Antibacterial Activity of Thai Seasonings Against *Staphylococcus aureus* in Aerosol Product

ฤทธิ์ต้านเชื้อแบคทีเรียสแตฟฟิโลคอคคัส ออเรียส ของเครื่องเทศไทย ในผลิตภัณฑ์ละอองพ่นฝอย

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Abstract

Staphylococcus aureus is a Gram-positive bacterium and flora presented on human skin. It is also a major human pathogen especially food-borne intoxication pathogen when the bacterium is contaminated from the hands into the intestinal tract by food. The research objective was to create innovative aerosols derived from Thai spices that would inhibit the growth of *S. aureus* on the hands. Ten types of Thai seasonings, particularly fingerroot (*Boesenbergia pandurata*), turmeric (*Curcuma longa*), chili (*Solanaceae capsicum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), onion (*Allium ascalonicum*), kaffir lime (*Citrus hystrix*), ginger (*Zingiber officinale*), greater galangal (*Alpinia galanga*), and pepper (*Piper nigrum*) were studied. Crude extracts of garlic (*Allium sativum*) bulbs and kaffir lime (*Citrus hystrix*) showed potential antimicrobial activities. The inhibition zone of extracted garlic and kaffir lime was 15 and 10 mm in diameters to inhibit *S. aureus* of Thailand Institute of Scientific and Technological Research; TISTR (*S. aureus* TISTR 1466). Ampicillin disk--10 µg and sterile distilled water were positive and negative control, respectively. The Minimal Inhibitory Concentration--MIC was used via the multiple broth microdilution method. MIC of extracted garlic (*A. sativum*) and kaffir lime (*C. hystrix*) were 12.5 and 25 mg/mL. Spread plate technique was estimated for Minimal Bactericidal Concentration--MBC while observing the bacterium with no growth. MBC of extracted garlic and kaffir lime were the same as MIC. Two potential extractions from Thai seasonings could be alternatives for development to use as hand sanitiser spray.

Keywords: antibacterial activity, Thai seasoning, *Staphylococcus aureus*

บทคัดย่อ

สแตฟฟิโลคอคคัส ออเรียส (*Staphylococcus aureus*) เป็นแบคทีเรียแกรมบวกซึ่งพบเป็นปกติบนผิวหนังของมนุษย์ และที่สำคัญแบคทีเรียชนิดนี้ยังสามารถก่อโรคจากสารพิษที่สร้างขึ้นในมนุษย์ได้ โดยเฉพาะโรคระบบทางเดินอาหารซึ่งเชื้อจะปนเปื้อนจากมือสู่อาหาร วัตถุประสงค์ของงานวิจัยเพื่อสร้างนวัตกรรมละอองพ่นฝอยจากเครื่องเทศของไทยที่สามารถยับยั้งการเจริญของเชื้อ สแตฟฟิโลคอคคัส ออเรียส บนมือได้ โดยการเก็บตัวอย่างเครื่องเทศของไทยจำนวน 10 ชนิด ได้แก่ กระชาย ขมิ้น พริก กระเทียม ตะไคร้ หัวหอม มะกรูด ขิง ข่า และพริกไทย การทดสอบพบว่าสารสกัดหยาบกระเทียม และใบมะกรูด มีประสิทธิภาพในการยับยั้งแบคทีเรีย สแตฟฟิโลคอคคัส ออเรียส สายพันธุ์ TISTR 1466 โดยมีขอบเขตการยับยั้ง 15 และ 10 มิลลิเมตร ตามลำดับ แอมพิซิลิน 10 ไมโครกรัม ถูกใช้เป็นตัวควบคุมเชิงบวก ส่วนน้ำกลั่นปราศจากเชื้อใช้เป็นตัวควบคุมเชิงลบ การทดสอบความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อ สแตฟฟิโลคอคคัส ออเรียสใช้วิธีการเจือจางในอาหารเลี้ยงเชื้อเหลวแบบหลายหลอด ค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อ สแตฟฟิโลคอคคัส ออเรียส ของสารสกัดหยาบจากกระเทียมและใบมะกรูด มีค่าเท่ากับ 12.5 และ 25 มิลลิกรัมต่อมิลลิลิตร การประเมินค่าความเข้มข้นต่ำสุดที่สามารถฆ่าเชื้อ สแตฟฟิโลคอคคัส ออเรียส ใช้เทคนิคการเกลี่ยเพลท โดยสังเกตจากเชื้อที่ไม่สามารถเจริญได้บนอาหารเลี้ยงเชื้อแข็ง ซึ่งมีค่าเท่ากันกับค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อ ได้ทั้งสารสกัดหยาบกระเทียมและใบมะกรูด สารสกัดจากเครื่องเทศของไทยทั้งสองชนิดมีประสิทธิภาพสามารถใช้เป็นทางเลือกเพื่อพัฒนาเป็นสเปรย์ยับยั้งการเจริญของเชื้อ สแตฟฟิโลคอคคัส ออเรียส บนมือได้

