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Full Length Research Paper

In vitro antibacterial activity of ethanol extracts of nine herbal formulas and its plant components used for skin infections in Southern Thailand

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The antibacterial activities of different Southern Thailand medicinal plant formulas as well as their medicinal plant 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*. Nine medicinal plant formulas consisting of 41 medicinal plants were chosen on the basis of their traditional use against skin infections. The preliminary antimicrobial activity of the ethanol extracts formula was carried out by disc diffusion test. The extracts which showed inhibitory effect were further investigated for minimum inhibitory concentration (MIC) using the broth microdilution method. 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. Results indicated that THR-SK004 and its medicinal components, *Metroxylon sagu* Rottb. and *Oroxylum indicum* Vent. exhibit 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 in the treatment of bacterial skin infections.

Key words: Antibacterial activity, medicinal plant formulas, medicinal plant, traditional Thailand medicine.

INTRODUCTION

Skin and soft tissue infections (SSTIs) represent common health care problems that range from simple uncomplicated superficial skin infections such as cellulitis, furuncles, superficial abscesses, and wound infections to life-threatening infections like necrotizing fasciitis or gas gangrene (Sarkar and Napolitano, 2010). Complicated SSTIs that involve deeper skin structures require significant surgical intervention and usually occur in the presence of significant co-morbidities (Kang et al., 2011). Certain conditions may influence to complicate SSTIs such as trauma, pre-existing skin conditions, diabetes mellitus, or immune suppression (Kanuck et al., 2006; Torralba and Quismorio, 2009). SSTIs in hospitalized patients are associated with considerable patient morbidity, mortality, and escalating healthcare expenditures, because of the need for additional surgery, antimicrobial therapy, and prolonged hospital stay (Lipsky et al., 2007). Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), *S. aureus*, and *Streptococcus pyogenes* (Lipsky et al., 2007; Lamagni et al., 2008; Tong et al., 2011) were frequently implicated as causes of SSTIs. In addition, cutaneous infections are caused by a variety of other microorganisms, including *Acinetobacter baumannii*, *Enterococcus* species, *Escherichia coli*, and *Pseudomonas aeruginosa* (Manfredi et al., 2002; Lee et al., 2009; Marioni et al., 2010). Antibacterial agents such as anti-staphylococcal penicillin,

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cephalosporins, trimethoprim-sulfamethoxa- zole, fluoroquinolones or rifampicin were employed as treatment options for SSTI (Moulin et al., 2008). However, the emergence of pandrug resistant bacteria has become a major problem; thereby leading to a continuous effort by the pharmaceutical industry to develop new antimicrobial agents for the treatment of the infections. The use of herbal medicines by traditional healers for the treatment of diseases remains the main stay of health care system. It is gaining increasing popularity especially among the rural population in the developing countries since it is an efficacious and cheap source of medical care. In low- and middle-income countries, for example, Nigeria, Thailand, and Turkey, the population depends on traditional medicine to treat wound infections. A wide range of medicinal plants, for example, *Ageratum conyzoides* (Oladejo et al., 2003), *Aloe vera* (Somboonwong et al., 2000), *Flabellaria paniculata* (Olugbuyiro et al., 2010), *Ocimium gratisimum* (Nweze and Eze, 2009), and *Prosopis africana* (Ezike et al., 2010) demonstrated pharmacological activities relevant to multiple aspect of wound healing property. Numerous Thailand ethnomedicinal plants generally used for the treatment of skin diseases, dermatitis, diarrhoea, wounds, and localized skin swellings have been documented as antibacterial, antifungal, antiviral, and antiprotozoan agents (Voravuthikunchai et al., 2004; Woradulayapinij et al., 2005; Sawangjaroen et al., 2006). Even though, Thailand medicinal plant formulas have long been used for healing dermatological conditions, there is no scientific evidence to prove their claims.

Therefore, in the present investigation, medicinal plant formulas and their herbal components employed in Southern Thai folk medicine for curing skin and wound infections were justified for their antibacterial activities against multidrug-resistant Gram-negative and Gram-positive bacteria.

