3, pp. 289-94, 2014.

[21] M. Liu, W. Peng, R. Qin, Z. Yan, Y. Cen, X. Zheng, X. Pan, W. Jiang, B. Li, X. Li, and H. Zhou "The direct anti-MRSA effect of emodin via damaging cell membrane," *Appl Microbiol Biotechnol*, vol. 99, no. 18, pp.7699-709, 2015.

[22] X. Y. Dai, W. Nie, Y. C. Wang, Y. Shen, Y. Li, and S. J. Gan "Electrospun emodin polyvinylpyrrolidone blended nanofibrous membrane: a novel medicated biomaterial for drug delivery and accelerated wound healing," *J Mater Sci Mater Med*, vol. 23, no. 11, pp. 2709-2716, 2012.

[23] X. Ding, B. Yin, L. Qian, Z. Zeng, Z. Yang, H. Li, Y. Lu, and S. Zhou "Screening for novel quorum-sensing inhibitors to interfere with the formation of Pseudomonas aeruginosa biofilm," *J Med Microbiol*. vol. 60, no. Pt 12, pp.1827-1834, 2011.

[24] L. Li, X, Song, Z. Yin, R. Jia, Z. Li, X. Zhou, Y. Zou, L. Li, L. Yin, G. Yue, G. Ye, C. Lv, W. Shi, and Y. Fu "The antibacterial activity and action mechanism of emodin from Polygonum cuspidatum against Haemophilus parasuis in vitro," *Microbiol Res*, vol. 186-187, pp. 139-145, 2016.

[25] F. Wallet, M. Roussel-Delvallez, and R. J. Courcol "Choice of a routine method for detecting methicillin-resistance in staphylococci," *J Antimicrob Chemother*, vol. 37, no. 5, pp. 901-909, 1996.

[26] M. W. Climo, R. L. Patron, and G. L. Archer "Combinations of vancomycin and beta-lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin," *Antimicrob Agents Chemother*, vol. 43, no. 7, pp. 1747-1753, 1999.

[27] J. D. Cha, J. H. Lee, K. M. Choi, S. M. Choi, and J. H. Park "Synergistic Effect between Cryptotanshinone and Antibiotics against Clinic Methicillin and Vancomycin-Resistant Staphylococcus aureus," *Evid Based Complement Alternat Med*. Vol. 2014, pp. 450572, 2014.

[28] J. H. Lee, Y. G. Kim, S. Y. Ryu, and J. Lee "Calcium-chelating alizarin and other anthraquinones inhibit biofilm formation and the hemolytic activity of *Staphylococcus aureus*," *Sci Rep*, vol. 14, no. 6, pp. 19267, 2016.

[29] L. K. Omosa, J. O. Midiwo, A. T. Mbaveng, S. B. Tankeo, J. A. Seukep, I. K. Voukeng, J. K. Dzotam, J. Isemeki, S. Derese, R. A. Omolle, T. Efferth, and V. Kuete "Antibacterial activities and structure-activity relationships of a panel of 48 compounds from

Kenyan plants against multidrug resistant phenotypes," *Springerplus*, vol. 27, no. 1, pp. 901, 2016.

[30] D. Dey, R. Ray, and B. Hazra "Antitubercular and antibacterial activity of quinonoid natural products against multi-drug resistant clinical isolates," *Phytother Res*, vol. 28, no. 7, pp.1014-1021, 2014.

[31] B. Périchon, and P. Courvalin "Synersism between beta-lactams and glycopeptides against VanA-type methicillin-resistant *Staphylococcus aureus* and heterologous expression of the vanA operon," *Antimicrob Agents Chemother*, vol. 50, no. 11, pp. 3622-3630, 2006.

[32] R. Qin, K. Xiao, B. Li, W. Jiang, W. Peng, J. Zheng, and H. Zhou "The combination of catechin and epicatechin gallate from Fructus crataegi potentiates beta-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro and in vivo," *Int J Mol Sci*, vol. 14, no. 1, pp. 1802-1821, 2013.

[33] J. G. Holler, H. C. Slotved, P. Mølgaard, C. E. Olsen, and S. B. Christensen "Chalcone inhibitors of the NorA efflux pump in *Staphylococcus aureus* whole cells and enriched everted membrane vesicles," *Bioorg Med Chem*, vol. 20, no. 14, pp. 4514-4521, 2012.