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Antibacterial of a Traditional Thai Herbal Recipe (THR 01) against *Staphylococcus epidermidis*

Thongrod Sirirat [1], Yincharoen Kanyatorn [2]

[1,2,] Department of Traditional Thai Medicine, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat Campus, Thung Song, Nakhon Si Thammarat, 80110, Thailand

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The experiment was conducted at the laboratory of the Department of Traditional Thai Medicine, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat Campus during October, 2015 to September, 2016. The aim of the study was to investigate the antibacterial effects of four ethanolic extracts from traditional Thai herbal recipe (THR 01) and herbal components, *Ocimum sanctum* Lin., *Rhinacanthus nasutus* Lin. and *Quisqualis indica* Lin. against *Staphylococcus epidermidis*, antibacterial activity was evaluated by the broth macro dilution method according to Clinical and Laboratory Standard Institute (CLSI, 2012) was carried out to obtain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Twofold serial dilution of the extracts was performed to obtain final concentrations ranging from 15.625 to 1,000 µg/ml. The bacterial inoculum (1ml) containing 106 CFU/ml was transferred into each test tube. Positive control with 1% DMSO and negative control without an inoculum added were included. The tested tube was then incubated at 37 ℃ for 18 h. The MIC values were observed at the lowest concentration of crude extracts that produced a complete suppression of bacterial growth. The MBC are performed with 3 concentrations at sub-MIC, MIC and over MIC values. 10 of suspension was sub-culturing on tryptic soy agar (TSA) plate. After incubation at 37 ℃ for 18 h, the plate was recorded MBC value at a concentration no colony of bacteria. The result showed that the antibacterial activity of methanol extracts traditional Thai herbal recipe (THR 01), its herbal components which were *Q. indica*, *R. nasutus* and *O. sanctum* against *S. epidermidis* isolated is considered to be a major virulence factor affecting their pathogensis in wound infections using broth microdilution. The data on the experimental were indicating the efficacy of a Thai traditional remedy, THR 01 and herbal components which were *Q. indica*, *R. nasutus* and *O. sanctum* that showed results for inhibiting *S. epidermidis* isolated from wound infections with the MIC were 1000 µg/ml, 500 µg/ml and 500 µg/ml, respectively and all extracts no killed bacteria for the MBC. The study indicated that *R. nasutus* and *Q. indica* had strong antibacterial activities against the tested isolates.

Keywords : recipe, *Staphylococcus epidermidis*, antibacterial

[1] **Corresponding author :** Thongrod, S.; **Email:** si-susu@hotmail.com

Introduction

Statistics from the Ministry of Public Health in 2015, skin disease is a common disorder ranked eighth compared to other infectious diseases. The total patient rate of 98.64 cases per 1,000 population and statistics from the patients at the Institute of Dermatology has a daily average of 800 people. (Institute of Dermatology, 2015) Thailand is a tropical country that can lead to pathogenic bacteria grow well. The most common bacterial skin infection is the group of Staphylococcus (Sutabhaha and Khantawa, 2011).

Staphylococcus epidermidis is a Gram-positive cocci in group of facultative anaerobic bacteria. *S. epidermidis* are common bacterial colonizers of the skin and mucous membranes such as the nose, mouth, ears and distal convoluted tubule of urinary system. (Namvar *et al*., 2014) According to previous study found, *S. epidermidis* is a bacterial species that forming in biofilm conditions more than other bacteria species. In *S. epidermidis*, biofilm formation is regarded as a major pathomechanism as it renders this pathogen highly resistant to antibiotic drugs. As a result, the treatment of *S. epidermidis* infectious diseases are difficult to treat (Thongrod, 2013; Chusria *et al*., 2016).

Herbal medicines have increased widespread interest in the search of alternative antibacterial agents because of the perception that they have a long history of use in folk medicine for the treatment of infectious diseases. The aim of this study was carried out to investigate the antibacterial activity of four ethanolic extracts from traditional Thai herbal recipe (THR 01) of Mr. Dunhaseed Wamak and its herbal components which are *Ocimum sanctum* L, *Rhinacanthus nasutus* L., *Quisqualis indica* L. and against *S. epidermidis* from wound infections.

Materials and methods

Preparation of crude extracts

Traditional Thai herbal recipe 01 (THR-01) consists of equal amounts (50 g) of their medicinal plant components, *Ocimum sanctum* L., *Rhinacanthus nasutus* L. and *Quisqualis indica* L. Single herbal component was used 50 g. The herbal powder was extracted (1:2, w/v) with 95% ethanol at room temperature for 7 days. After filtration, 95% ethanol was removed with a rotatory evaporator, kept at 55 ℃ until they were completely dry and stored in a sterile eppendorf at 4 ℃. Extraction yield (%, w/w) was calculated as the ratio of the weight of the extract to the weight of the crude herb powder. The crude extract was dissolved with dimethylsulfoxide (DMSO) at concentration 40 mg/ml.