MATERIALS AND METHODS

Southern Thailand herbal formulas and preparation of extracts

Nine Thailand medicinal formulas, selected on their traditional uses as anti-skin infectious agents were studied (Table 1). With the exception of THR-SK007, the formulas consist of equal amounts (100 g) of their medicinal plant components. THR-SK007 contains 200 g of *Cissampelos pareira*, 100 g of *Cinnamomum iners*, 100 g of *Ixora finlaysoniana*, and 100 g of *Morus alba*. The formula powder was extracted (1:2, w/v) with 95% ethanol at room temperature for 7 days. Similarly, the herbal component powder was separately extracted under this condition. After filtration, excess solvent was removed with a rotatory evaporator, kept at 55°C until they were completely dry and stored in a sterile screw-capped bottle at -20°C. Extraction yield (%, w/w) was calculated as the ratio of the weight of the extract to the weight of the crude herb powder.

Selected bacterial strains

A panel of skin and wound pathogens and their antibiotic resistant profiles were described in Table 2. Multi-drug resistant clinical isolates were kindly provided by Natural Product Research Center, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand. *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *A. baumannii* ATCC 19606 were included as quality control strains. Except for *S. pyogenes* NPRC 101, the bacteria were grown on tryptic soy agar (TSA) at 37°C for 18 to 24 h. *S. pyogenes* was cultured on brain heart infusion agar (BHIA) at 37°C with 5% CO_2 for 24 to 48 h. Antibacterial susceptibility patterns of the clinical isolates conducted by disc diffusion method (CLSI, 2009a) were obtained from Natural Product Research Center, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand.

Antibacterial activities of Southern Thailand medicinal plant formulas

Paper disc agar diffusion method (CLSI, 2009a) was used for primary screening of antibacterial activity of the formula extracts. An aliquot of 10 µl of 250 mg/ml of each extract was individually applied to sterile filter paper discs (Whatman no. 1; 6 mm in diameter). The discs were placed on the surface of Mueller Hinton agar (MHA) plates or BHIA previously seeded with the culture of the tested bacteria (10^8 CFU/ml). Antibiotic susceptibility discs including penicillin G (10U/disc) and ciprofloxacin (5 µg/disc) were used as controls, while 10 µl of dimethyl sulfoxide (DMSO) was included as negative control. The plates were then incubated for 24 h at 37°C, except for *S. pyogenes* seeded plate that was incubated under 5% CO_2 condition. The antibacterial activity was evaluated by measuring the diameter of inhibition zones. The experiment was performed in duplicate and the means of inhibition zone diameters were calculated.

Assessment of minimum inhibitory concentration (MIC)

A modified broth microdilution method according to Clinical and Laboratory Standard Institute (CLSI, 2009b) was carried out to obtain the MICs and minimum bactericidal concentrations (MBCs) of the effective Southern Thailand herbal formulas and their herbal component extracts. Two-fold serial dilution of the extracts was performed to obtain concentrations ranging from 16 to 1,000 µg/ml. The bacterial inoculum (100 µl) containing 10^6 CFU/ml was added to each well. Positive control with 1% DMSO and negative control without an inoculum added were included. The microtiter plates were then incubated at 37°C for 18 h. The MIC values were observed at least in duplicate as the lowest concentration of plant extracts that produced a complete suppression of bacterial growth. Minimum bactericidal concentrations are performed with the extracts that gave significant MIC values by sub-culturing on fresh MHA. The effective formula was selected as the test material that produced the lowest MIC value.

RESULTS

The selection of the medicinal plant formulas for this study was based on their traditional use for the treatment of skin and wound infections. The percentage yield of the extracts was calculated as shown in Table 1. The formula extracts from 95% ethanol exhibited a wide range of yield from 0.29 to 3.57% (w/w). The maximum yield for the formula extract was obtained from THR- SK004 (3.57%), while *Plumeria obtusa*, the herbal component of THR-SK001 and THR-SK009, gave the highest extraction

Table 1. Herbal components, tradition uses, and extraction yields of selected Southern Thailand herbal recipes for skin infectious treatments.

