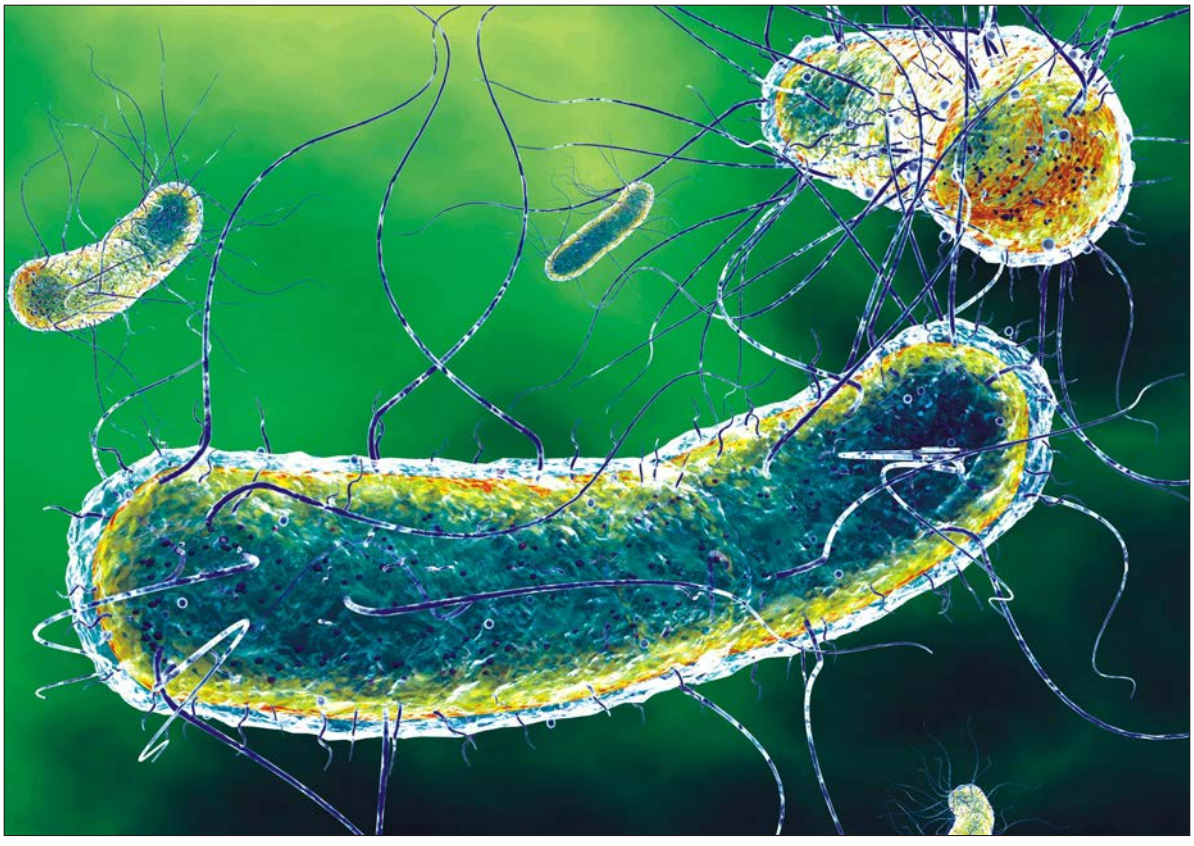

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Antibacterial Activity of *Malvastrum coromandelianum* Garcke Against Methicillin-Sensitive and Methicillin-Resistant Strains of *Staphylococcus aureus*

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Abstract: Crude water extract of aerial part of *Malvastrum coromandelianum* Garcke (ME) was studied for anti-*Staphylococcus aureus* activity against *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and 6 clinical isolates of each group of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). ME exhibited moderate anti-bacterial activity against MSSA (Minimum Inhibitory Concentration (MICs) = 2.5-5 mg mL^{-1}, Minimum Bactericidal Concentration (MBCs) = 10-80 mg mL^{-1}), most of MRSA (MICs = 2.5-5 mg mL^{-1}, MBCs = 10-20 mg mL^{-1}) except for MRSA strain No. 6 (MBC >160 mg mL^{-1}), *S. aureus* ATCC 25923 (MIC = 5 mg mL^{-1}, MBC = 20 mg mL^{-1}) and *S. aureus* ATCC 29213 (MIC = 5 mg mL^{-1}, MBC = 10 mg mL^{-1}). These results suggest that ME potentially benefits as alternative remedy for MRSA.

Key words: Anti-*Staphylococcus aureus*, *Malvastrum coromandelianum*, antimicrobial, Methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA)

INTRODUCTION

Malvastrum coromandelianum Garcke, or Threelobe false-mallow is a shrub belong to Malvaceae Family. It has been reported hypoglycemic activity, antipyretic activity, affecting smooth muscle activity and ulceroprotective activity (Andrade-Cetto and Heinrich, 2005; Dahanukar *et al*., 2000).

Staphylococcus aureus cause serious community-acquired and nosocomial infection (Chambers, 1997). Epidemiological studies on high-level methicillin-resistant *S. aureus* (MRSA), which is resistant to numerous antibiotics and antiseptics, revealed nosocomial out breaks with clones dissemination nationally and internationally. In 1961, there were reports from the United Kingdom on *S. aureus* that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) and MRSA isolates were soon recovered from other European countries and later from Japan, Australia and the United States (Enright *et al*., 2002). MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and community. The methicillin-resistance gene (mecA) encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species.