คำสำคัญ: ฤทธิ์ต้านแบคทีเรีย เครื่องเทศไทย สแตฟฟิโลคอคคัส ออเรียส

Introduction

Staphylococcus aureus is a Gram-positive bacterium. It is a major human pathogen that causes a wide range of clinical infections and leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections (Tong, Davis, Eichenberger, Holland & Fowler, 2015). Hands are the most common vehicle for the transmission pathogens. Hand hygiene is the leading measure for preventing the spread of antimicrobial resistance and reducing health care-associated infections (Castro et. al., 2016). Some of Thai medicinal plants can inhibit *S. aureus* infections on skin such as pomegranate (*Punica granatum*) (Chansakaow, Leelapornpisid, Yosprasit & Tharavichitkul, 2005). So, Thai traditional food has a variety of seasoning preparations to cook. Some of them could be antimicrobial on skin and developed to hand sanitizer spray.

Objective

Research objective was to create innovative aerosols derived from Thai seasonings which inhibit the growth of *S. aureus* on hands.

Materials and Methods

Screening and Extraction of Thai Seasoning

Ten types of Thai seasonings, particularly fingerroot (*Boesenbergia pandurata*), turmeric (*Curcuma longa*), chili (*Solanaceae capsicum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), onion (*Allium ascalonicum*), kaffir lime (*Citrus hystrix*), ginger (*Zingiber officinale*), greater galangal (*Alpinia galanga*), and pepper (*Piper nigrum*) were collected from Sura Nakhon market at Nakhon Ratchasima Province, Thailand. Screening of samples were following as; fresh samples were washed by tap water, chopped, blended and filtrated through sterile fabric sheets. Each aqueous solution was tested for inhibition of *S. aureus* on agar plate. The strongest inhibitors were species that were selected for further examination. The selected Thai seasonings were washed, chopped, dried at 60 °C in hot air oven and blended to fine powder. One-hundred and twenty-five grams of each powder was soaked in 500 mL of 95% ethanol for a week, then filtrated though paper filter with Whatman No.1. The evaporation of the solvent was poured the supernatant onto ceramic cups then put on hotplate until dry. Crude extracts were collected, weighed and kept at 4 °C until use. The crude extracts were examined for antibacterial activity.

Percentage yield

Percentage yield of all extracts was determined by following formula:

Yield (%) = (Extracted weight/Dried Sample weight) × 100

Bacterial Preparation

S. aureus of Thailand Institute of Scientific and Technological Research (TISTR) 1466; (*S. aureus* TISTR1466) was used as reference strain. The bacterium was cultured in 10% NaCl trypticase soy broth (TSB, Difco, U.S.A.) and incubated at 37 °C for 24 h, then streaked on 10% NaCl trypticase soy agar (TSA, Difco, U.S.A.) and incubated in the same conditions. The single colony was transferred to 10% NaCl TSB and incubated at 37 °C for 16-18 h for antimicrobial test.