Herbal recipe and their herbal component	Botanical family	Plant part	Yield (%)
THR-SK001 (A)[a]: Used for treatment of skin rashes with fever			1.9
Amaranthus spinosus L.	Amaranthaceae	Whole plant	1.3
Amaranthus lividus L.	Amaranthaceae	Whole plant	1.15
Flacourtia rukam Zoll. & Moritzi	Flacourtiaceae	Wood	0.98
Artemisia annua L.	Compositae (Asteraceae)	Whole plant	1.21
Ficus pubigera Wall	Moraceae	Wood	1.31
Bridelia ovata Decne	Euphorabiaceae	Leaf	1.26
Derris scandens (Roxb.) Benth	Papilionaceae	Stem	1
P. obtusa L.	Apocynaceae	Wood	4.4
Tribulus cistoides L.	Zygophyllaceae	Whole plant	2.26
THR-SK002 (A): Used for treatment of skin rashes			1.38
Ocimum tenuiflorum L.	Lamiaceae	Whole plant	2.86
Xantonnea parvifolia Craib	Rubiaceae	Whole plant	0.76
Ziziphus oenoplia (L.) Mill. var. *oenoplia*	Rhamnaceae	Stem	0.3
Ricinus communis L.	Euphorbiaceae	Root	0.76
Dendrotrophe buxifloia (Blume) Miq.	Santaliaceae	Stem	ND
THR-SK003 (A): Used for treatment of chronic skin infections in children			0.42
Smilax spp.	Smilacaceae	Rhizome	1.7
Tinospora crispa (L.) Miers ex Hook.f. & Thoms.	Menispermaceae	Stem	1.38
THR-SK004 (B): Used for treatment of wound infections			3.57
M. sagu Rottb.	Palmae	Whole plant	3.85
O. indicum Vent.	Bignoniaceae	Bark	1.23
Lindenbergia philippensis (Cham.) Benth.	Scrophulariaceae	Whole plant	0.95
THR-SK005 (C): Used for treatment of chronic skin infections			2.16
Datura fastuosa L.	Solanaceae	Seed	1.33
Pandanus humilis Lour.	Pandanaceae	Leaf	0.45
Hydonocarpus anthelminthicus Pierre ex Laness.	Flacourtiaceae	Fruit	2.68
H. odorata Roxb.	Dipterocarpaceae	Resin	3.03
Caesalpinia bonduc (L.) Roxb.	Leguminosae- Caesalpinioideae	Fruit	0.98
E. pursaetha DC	Mimosae	Fruit	1.81

[a]The recipes are kindly suggested by Southern folk healers including Mr. Nhom Ratchgaew (A), Mr. Earn Thongsongsi (B), Mr. Rhawwan Watchjirasophon (C), Mr. Gard Eardmong (D), and Mr. Lim Rugtong (E), [b]ND; not determined

Table 1. Contd.

THR-SK006 (D): Used for treatment of skin inflammation and skin swelling			1.42
Oryza sativa L.	Graminae (Poaceae)	Seed	0.2
Curcuma longa L.	Zingiberaceae	Rhizome	2.18
Callicarpa candicans (Burm.f.) Hochr.	Labiatae (Laminaceae)	Stem	1.68
THR-SK007 (A): Used for treatment of an abscess			0.29
C. pareira L.var. hirsutus (Buch.ex.DC) Ferman	Menispermaceae	Root	ND
C. iners Reinw. ex Blume	Lauraceae	Wood	0.8
I. finlaysoniana Wall. ex. G. Don.	Rubiaceae	Root	ND
M. alba L.	Maraceae	Whole plant	0.33
THR-SK008 (E): Used for treatment of chronic wound infections			0.75
Triumfetta rhomboidea Jacq.	Tiliaceae	Whole plant	ND
Ficus racemosa Linn.	Moracea	Bark	1
Barringtonia acutangula (L.) Gaertn.	Leguminosae	Wood	0.45
Caesalpinia enneaphylla Roxb.	Ceasalpiiiaceae	Wood	ND
Sida rhombifolia L.	Malvaceae	Root	0.56
Ceiba pentandra (L.) Gaertn.	Bombaceae	Root	0.45
THR-SK009 (C): Used for treatment of wound or skin inflammation			1.42
Albizia myriophylla Benth.	Leguminosae	Root	1.83
P. obtusa L.	Apocynaceae	Wood	4.4
Leea indica (Burm.f.) Merr.	Leeaceae	Leaf/Wood	0.33

[a]The recipes are kindly suggested by Southern folk healers including Mr. Nhom Ratchgaew (A), Mr. Earn Thongsongsi (B), Mr. Rhawwan Watchjirasophon (C), Mr. Gard Eardmong (D), and Mr. Lim Rugtong (E), [b]ND; not determined

yield (4.40%).

