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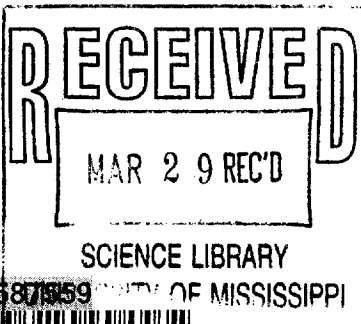
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Ethnopharmacological communication

Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria

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Abstract

Propionibacterium acnes and *Staphylococcus epidermidis* have been recognized as pus-forming bacteria triggering an inflammation in acne. The present study was conducted to evaluate antimicrobial activities of Thai medicinal plants against these etiologic agents of acne vulgaris. Crude extracts were tested for antimicrobial activities by disc diffusion and broth dilution methods. The results from the disc diffusion method showed that 13 medicinal plants could inhibit the growth of *Propionibacterium acnes*. Among those, *Senna alata*, *Eupatorium odoratum*, *Garcinia mangostana*, and *Barleria lupulina* had strong inhibitory effects. Based on a broth dilution method, the *Garcinia mangostana* extract had the greatest antimicrobial effect. The MIC values were the same (0.039 mg/ml) for both bacterial species and the MBC values were 0.039 and 0.156 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis*, respectively. In bioautography assay, the *Garcinia mangostana* extract produced strong inhibition zones against *Propionibacterium acnes*. Antimicrobial activity from fractions of column chromatography revealed one of the active compounds in *Garcinia mangostana* could be mangostin, a xanthone derivative. Taken together, our data indicated that *Garcinia mangostana* had a strong inhibitory effect on *Propionibacterium acnes* and *Staphylococcus epidermidis*. Therefore, this plant would be an interesting topic for further study and possibly for an alternative treatment for acne.
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Keywords: *Acne vulgaris*; *Propionibacterium acnes*; *Staphylococcus epidermidis*; Antimicrobial activity

1. Introduction

Acne vulgaris is the most common skin disease that affects areas containing the largest oil glands, including the face, back, and trunk (Leydon, 1997). Normal skin commensals including *Propionibacterium acnes*, *Propionibacterium granulosum*, *Staphylococcus epidermidis* and *Malassezia furfur*, proliferate rapidly during puberty and are often involved in the development of acne (Hamnerius, 1996). *Propionibacterium acnes* has been described as an obligate

anaerobic organism. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. On contrary, *Staphylococcus epidermidis*, an aerobic organism, usually involves in superficial infections within the sebaceous unit (Burkhart et al., 1999).

For many years, antibiotics have been used to treat acne vulgaris, however, antibiotic resistance has been increasing in prevalence within the dermatologic setting (Swanson, 2003). The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem

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the minimal bactericidal concentration (MBC) was defined as the lowest concentration of the compound to kill the microorganisms.

2.4. Phytochemical screening

Crude plant extracts were subjected to column chromatography (Silica gel 60, Merck KGaA, Darmstadt, Germany) and eluted with chloroform, ethylacetate and methanol. Separated components were collected as 12 fractions. The components were then identified by thin-layer chromatography (TLC). Silica gel GF₂₅₄ plates (Merck KGaA, Darmstadt, Germany) were developed with CHCl₃:EtOAc:MeOH (8:1:1), and the components were separated into R_f values when visualized under visible and UV lights (254 and 366 nm).

2.5. Bioautography

Bioautography was performed with bacterial cultures exhibiting high sensitivity to the extracts. Developed TLC plates were carefully dried for complete removal of solvent, overlaid with agar containing an aliquot of an overnight culture and incubated at 37 °C. The plates were run in duplicate; one set was used as the reference chromatogram and the other was used for bioautography. The areas of inhibition were compared with the R_f of the related spots on the reference TLC plate.

3. Results and discussion

In the present study, 19 medicinal plant extracts were examined for antimicrobial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*. The results showed that 13 extracts could effectively inhibit the growth of *Propionibacterium acnes*. Among those, the extracts of *Senna alata*, *Eupatorium odoratum*, *Garcinia mangostana*, and *Barleria lupulina* showed strong inhibitory effects (zone of inhibition ≥ 15 mm). Interestingly, *Hibiscus sabdariffa*, *Garcinia mangostana*, *Eupatorium odoratum*, and *Senna alata* extracts showed promising antibacterial activities against both *Propionibacterium acnes* and *Staphylococcus epidermidis*. The remaining 15 plant extracts had no detectable activity against *Staphylococcus epidermidis*.