Antibacterial Test

Paper disk diffusion method was used for the following. The suspension of *S. aureus* TISTR 1466 in TSB was mixed and compared to 0.5 McFarland. The final inoculum was adjusted to 108 Colonies Forming Unit--CFU of preparation. The bacterium was swabbed on TSA using a sterile cotton swab. Ampicillin 10 µg and sterile distilled water was used as positive and negative control, respectively. Extracted Thai seasonings (100mg/mL) were dropped on the sterile paper disk. The paper disks were made from sterile filter papers (Whitman No.1) which were the same size as the antibiotic disk diameter. TSA duplication plates were incubated at 37 °C for 24 h. The inhibition zones diameter was measured in millimeters.

The Minimal Inhibitory Concentration--MIC and the Minimal Bactericidal Concentration--MBC were studied. Multiple broth dilution method was used for the determination of MIC. Two-fold dilution of each extract substance was made in TSB tubes. The inoculum was added into the dilution tubes with a proportion of 1:1 then incubated at 37 °C for 24 h. The turbidity was indicated as bacterial growth. The lowest concentration tube of

the extracted and no growth of the bacterium (clear broth) was MIC according to Phaiboon, Pulbutr, Sungthong and Rattanakiat (2019). Spread plate technique on TSA was tested for MBC while observed of bacterium with no growth tubes.

Innovative Aerosols

The potentials extracted were developed as hand sanitizer spray. The MIC of each extract were calculated and mixed with sterile distilled water then placed into sterile glass containers, well-mixed and ready to use.

Results

Screening of Thai Seasoning and Antibacterial Activities

One-hundred and twenty-five grams of each powder was soaked in 500 mL of 95% ethanol for a week. The inhibition zone diameter of plant extracts against test the bacterium strain shown in Table 1. Extracted garlic (*A. sativum*) and kaffir lime (*C. hystrix*) was selected for antimicrobial activities. The inhibition zone of extracted garlic and kaffir lime was 15 mm and 10 mm in diameter, respectively. The inhibition zone against *S. aureus* TISTR 1466 shown as Figure 1.

Table 1.

The inhibition zone diameter of plant extracts against S. aureus TISTR 1466

Scientific name	General Name	Extracted part	Yield (%)	Inhibition zone diameter (mm)
Boesenbergia pandurata	Fingerroot	Roots	1.8	0
Curcuma longa	Turmeric	Roots	1.2	7
Solanaceae capsicum	Chili	Fruits	2.2	0
Allium sativum	Garlic	Bulbs	1.1	15
Cymbopogon citratus	Lemongrass	Stalks	2.4	0
Allium ascalonicum	Onion	Bulbs	1.4	0
Citrus hystrix	Kaffir lime	Leaves	1.6	10
Zingiber officinale	Ginger	Roots	2.0	0
Alpinia galanga	Greater galangal	Roots	1.6	6
Piper nigrum	Pepper	Berries	1.2	0
Positive control (Ampicillin 10 µg)				21
Negative control (Distilled water)				0

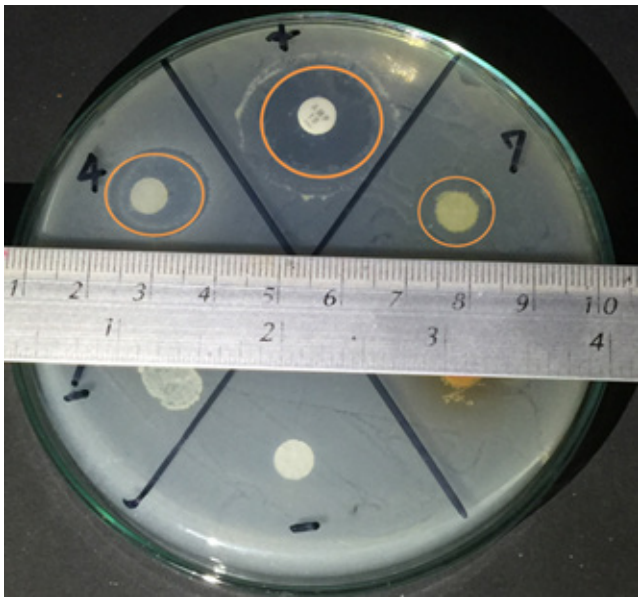

Figure 1. The inhibition zone of positive control (+, ampicillin 10 µg), negative control (-, sterile distilled water), garlic extracted (4) and kaffir lime extracted (7)

Determination of MIC and MBC

Multiple broth dilution method and spread plate technique were used for the determination of MIC and MBC (100mg/mL) of the extracts. MIC of garlic (*A. sativum*) and kaffir lime (*C. hystrix*) were 12.5 and 25 mg/mL, respectively. The results the inhibition of *S. aureus* TISTR 1466 from both Thai seasoning at low concentration.