As shown in Table 2, the antibacterial activities of the formula extracts were further investigated against a panel of clinically isolated multidrug-resistant (MDR) SSTI pathogens including MRSA, MDR *E. coli*, MDR *P. aeruginosa*, and MDR *A. baumannii*. Preliminary test for antibacterial activity of the ethanol extracts of the formulas against the pathogens is as shown in Table 3. With the exception of THR-SK007, all the formula extracts revealed the antibacterial activity in the disc diffusion assay with inhibition zones ranging from 7.5 to 21.0 mm.

THR-SK004 and THR-SK005 extracts displayed broad spectrum activity against 85% of the test strains including MRSA, MDR *P. aeruginosa*, and MDR *A. baumannii*. THR-SK003 demonstrated narrow spectrum activity against only Gram-positive bacteria. Individual medicinal plant extracts (n=11) which are the herbal components of the effective formulas (THR-SK003, 004, and 005) were subjected to determination of their potency against the test pathogens by the disc diffusion assay method. Among the evaluated herbs, five extracts were solely inactive against the tested strains. *Smilax* species, *Metroxylon sagu*, *Oroxylum indicum*, and *Hopea odorata* exhibited potent antibacterial activity against all the tested Gram-positive pathogens. Their inhibition zones were in the range of 8.3 to 23.5 mm (Table 4).

Table 2. A panel of skin pathogenic bacteria and their antimicrobial resistance profile.

Test strain	Antibiotic resistance profile
Methicillin-resistant *S. aureus* NPRC R001	Resistant to oxacillin, penicillin, teicoplanin, gentamycin, erythromycin, tetracycline, ciprofloxacin, clindamycin, and sulfamethoxazole/trimethoprim
Methicillin-susceptible *S. aureus* NPRC S001	Susceptible to all tested antibiotics including oxacillin, penicillin, teicoplanin, gentamycin, erythromycin, tetracycline, ciprofloxacin, clindamycin, vancomycin, and sulfamethoxazole/trimethoprim
Clinical isolated coagulase positive staphylococci NPRC 301	Resistant to erythromycin and sulfamethoxazole/trimethoprim
Clinical isolated coagulase negative staphylococci NPRC 501	Intermediate to clindamycin
S. pyogenes NPRC 101	Susceptible to all tested antibiotics including penicillin and erythromycin
Multidrug-resistant *E. coli* NPRC EC05	Resistant to cefotaxime, ciprofloxacin, and levofloxacin
Multidrug-resistant *A. baumannii* NPRC AB013	Resistant to ceftazidime, cefotaxime, imipenem, meropenem, amikacin, tobramycin, ciprofloxacin, and levofloxacin
Multidrug-resistant *A. baumannii* JVC 1053	Resistant to levofloxacin, aztreonam, ciprofloxacin, and sulfamethoxazole/trimethoprim
Multidrug-resistant *P. aeroginosa* NPRC PA03	Resistant to amikacin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, cefalotin, sulfamethoxazole/trimethoprim, gentamycin, imipenem, sulperazone, tazocin, ertapenem, and meropenem

Table 3. Antibacterial activity of ethanol extracts of Southern Thailand herbal recipes (2.5 mg/disc) against skin pathogenic bacteria[a].

