



รายงานการวิจัยฉบับสมบูรณ์

โครงการ ฤทธิ์ต้านเชื้อ Methicillin-resistant *Staphylococcus aureus*

ของตำรับยาแผนไทยในภาคใต้สำหรับรักษาโรคผิวหนัง

โดย ดร. ศศิธร ชูศรี และคณะ

กุมภาพันธ์ 2555

สัญญาเลขที่ TTM540049S

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คณะผู้วิจัย

ดร. ศศิธร ชูศรี

คณะกรรมการแพทย์แผนไทย มหาวิทยาลัยสงขลานครินทร์

ดร. สุรศักดิ์ สัมสุวรรณ

คณะกรรมการแพทย์แผนไทย มหาวิทยาลัยสงขลานครินทร์

ดร. เกศริน มณีบุญ

คณะกรรมการแพทย์แผนไทย มหาวิทยาลัยสงขลานครินทร์

ABSTRACT

The antibacterial activities of different southern Thai medicinal plant formulas as well as their components were tested against skin and wound pathogens, including *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, multidrug-resistant (MDR) *Escherichia coli*, MDR *Pseudomonas aeruginosa*, and MDR *Acinetobacter baumannii*. Eight formulas were found to inhibit the growth of at least one of the tested pathogens with inhibition zones ranging from 7.5 to 21.0 mm. THR-SK004 and THR-SK005 extracts displayed broad spectrum activity against both Gram-positive and Gram-negative. Our results indicated that THR-SK004 and its medicinal components, *Metroxylon sagu* Rottb. and *Orxylum indicum* Vent. exhibited good antibacterial activity with MIC values in the range of 30 to 1,000 µg/mL. This antibacterial property tends to support the use of traditional medicine for the treatment of bacterial skin infections. Water and ethanol extracts of traditional Thai herbal formulas including, THR-SK004, THR-SK010, and THR-SK011 were tested for their antibacterial potency against the most common cause of wound infection pathogens; methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA). Among the tested extracts, the ethanol extract of THR-SK010 that consists of four herbs: *Curcuma longa* L., *Areca catechu* L., *Oryza sativa* L., and *Garcinia mangostana* L. possessed promising antibacterial activities with the minimum inhibitory concentrations (MICs) of 4 and 8 µg/ml against MRSA and MSSA isolates, respectively. The ethanol extract exhibited bacteriostatic activity demonstrated by time kill assay.

Development of biofilm is a key mechanism involved in *Staphylococcus* spp. virulence during infections. We aimed to investigate anti-biofilm formation and mature biofilm eradication ability of the ethanol and water extracts of Thai traditional herbal recipes including THR-SK004, THR-SK 010, and THR-SK 011 against *S. epidermidis*. A biofilm

forming reference strain, *S. epidermidis* ATCC 35984 was employed as a model for searching anti-biofilm agents by MTT reduction assay. The results revealed that the ethanol extract of THR-SK004 (THR-SK004E) inhibited the formation of *S. epidermidis* biofilm on polystyrene surfaces. Furthermore, treatments with the extract efficiently inhibited the biofilm formation of the pathogen on glass surfaces determined by scanning electron microscopy and crystal violet staining. In addition, THR-SK010 ethanol extract (THR-SK010E; 0.63-5 $\mu\text{g}/\text{mL}$) could decrease 30 to 40% of the biofilm development. Almost 90% of a 7-day-old staphylococcal biofilm was destroyed after treatment with THR-SK004E (250 and 500 $\mu\text{g}/\text{mL}$) and THR-SK010E (10 and 20 $\mu\text{g}/\text{mL}$) for 24 h. Therefore, our results clearly demonstrated THR-SK004E could prevent the staphylococcal biofilm development whereas both THR-SK004E and THR-SK010E possessed remarkable eradication ability on the mature staphylococcal biofilm.

Anti-inflammatory activities of the extracts were assessed by detection of the inhibition of NO production in LPS-stimulated RAW 264.7 cells. Anti-oxidant activities of the extracts were measured using DPPH, super oxide, and hydroxyl radicals scavenging assays. Moreover, *in vitro* cytotoxic effects of the formula extracts were carried out on Vero cells. THR-SK010 ethanol extract additionally offered the highest anti-inflammatory activity ($\text{IC}_{50}=71.06 \mu\text{g}/\text{mL}$) as well as DPPH and hydroxyl radical scavenging activities ($\text{IC}_{50}=19.24$ and $13.58 \mu\text{g}/\text{mL}$). All the tested formulas were found to be non cytotoxic or with very low toxicity on Vero cells.

The antibacterial potency, anti-inflammatory, and antioxidant activities provide scientific information that support the topical use of THR-SK010 for wound treatment in the traditional medicine of southern Thailand.

ACKNOWLEDGEMENTS

I would like to express my deep and sincere gratitude to my mentor, Assoc. Prof. Dr. Supayang Voravuthikunchai for her wide knowledge, thoughtfulness supervision, and endless stimulation have been of great value for me.

I am also deeply indebted to my colleagues, Dr. Surasak Limsuwan and Dr. Katesarin Maneenoon for their kindly collaboration, comments, and suggestion in this research and in the preparation of this document as well as my students (TTM 03 and TTM 04) at Bacteriology Research Laboratory, Faculty of Traditional Thai Medicine, Prince of Songkla University for all their hard works. My special application is expressed my friends at Natural Product Research Center, Faculty of Science, Prince of Songkla University for their friends, helps, and supports.

This work was supported by Grants for Development of New Faculty Staff, The Annual Income Budget of Prince of Songkla University (TTM540049S, fiscal year 2011-2013)

Sasitorn Chusri

February 2012