Hand Sanitizer Spray

The potentials extracted of garlic (*A. sativum*) and kaffir lime (*C. hystrix*) were developed as hand sanitizer spray. The products were calculated from MIC and MBC at 12.5 and 25 mg/mL for garlic and kaffir lime.

Discussion

S. aureus is a leading cause of skin and soft-tissue infections and food poisoning (Ferreira et. al, 2021; Castro et. al, 2016). Hand hygiene is the most successful intervention for infection control (Espadale et. al, 2018). The bacterium culture positivity was found to decrease significantly with increasing handwashing frequency (Genc & Arikan, 2020). Many plants were used for inhibition of *S. aureus* on human skin with extracted by several extracting procedures with various solvents. Thai medicinal plants such as *Acanthus ilicifolius* Linn. var. *ilicifolius; Argyreia nervosa; Punica granatum* L. var. *granatum; Terminalia chebula* Retz. var. *chebula* and *Zanthoxylum myriacanthum* were studied on *S. aureus* inhibition (Chansakaow et. al, 2005). *Punica granatum; Cinnamomum camphora; Curculigo orchioides*; and *Curcuma longa* from Nepal were inhibited the bacterium also (Marasini et. al, 2015; Sajjad et. al, 2015). In this studied, 95% ethanol is easy to find in all laboratories, low cost and good solvent to give a good extract for many plants when compared to distilled water (Mongkoltrirat, Kerdchoechuen & Laohakunjit, 2013). Ethanolic extract of garlic bulbs and kefir lime leaves gave a strong inhibition to *S. aureus*

TISTR 1466. For other researches, fresh garlic extract inhibits *S. aureus* biofilm formation under chemopreventive and chemotherapeutic conditions (Ratthawongjirakul & Thongkerd, 2015). Garlic also used as a natural alternative to chlorhexidine for oral infections (Fahim, Himratul-Aznita & Abdul-Rahman 2020) but not hand sanitizer yet. Moreover, garlic aqueous extract was used for *S. aureus* inhibition in hamburger that can increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods (Mozaffari Nejad, Shabani, Bayat & Hosseini, 2014) same as essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms (Hongpattarakere, Chanthachum & Chanthaphon, 2008). Essential oil extracted from *Citrus hystrix* (Kaffir Lime) peels were the most susceptible to S. aureus with MIC 1.0-8.0 mg/mL (Sreepian, Sreepian, Chanthong, Mingkhwancheep & Prathit 2019). However, an innovation of the study will be helped to decreased and inhibited of S. aureus on hands contamination for a very good hand hygiene and easy to use. For further experiment of garlic extract spray shall be reduce its odor by adding some perfume. Garlic bulb and kaffir lime leaves extracted, they could be developed to alternative for Thai medicine or mixed in skin cosmetics or others products in the future.

Conclusion

Two of ten Thai seasonings were selected for inhibition of S. aureus TISTR 1466. The strong inhibitors against the bacterium were garlic (*A. sativum*) and kaffir lime (*C. hystrix*) extracted by 95% ethanol for a week. The inhibition zones were 15 and 10 mm, respectively. MIC and MBC of the extracts were 12.5 mg/mL for garlic and 25 mg/mL mg/mL for kaffir lime. Both crudes extracted could be used as hand sanitizer spray preparation to inhibit *S. aureus*.