Thailand herbal recipe	Inhibition zone (Mean values ± SD)													
	MRSA NPRC R001	MSSA NPRC S001	CNS NPRC 501	CPS NPRC 301	*S. pyogenes* NPRC 101	*S. aureus* ATCC 25923	*S. epidermidis* ATCC 35984	*S. epidermidis* ATCC 12228	MDR- *E. coli* NPRC EC05	MRD- *P. aeruginosa* NPRC PA03	MDR- *A. baumannii* JVC 1053	MDR- *A. baumannii* NPRC AB013	*E. coli* ATCC 25922	*A. baumannii* ATCC 19606
THR-SK001	11.1±0.9	7.5±0.7	- [b]	-	-	9.5±0.7	-	-	- [b]	-	9.9±0.9	8.4±0.5	-	8.4±0.5
THR-SK002	10.9±2.0	7.5±0.7	9.0±1.4	-	9.5±0.7	9.4±0.5	-	-	-	-	9.5±0.6	7.6±0.5	-	8.4±0.5
THR-SK003	13.5±3.1	10.3±1.6	10.9±1.7	10.0±0.0	12.0±1.4	9.3±1.0	11.3±3.9	11.5±1.0	-	-	-	-	-	-
THR-SK004	19.3±1.0	17.6±1.9	18.8±1.0	18.0±1.8	13.5±3.5	18.0±1.7	21.0±2.3	19.0±1.8	-	8.6±1.4	8.9±1.3	8.4±1.1	-	9.0±0.7
THR-SK005	16.0±0.8	12.8±1.5	10.8±2.1	11.0±1.4	10.8±1.1	11.0±0.9	17.5±0.7	9.5±1.3	-	9.1±0.9	10.8±1.0	8.1±1.0	-	8.9±0.5
THR-SK006	-	-	-	-	-	-	-	8.5±0.7	-	-	7.8±0.4	-	-	-
THR-SK007	-	-	-	-	-	-	-	-	-	-	-	-	-	-
THR-SK008	9.3±1.3	10.0±1.4	8.3±0.5	-	-	8.5±0.4	8.5±0.7	9.0±1.4	-	-	9.6±1.1	9.5±0.7	-	8.9±1.5
THR-SK009	9.5±0.4	-	-	-	11.0±0.0	9.3±1.2	-	11.5±0.7	-	-	8.1±0.3	8.3±0.4	-	-
Penicillin G (10 U/disc)	8.5±0.7	39.0±1.4	25.0±1.4	41.5±2.1	52.5±3.5	49.0±1.4	21.0±1.4	20.5±0.7	-	-	-	-	-	-
Ciprofloxacin (5 µg/disc)	-	-	-	-	-	-	-	-	9.5±0.7	-	-	-	36.0±1.4	25.0±1.4

[a]The tested skin pathogens were methicillin resistant *S. aureus* (MRSA), methicillin susceptible *S. aureus* (MSSA), *Staphylococcus epidermidis* (*S. epidermidis*), coagulase positive staphylococci (CPS), coagulase negative staphylococci (CNS), *Streptococcus pyogenes*, multidrug-resistant *Acinetobacter baumannii*, multidrug-resistant *Pseudomonas aeruginosa*, and multidrug-resistant *Escherichia coli*. [b]No inhibition zone

Table 4. Potency of ethanol extracts of Southern Thailand herbal recipes and their herbal components on skin pathogenic bacteria[a].

Thai herbal recipe/ Medicinal plant	Inhibition zones (Mean values ± SD)													
	MRSA NPRC R001	MSSA NPRC S001	CNS NPRC 501	CPS NPRC 301	*S. pyogenes* NPRC 101	*S. aureus* ATCC 25923	*S. epidermidis* ATCC 35984	*S. epidermidis* ATCC 12228	MDR- *E. coli* NPRC EC05	MRD- *P. aeruginosa* NPRC PA03	MDR- *A. baumannii* JVC 1053	MDR- *A. baumannii* NPRC AB013	*E. coli* ATCC 25922	*A. baumannii* ATCC 19606
THR-SK003	13.5±3.1	10.3±1.6	10.9±1.7	10.0±0.0	12.0±1.4	9.3±1.0	11.3±3.9	11.5±1.0	-[a]	-	-	-	-	-
Smilax spp.	12.0±0.8	9.6±0.5	15.5±2.6	9.8±1.0	9.5±0.7	11.3±0.3	10.0±1.2	12.5±1.1	-	-	-	-	-	-
Tinospora crispa	-[b]	-	-	-	-	-	-	-	-	-	-	-	-	-
THR-SK004	19.3±1.0	17.6±1.9	18.8±1.0	18.0±1.8	13.5±3.5	18.0±1.7	21.0±2.3	19.0±1.8	-	8.6±1.4	8.9±1.3	8.4±1.1	-	11.3±0.7
M.sagu	21.3±1.3	18.3±1.0	23.5±1.3	20.5±0.6	13.3±1.0	20.4±0.5	20.3±0.5	22.8±1.7	9.5±0.6	14.1±1.0	11.3±0.5	11.4±0.5	-	-
O. indicum	12.3±0.5	11.3±1.0	13.3±1.0	10.8±0.5	8.25±1.0	8.6±0.8	11.3±1.5	12.4±0.6	-	-	8.0±0.8	8.0±0.8	-	-
Lindenbergia philippensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
THR-SK005	16.0±0.8	12.8±1.5	10.8±2.1	11.0±1.4	10.8±1.1	11.0±0.9	17.5±0.7	9.5±1.3	-	9.1±0.9	10.8±1.0	8.1±1.0	-	8.9±0.5
Datura fastuosa	11.0±0.0	8.5±0.7	8.5±0.7	-	10.5±0.7	-	-	-	-	-	-	-	-	-
Pandanus humilis	-	-	-	-	8.8±1.0	-	-	-	-	-	-	-	-	-
Hydonocarpus anthelminthicus	-	-	-	-	-	-	-	-	-	9.0±1.8	-	-	-	-
H. odorata	9.0±0.0	14.8±1.5	18.3±03	15.3±1.5	9.0±0.0	8.3±0.4	14.3±0.5	17.8±1.7	-	-	9.5±0.6	9.5±0.6	-	-
Caesalpinia bonduc	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. pursaetha	15.5±0.7	15.5±0.7	19.1±0.9	11.9±1.9	-	14.3±1.0	24.3±1.3	17.5±1.3	-	8.5±0.7	-	9.7±0.5	-	9.8±0.4