Evaluation of antibacterial activity

Antibacterial activity was evaluated by the broth macrodilution method according to Clinical and Laboratory Standard Institute (CLSI, 2012) was carried out toobtain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Twofold serial dilution of the extracts was performed to obtain final concentrations ranging from 15.625 to 1,000 µg/ml. The bacterial inoculum (1 ml) containing 106 CFU/ml was transferred into each test tube. Positive control with 1% DMSO and negative control without an inoculum added were included. The tested tube were then incubated at 37 ℃ for 18 h. The MIC values were observed at the lowest concentration of crude extracts that produced a complete suppression of bacterial growth.

The MBC are performed with 3 concentrations at sub-MIC, MIC and over MIC values. 10 of suspension was sub-culturing on tryptic soy agar (TSA) plate. After incubation at 37 ℃ for 18 h, the plate was recorded MBC value at a concentration no colony of bacteria. All experiments were carried out in triplicate.

Results

Antibacterial activity of plant

THR-01 extracts and its herbal components which are *Ocimum sanctum* L., *Rhinacanthus nasutus* L., *Quisqualis indica* L. and exhibited different inhibition levels against *S. epidermidis* as shown in Table 1. The ratio of MBC to the macrobroth-determined MIC was 15.625 to 1,000 µg/ml. The MIC for the ethanolic extracts present *R. nasutus* L. and *Q. indica* L. showed the strongest antibacterial activity with MIC values of 500 µg/ml secondly THR-01 extracts showed activity with MIC values of 1,000 mg/ml while *O. sanctum* L. in this study no inhibitory activity against bacteria tested and all extracts no killed bacteria for the MBC as shown in Table 2.

Table 1. Minimum inhibitory concentrations (MIC) of ethanolic extract against *S. epidermidis* determined by macro-broth dilution methods

	Concentration of ethanol extract (µg/ml)						
Herbal components	**1,000**	**500**	**250**	**125**	**62.5**	**31.25**	**15.62**
Ocimum sanctum Linn.	-	-	-	-	-	-	-
Rhinacanthus nasutus Linn.	-	+	-	-	-	-	-
Quisqualis indica Linn.	-	+	-	-	-	-	-
THR 01	+	-	-	-	-	-	-

Table 2. Minimum bactericidal concentrations (MBC) of ethanolic extracts against *S. epidermidis*

Herbal components	MIC and MBC (µg/ml)					
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Rhinacanthus nasutus Linn.	1,000	-	500	-	250	-
Quisqualis indica Linn.	1,000	-	500	-	250	-
THR 01	1,000	-	500	-	-	-

Discussion

S. aureus and *S. epidermidis* are the most common bacterial skin infection is the group of Staphylococcus. The peppermint oil (10 lL) exhibited greater zone of inhibition against *S. aureus, S. pyogenes*, and *K. pneumonia* than the positive control gentamycin (10 lL of 10 lg/mL concentration) were reported by Singh *et al*., (2015) while *Ocimum sanctum* L. in this study no inhibitory activity against bacteria tested although, both of the extracts has contain essential oils as well. The silver nanoparticles synthesized by *S. tricobatum, O. tenuiflorum* extracts were found to have highest antimicrobial activity against *S. aureus* (30 mm) and *E. coli* (30 mm) respectively, these reported Logeswari *et al*., (2015). The extract of *O. sanctum* significantly decreased the antihealing activities of dexamethason in all the wound models. The results indicated that the leaf extract promotes wound healing significantly and ability to overcome the wound healing suppressing action of dexamethasone (Udupa *et al*., 2006). The results agree with reports based on local use of common diseases and ethnobotanical knowledge, an attempt has been made to assess the antibacterial properties of selected medicinal plants such as *O. sanctum* (Tulsi) and *Origanum majorana* (Ram Tulsi). The plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria. The most susceptible bacteria were *B. subtilis*, followed by *S. aureus*, while the most resistant bacteria were *E. coli*, followed by *Shigella dysenteriae, Klebsiella pneumoniae* and *Salmonella typhi* (joshi *et al*., 2009). The aqueous extract of *Zingiber officinale* was active against *P. aerugenosa* and *S. aureus*, with highest activity against the former at a concentration of 200 g (Sulaiman *et al*., 2014).

The antifungal activity of rhinacanthin-rich extract (HRn) against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Microsporum gypseum* was evaluated and compared with those of the ethyl acetate extract and standard rhinacanthins. The result showed that the antifungal activity of HRn was better than that of the ethyl acetate extract. The antifungal activity of HRn was equal to that of rhinacanthin-C. This may be due to a synergistic effect of all the three rhinacanthins is rhinacanthin-C, rhinacanthin-D, and rhinacanthin-N in *Rhinacanthus nasutus* leaves on the antifungal activity (Panichayupakaranant *et al*., 2009). Antimicrobial

activities, evaluation of the Rhinacanthins-rich Rhinacanthus nasutus (RRn) extract and rhinacanthin-C against *S. mutans*, *P. acnes*, *H. pylori*, *S. aureus*, *S. epidermidis*. The RRn extract exhibited potent bactericidal activity against Gram-positive anaerobic bacteria including *S. mutans* and *P. acnes* with MBC values of 4 and 32 mg/ml, respectively. The extract also showed moderate bactericidal activity against Gram positive aerobic bacteria including *S. aureus* and *S. epidermidis* with the MBC values of 256 and 512 mg/ml, respectively. (Puttarak *et al.*, 2010) as same as in this study, *Rhinacanthus nasutus* L. showed the strongest antibacterial activity, although the MIC is less than any previous study.