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In vitro antimicrobial screening in this study permits the selection of crude plant extract with potentially properties to be used for further chemical and pharmaceutical studies. The water extract from *M. coromandelianum* Garcke was selected for antimicrobial activity testing against clinical isolated of *S. aureus*, including MRSA strains. The objective of this study was to assess the *in vitro* anti-staphylococcal activity of the water extract from plant, *M. coromandelianum* Garcke.

MATERIALS AND METHODS

Plant Material

Malvastrum coromandelianum Garcke (Malvaceae) was identified by Royal Forest department of Thailand and cultivated on January 2006 in open field at Singburi province, Thailand. Aerial part of plant was collected in the beginning of June 2006 and the following extraction processes were finished in the end of June 2006. The antimicrobial investigation in this article was done on December of 2006.

Extraction

The extraction method was modified from Chinese traditional method (Lang and Wai, 2003). Dried aerial parts were collected and 10 kg of dried plant was extracted by boiling in 100 L of water for 30 min. The extraction was repeated twice. The pooled filtrates were spray-dried. The yield of spray-dried extract powder was 8-10% w/w of raw material. In this study used one batch of extraction and HPLC chemical finger print was determined for each experiment to confirm the same quality of ME. Tested microorganisms

Twelve clinical isolates of *S. aureus* (supplied by Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand) were included in this study. The tested isolates were divided into 2 groups, 6 of each, according to susceptibility to methicillin where MRSA referred to methicillin-resistant strains and MSSA referred to methicillin-susceptible strains. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were also used as reference strains.

Antimicrobial Assay

MICs of crude water extract of ME were determined by agar dilution method (Merck) (Lorian, 1996) while MBCs were determined by broth macro-dilution method were (Lorian, 1996) and reference antibiotics Oxacillin (Sigma Chemical Co., St.Louis, USA). Inoculates were prepared in the same medium at density adjusted to 0.5 McFarland turbidity standard (10^8 colony-forming units (cfu mL^{-1}) and two fold dilution for the broth macro-dilution procedure. The inoculated tube were incubated at 37°C and the MICs were recorded after 24 h of incubation. The MIC was defined as the lowest concentration of ME or Oxacillin at which the microorganism tested did not showed visible growth while MBC was defined as the minimum bactericidal concentration with negative subcultures on agar medium. Values were means of triplicate.

RESULTS AND DISCUSSION

Methicillin-resistant *S. aureus* (MRSA) is increased and caused of nosocomial infection problem in many countries (Abrahamian and Snyder, 2007). Recently, only few antimicrobial agent including vancomycin and teicoplanin are still effective against MRSA. Thus, this pathogen can cause serious infection in various body systems in patients in particular the ICU patients.

The screening of plant extract to inhibit growth of MRSA is during interesting. Agar dilution and broth macro dilution were selected to evaluated *in vitro* antimicrobial activity tests in this study. Due to the red brown color of ME the MICs from only broth macro dilution was difficult to observed.

Table 1: Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBCs) of ME against strains of *S. aureus*, standard antibacterial agent: oxacillin

	MIC		MBC	
Bacteria	ME (mg mL^{-1})	Oxacillin (μg mL^{-1})	ME (mg mL^{-1})	Oxacillin (μg mL^{-1})
S. aureus ATCC 25923	5.0	0.25	20	0.50
S. aureus ATCC 29213	5.0	0.25	10	1.00
MSSA 1	5.0	0.25	10	2.00
MSSA 2	2.5	0.25	80	1.00
MSSA 3	5.0	0.50	80	32.00
MSSA 4	5.0	0.25	10	8.00
MSSA 5	5.0	0.50	20	16.00
MSSA 6	5.0	0.50	20	0.50
MRSA 1	5.0	>256	10	>256
MRSA 2	2.5	>256	10	>256
MRSA 3	2.5	>256	10	>256
MRSA 4	2.5	>256	20	>256
MRSA 5	2.5	>256	20	>256
MRSA 6	5.0	>256	>160	>256

Therefore, agar dilution method was used to determine MICs of ME while MBCs was from the broth macro dilution method. The water boiling extraction methods which have been used for thousands of years in China to prepare herbal remedies (Lang and Wai, 2003) was used in this study. Furthermore, water was no toxic drug vehicle and less interfering effect on experiment. The record of using of plant in family Malvaceae as medicinal plant has been report such as in Turkey (Abu-Shanab *et al*., 2003), New Zealand (Bloor, 1995) and Iran (Sharhidi Bonjar, 2004). The plant in Malvaceae has been tested against MRSA for *Althaea officinalis* and showed no inhibition effect against MRSA (Abu-Shanab *et al*., 2006).

Crude water extract of *M. coromandelianum* Garcke showed antibacterial activity against MSSA (MICs = 2.5-5 mg mL^{-1}, MBCs = 10-80 mg mL^{-1}) and MRSA (MICs = 2.5-5 mg mL^{-1}, MBCs = 10-20 mg mL^{-1}) as well as *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213. Although ME did not showed bactericidal activity to all tested strains of MRSA (MRSA No. 6; MBC >160 mg mL^{-1}) but it did show inhibitory effect at considerable low concentration for crude water extract against most of tested MRSA (Table 1).

This study is the first reported of antibacterial activity of *M. coromandelianum* water extract. In addition the results from this study provide very useful information concerning new natural drug discovery.

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