[a]The tested skin pathogens were methicillin resistant *Staphylococcus aureus* (MRSA), methicillin susceptible *S. aureus* (MSSA), *Staphylococcus epidermidis* (*S. epidermidis*), coagulase positive staphylococci (CPS), coagulase negative staphylococci (CNS), *Streptococcus pyogenes*, multidrug resistant *Acinetobacter baumannii*, multidrug resistant *Pseudomonas aeruginosa*, and multidrug resistant *Escherichia coli.* [b]No inhibition zone

Except for *E. coli* and MDR *E. coli*, *M. sagu* demonstrated the strongest antibacterial activity on both Gram-positive and Gram-negative pathogens including MDR stains.

The MIC/MBC values for the ethanol extracts of three herbal formulas as well as five effective medicinal components against MRSA, methicillin susceptible *S. aureus* (MSSA), coagulase positive staphylococci (CPS), and three reference staphylococci strains are shown in Table 5. Among the estimated extracts, five extracts (THR-SK003, *Smilax* spp., THR-SK005, *H. odorata*, and *Entada pursaetha*) were inactive against the strains at the test concentration. Of these extracts, three extracts of THR-SK004 and its herbal composition, *M. sagu*, and *O. indicum* exhibited potent anti-staphylococcal activity with MIC and MBC value at 32 to 500 µg/ml and 500 to >1,000 µg/ml, respectively.

DISCUSSION

Traditional plant based formulas have been commonly employed for the treatment of skin and soft tissue infections, diarrhoea, gastritis, and peptic ulcer diseases in several countries including Thailand (Zhu et al., 2002; Bussmann et al., 2010b; Kondo et al., 2011; Tam et al., 2011). In this work, nine formula ethanol extracts topically applied for healing skin diseases that were obtained from five Southern Thailand folk healers have been discussed for their antibacterial properties against skin and wound pathogens.

As previously proposed by Rios and Recio (2005), the antibacterial activity is very interesting and will be considered as potentially useful therapeutically in the case of concentrations below 100 µg/ml for crude plant extracts and 10 µg/ml for isolated phytochemical compounds. This study has shown that the ethanol extracts of THR-SK004 and its herbal constituents, *M. sagu* and *O.*

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanol extracts of the effective Southern Thailand herbal recipes and herbal components against *Staphylococcus* spp.[a].

