รายงานการวิจัยฉบับสมบูรณ์

โครงการ ฤทธิ์ด้านเชื้อ Methicillin-resistant Staphylococcus aureus

ของตัวรับยาแผนไทยในภาคใต้สำหรับรักษาโรคผิวหนัง

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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 investigated 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 µg/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 µg/mL) and THR-SK010E (10 and 20 µg/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 (IC₅₀=71.06 µg/mL) as well as DPPH and hydroxyl radical scavenging activities (IC₅₀=19.24 and 13.58 µg/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.
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