The in vitro study revealed that methanol extract was more effective than aqueous extract. Leaf extracts of *Quisqualis indica* L. and *Achyranthes aspera* L. was reported to be more effective on fungal species and on contrary leaf extracts of *Calotropis procera* Ait. and *Quisqualis sanctum* L. was found more effective on *B. subtilis*, *E. coli* and *Pseudomonas aeruginosa* (Sanguri, *et al.*, 2012). Diphenylpropanoids from *Q. indica* L. were tested for their anti-staphylococcal activity against a total of five multidrug-resistant (MDR) and methicillin-resistant *Staphylococcus aureus* strains and the minimum inhibitory concentrations (MICs) were in the range of 128-256 μg/ml. (Jahan *et al.*, 2009)

Conclusion

The results of this study showed that a Thai traditional herbal recipe THR 01 and its major constituent, *Rhinacanthus nasutus* L., *Quisqualis indica* L. have an antibacterial activity and supports the traditional usage of the recipe for dermatitis.

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References

Chusri, S., Tongrod, S., Saising, J., Mordmuang, A., Limsuwan, S., Sanpinit, S. and Voravuthikunchai, S. P. (2016). Antibacterial and anti-biofilm effects of a polyherbal formula and its constituents against coagulase-negative and-positive staphylococci isolated from bovine mastitis. Journal of Applied Animal Research, 45(1), 364-372.

Clinical and Laboratory Standards Institute; CLSI. (2012). Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard-ninth edition. 32(2): 16-18.Institute of Dermatology, Ministry of Public Health,

Thailand. Statistics of Disease. (2015). Available from: http://pr.moph.go.th/iprg/include/admin_hotnew/show_hotnew.php?idHot_new=75874. (Online).

Jahan, F. N., Rahman, M. S., Rahman, M. M., Gibbons, S., Masud, M. M., Sadhu, S. K. and Rashid, M. A. (2009). Diphenylpropanoids from *Quisqualis indica* Linn. and their Anti-staphylococcal Activity. Latin American Journal of Pharmacy, 28(2), 279-83.

Joshi, B., Lekhak, S., and Sharma, A. (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. Kathmandu university journal of science, engineering and technology, 5(1), 143-150.

Logeswari, P., Silambarasan, S., and Abraham, J. (2015). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property.Journal of Saudi Chemical Society, 19(3), 311-317.

Namvar, A. E., Bastarahang, S., Abbasi, N., Ghehi, G. S., Farhadbakhtiarian, S., Arezi, P., and Chermahin, S. G. (2014). Clinical characteristics of *Staphylococcus epidermidis*: a systematic review. GMS Hygiene and Infection Control, 9(3).

Puttarak, P., Charoonratana, T. and Panichayupakaranant, P. (2010). Antimicrobial activity and stability of rhinacanthins-rich Rhinacanthus nasutus extract. Phytomedicine, 17(5), 323-327.

Panichayupakaranant, P., Charoonratana, T., and Sirikatitham, A. (2009). RP-HPLC analysis of rhinacanthins in *Rhinacanthus nasutus*: validation and application for the preparation of rhinacanthin high-yielding extract. Journal of chromatographic science, 47(8), 705-708.

Singh, R., Shushni, M. A. and Belkheir, A. (2015). Antibacterial and antioxidant activities of Mentha piperita L. Arabian Journal of Chemistry, 8(3), 322-328.

Sutabhaha and Khantawa. (2011). Antibacterial and antifungal activities Fak Khaao leaf extract. Bulletin of Chiang Mai Associated Medical Sciences. 44(1), 31-37.

Sulaiman, F. A., Kazeem, M. O., Waheed, A. M., Temowo, S. O., Azeez, I. O., Zubair, F. I., and Adeyemi, O. S. (2014). Antimicrobial and toxic potential of aqueous extracts of *Allium sativum*, *Hibiscus sabdariffa* and *Zingiber officinale* in Wistar rats. Journal of Taibah University for Science, 8(4), 315-322.

Thongrod, S., (2013). Antibacterial mechanisms of a traditional Thai herbal recipe against Staphylococci Isolated from bovine mastitis. 81-83.

Udupa, S. L., Shetty, S., Udupa, A. L., and Somayaji, S. N. (2006). Effect of *Ocimum sanctum* Linn. on normal and dexamethasone suppressed wound healing. Indian journal of experimental biology, 44(1), 49.

Sanguri, S., Kapil, S., Gopinathan, P., Pandey, F. K. and Bhatnagar, T. (2012). Comparative screening of antibacterial and antifungal activities of some weeds and medicinal plants leaf extracts: An in-vitro study. Elixir Applied Botany, 47, 8903-8905.

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