Thai herbal recipe /Medicinal plant	MIC/MBC (µg/ml)					
	MRSA NPRC R001	**MSSA NPRC S001**	**CPS NPRC 301**	***S. aureus* ATCC 25923**	***S. epidermidis* ATCC 35984**	***S. epidermidis* ATCC 12228**
THR-SK003	>1,000/NA[b]	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA
Smilax spp.	1,000/1,000	1,000/1,000	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA
THR-SK004	125/>1,000	31.3/1,000	62.5/1,000	62.5/1,000	62.5/1,000	125/500
M. sagu	62.5/500	31.3/500	31.3/500	31.3/500	31.3/500	31.3/500
O. indicum	125/500	250/500	125/500	125/500	250/500	250/500
THR-SK005	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA
H. odorata	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA
E. pursaetha	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA	>1,000/NA

[a]The tested *Staphylococcus* spp. were methicillin resistant *Staphylococcus aureus* (MRSA), methicillin susceptible *S. aureus* (MSSA), *Staphylococcus epidermidis* (*S. epidermidis*), coagulase positive staphylococci (CPS), and coagulase negative staphylococci (CNS). [b]NA; non applicable.

indicum could be effective agents for the topical treatment of SSTIs. THR-SK004 was the strongest antibacterial agent that completely inhibits the growth of Gram-positive and Gram-negative pathogens including MDR stains, while almost the entire formula extracts exhibited antimicrobial ability against at least one test strains.

The greater resistance of Gram negative bacteria to the formula extract was found which is similar to the activity of numerous plant extracts (Zaidan et al., 2005; Trakulsomboon et al., 2006; Bussmann et al., 2010b) since there are differences in cell wall structure between Gram-positive and Gram-negative bacteria. It should be noted that there was no significance in MIC values of THR-SK004 and *M. sagu* which exhibited potent anti-MRSA activity.

Interest in medicinal plant extracts that exhibited antimicrobial activities as well as their active principles which is responsible for the actions has increased. However, there is limited information on herbal formulas that have been studied, especially the dermatological remedies. Pikutbenjakul is the only Thailand herbal formula that was recently recorded for its antibacterial potency (Kondo et al., 2011). *Plumbago indica* was shown to be the most effective component of Pikutbenjakul inhibiting all tested strains, whereas Pikutbenjakul demonstrated low activity against the bacteria. The MIC values of THR-SK004 reported in this work were higher than those obtained from some Thailand medicinal plants (Wannissorn et al., 2005; Limsuwan and Voravuthikunchai, 2008; Limsuwan et al., 2009). But, they were in range or lower than concentrations reported from Thailand formula, Pikutbenjakul and several reported traditional medicinal plants (Voravuthikunchai et al., 2004; Zaidan et al., 2005; Trakulsomboon et al., 2006; Bussmann et al., 2010a). *M. sagu* was shown to be the most effective herbal component of THR-SK004. This plant is widely distributed in the coastal areas of South East Asia and has great potential for starch production in Thailand and Malaysia (Ahmad et al., 1999). It is one of the recorded medicinal plants in the 17th century, Ambonese Herbal by George Everhard Rumphius (Buenz et al., 2005). Moreover, the plant was recorded as medicinal plant in Papua New Guinea for the treatment of headaches, burn, diarrhoea, and infected sores (Holdsworth, 1977). Its phytochemical constituents such as catechin and flavonoids have been reported, but there is no information about the biological activity of this species (Onsa et al., 2000).

The most effective formula, THR-SK004 is traditionally used by Thailand folk healer to treat wound infections. It is well-recognized that wound healing process consists of three phases, including inflammatory, proliferation, and remodeling phases which are different and overlap. A plant-based remedy should affect at least two different processes or bioassays relevant to wound healing before it can be said to have some scientific support for its traditional use (Houghton et al., 2005). Although, the antimicrobial activity reported here lends some support to the use of THR-SK004 in the topical treatment of wounds. The potency of the formula to stimulate fibroblast growth and protect the cells from hydrogen peroxide-induced injury that could play a role in its effect on tissue repair should be further examined. According to the present results, we can conclude that the traditional Thai herbal formula, THR-SK004 generally applied for wound infectious treatments demonstrated broad antibacterial activity against both Gram-negative and Gram-positive bacteria. This is the first potential of the scientific information that proved the therapeutic Southern traditional Thailand herbal formulas. However, other biological activities which involve different processes of wound healing and active principles of the traditional herbal

formula should be additionally established. The substantiation of folk remedies for common illness healing is extremely important both for the preservation of traditional medical knowledge and the application as alternative health care solutions.

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