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References

Castro, A., Komora, N., Ferreira, V., Lira, A., Mota, M., Silva, J., & Teixeira, P. (2016). Prevalence of *Staphylococcus aureus* from nares and hands on health care professionals in a Portuguese Hospital. *Journal of Applied Microbiology, 121*(3), 831–839. https://doi.org/10.1111/jam.13186

Chansakaow, S., Leelapornpisid, P., Yosprasit, K., & Tharavichitkul, P. (2005). Antibacterial activity of Thai medicinal plant extracts on the skin infectious microorganisms. *Acta horticulturae, 678*, 153-157. https://doi.org/10.17660/ActaHortic.2005.678.21

Hongpattarakere, T., Chanthachum, S., & Chanthaphon, S. (2008). Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. *Songklanakarin Journal of Science and Technology (SJST), 30*, 125-131. (in Thai)

Espadale, E., Pinchbeck, G., Williams, N. J., Timofte, D., McIntyre, K. M., & Schmidt, V. M. (2018). Are the hands of veterinary staff a reservoir for antimicrobial-resistant bacteria? a randomized study to evaluate two hand hygiene rubs in a veterinary hospital. *Microbial Drug Resistance. 24*(10), 1607-1616. doi: 10.1089/mdr.2018.01831607

Fahim, A., Himratul-Aznita, W. H., & Abdul-Rahman, P. S. (2020). Allium-sativum and bakuchiol combination: A natural alternative to Chlorhexidine for oral infections?. *Pakistan journal of Medical Sciences, 36*(2), 271–275. https://doi.org/10.12669/pjms.36.2.1457

Ferreira, C., Costa, S. S., Serrano, M., Oliveira, K., Trigueiro, G., Pomba, C., & Couto, I. (2021). Clonal lineages, antimicrobial resistance, and pvl carriage of *staphylococcus aureus* associated to skin and soft-tissue infections from ambulatory patients in Portugal. *Antibiotics, 10*(4), 345. https://doi.org/10.3390/antibiotics10040345

Genc, O., & Arikan, I. (2020). The relationship between hand hygiene practices and nasal *Staphylococcus aureus* carriage in healthcare workers. *La Medicina del lavoro, 111*(1), 54–62. https://doi.org/10.23749/mdl.v111i1.8918

Marasini, B. P., Baral, P., Aryal, P., Ghimire, K. R., Neupane, S., Dahal, N., Singh, A., Ghimire, L., & Shrestha, K. (2015). Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. *BioMed Research International, 2015*, 265425. https://doi.org/10.1155/2015/265425

Mongkoltrirat, S., Kerdchoechuen, O., & Laohakunjit, N. (2013). Antioxidant activity of garlic and onion extracts. *Agricultural Science Journal, 44*(2), 585-588. (in Thai)

Mozaffari Nejad, A. S., Shabani, S., Bayat, M., & Hosseini, S. E. (2014). Antibacterial effect of garlic aqueous extract on *Staphylococcus aureus* in Hamburger. *Jundishapur Journal of Microbiology, 7*(11), e13134. https://doi.org/10.5812/jjm.13134

Phaiboon, N., Pulbutr, P., Sungthong, B., & Rattanakiat, S. (2019). Effects of the ethanolic extracts of Guava Leaves, Licorice Roots and Cloves on the Cariogenic Properties of *Streptococcus mutans*. *Pharmacognosy Journal, 11*(5), 1029-1036. doi:10.5530/pj.2019.11.162

Ratthawongjirakul, P., & Thongkerd, V. (2015). Fresh garlic extract inhibits *Staphylococcus aureus* biofilm formation under chemopreventive and chemotherapeutic conditions. *Songklanakarin Journal of Science and Technol, 38*(4), 381-389. (in Thai)

Sajjad, W., Sohail, M., Ali, B., Haq, A., Din, G., Hayat, M., Khan, I., Ahmad, M., & Khan, S. (2015). Antibacterial activity of *Punica granatum* peel extract. *Mycopath, 13*(2), 105-111. https://bit.ly/3u9AL3y

Sreepian, A., Sreepian, P. M., Chanthong, C., Mingkhwancheep, T., & Prathit, P. (2019). Antibacterial activity of essential oil extracted from *Citrus hystrix* (Kaffir Lime) peels: An in vitro study. *Tropical Biomedicine, 36*(2), 531–541. https://www.msptm.org/files/Vol36No2/531-541-Sreepian-A.pdf.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G., Jr (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews, 28*(3), 603–661. https://doi.org/10.1128/CMR.00134-14