

## Synergistic effect of emodin in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*

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### 메티실린에 내성을 가진 포도상구균에 대하여 에모딘을 포함한 암피실린 또는 옥사실린의 상승효과

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#### Objectives

The pharmacological tools available to cure MRSA being limited today, new agents to treat MRSA-associated infections are greatly needed. The treatment guidelines for these infections recommend combinations of antibiotics against these pathogenic organisms. *Rheum palmatum*, popularly known as Daehwang, has traditionally been used as an oriental folk medicine. The emodin (EM) is biologically active and naturally occurs in anthraquinone found in *Rheum palmatum* and related plants such as rhubarb. Thus, here we present a study that shows the antimicrobial activity of EM against MRSA and Methicillin sensitive *Staphylococcus aureus* (MSSA) strains, as well as its ability to lower the MICs of  $\beta$ -lactam antibiotics.

#### Materials and Methods

**Bacterial strains and growth conditions** : Among the 17 *S. aureus* strains used in this study, 15 clinical isolates (MRSA) were obtained from 15 different patients at Wonkwang University Hospital (Iksan, South Korea). The other two strains were *S. aureus* ATCC 33591 (methicillin-resistant strain) and *S. aureus* ATCC 25923 (methicillin-susceptible strain).

**Determination of antibacterial activity by the disc diffusion method** : Sterile paper discs were loaded with 20  $\mu$ L of EM dissolved in dimethyl sulfoxide. The bacterial suspensions were diluted to match the 0.5 McFarland standard scale, and were further diluted to obtain the final inoculum. The MHA was poured into Petri dishes and inoculated with 100  $\mu$ L of the suspension containing  $1.5 \times 10^8$  cfu/mL of bacteria. AM and OX were used as positive control, and the discs treated with 50% DMSO were used as negative controls. The plates were placed in an incubator at 37°C for 24 h. The inhibition zone diameter around each of the discs was measured and recorded at the end of the incubation period.

**Determination of minimal inhibitory concentrations (MICs)** : The EM and the two antimicrobial agents were dissolved in MHA with 10% DMSO. Agar dilution method was used to determine the MICs of ampicillin, oxacillin or emodin. All strains suspensions were adjusted to the 0.5 McFarland standards.

Final inoculums were adjusted to the  $10^6$  cfu/spot.

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**Determination of in vitro combinations :** The antimicrobial combination assayed included EM plus AM or OX. Serial dilutions of the EM with AM or EM with OM antimicrobial agents were mixed in cation-supplemented MHA. The final bacterial concentration after inoculation was  $5 \times 10^6$  cfu/mL. The MIC was defined as the lowest concentration of drug alone or in combination inhibiting the visible growth. The in vitro interaction was quantified by determining the fractional inhibitory concentration (FIC). The FIC index was determined using the following formula: FIC index = FICA + FICB = [A]/MICA + [B]/MICB

## Results

**Table 1.** The antimicrobial activity (as inhibition zone diameters) of EM, AM and OX against *S. aureus* strains

<i>S. aureus</i> strain	Zone of inhibition (mm)			
	EM		AM <sup>†</sup>	OX
	10 µg	50 µg	10 µg	1 µg
MSSA ATCC 25923	12	19	30	16
MRSA ATCC 33591	26	30	20	– <sup>‡</sup>
DPS1*	15	18	13	–
DPS2	18	23	14	7
DPS15	21	23	18	–

\* AM resistance  $\leq 28$  mm, OX resistance  $\leq 10$  mm.

<sup>‡</sup> –, absence of inhibition. ⇨ ⇨

**Table 2.** The MICs of EM, AM, OX against *S. aureus* strain.

<i>S. aureus</i> Strain	MIC (µg/ml)		
	EM	AM	OX
MSSA ATCC 25923	25	0.9	1.9
MRSA ATCC 33591	25	62.5	1000
DPS1*	25	31.25	500
DPS2	1.56	31.25	1000
DPS15	1.56	125	500

**Table 3.** Result of the combined effect of EM and AM against *S. aureus*.

<i>S. aureus</i> Strain	MIC emodin (µg/ml) <sup>†</sup>				FICI <sup>‡</sup>
	Alone <sup>¶</sup>	With AM <sup>§</sup>	Alone	With EM	
MSSA ATCC 25923	25	6.25	0.9	0.22	0.5
MRSA ATCC 33591	25	6.25	62.5	15.62	0.5
Clinical isolates DPS 1*	25	6.25	31.25	7.81	0.5
DPS 2	1.56	0.39	31.25	7.81	0.5
DPS 15	1.56	0.39	125	15.31	0.37

<sup>‡</sup> FICI, Fractional Inhibitory Concentration Index: <0.5, synergy; 0.5-0.75, partial synergy; 0.76-1.0, additive effect; >1.0-4.0, indifference; and >4.0, antagonism (Choi et al., 2008).

<sup>¶</sup> Alone, alone compound.

<sup>§</sup> With AM, EM + AM.

**Table 4.** Result of the combined effect of EM and OX against *S. aureus*.

<i>S. aureus</i> Strain	MIC emodin (µg/ml) <sup>†</sup>				FICI <sup>‡</sup>
	Alone <sup>¶</sup>	With OX <sup>§</sup>	Alone	With EM	
MSSA ATCC 25923	25	6.25	1.9	0.47	0.5
MRSA ATCC 33591	25	6.25	1000	250	0.5
Clinical isolates DPS 1*	25	6.25	500	125	0.5
DPS 2	1.56	0.39	1000	250	0.5
DPS 15	1.56	0.39	500	125	0.5