Subsequent experiments were conducted to determine inhibitory concentrations of all selected plant extracts. *Garcinia mangostana* showed the greatest antimicrobial effect. The MIC values against both organisms were equal (0.039 mg/ml) and the MBC values were 0.039 and 0.156 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis*, respectively (Table 1). The same amount of MIC and MBC obtained from this plant against *Propionibacterium acnes* suggested that *Garcinia mangostana* extract could possibly act as a bactericidal agent to this microorganism. In addition, the *Houttuynia cordata* extract also showed good antimicrobial effects against *Propionibacterium*

acnes with a MIC of 0.039 mg/ml but a high concentration was required to kill both *Propionibacterium acnes* and *Staphylococcus epidermidis* as compared to the *Garcinia mangostana* extract. Two plant extracts, *Senna alata* and

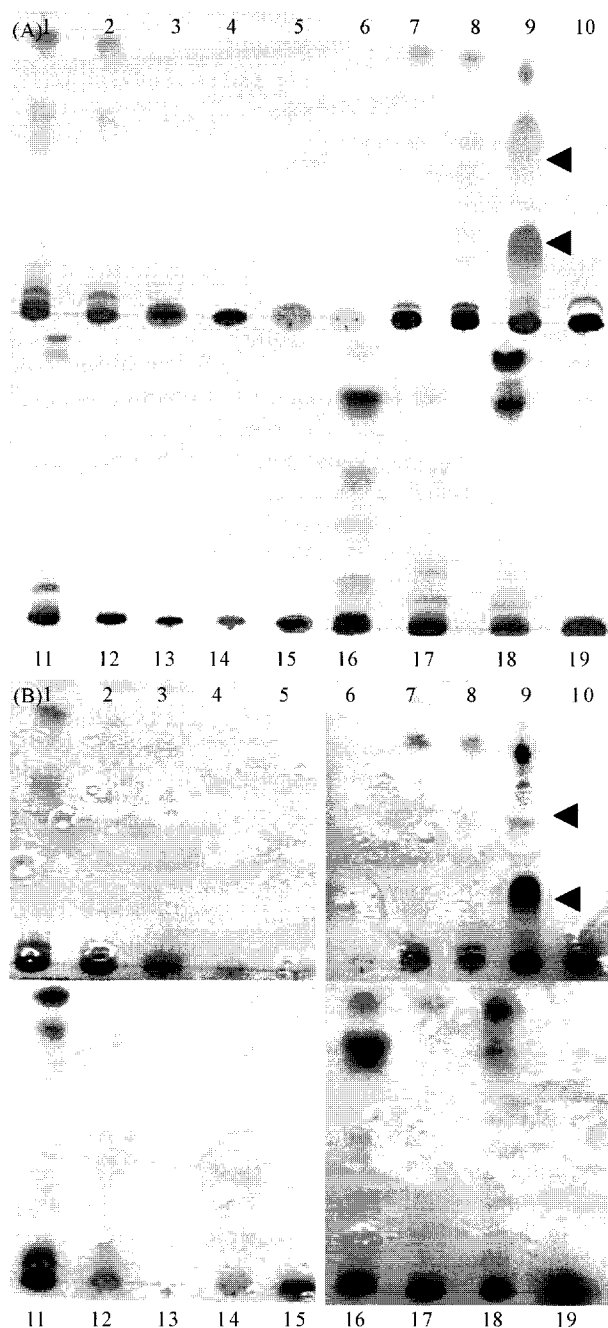


Fig. 1. TLC fingerprints (A) of crude extracts with chloroform: ethylacetate and bioautography (B) against *Propionibacterium acnes*: lane 1, *Andrographis paniculata*; lane 2, *Azadirachta indica*; lane 3, *Barleria lupulina*; lane 4, *Carthamus tinctorius*; lane 5, *Centella asiatica*; lane 6, *Clinacanthus nutans*; lane 7, *Cymbopogon citrates*; lane 8, *Eupatorium odoratum*; lane 9, *Garcinia mangostana*; lane 10, *Hibiscus sabdariffa*; lane 11, *Houttuynia cordata*; lane 12, *Lawsonia inermis*; lane 13, *Lycopersicon esculentum*; lane 14, *Murdannia loriformis*; lane 15, *Psidium guajava*; lane 16, *Senna alata*; lane 17, *Senna occidentalis*; lane 18, *Senna siamea*; lane 19, *Tagetes erecta*. Arrows indicate active reactions by areas of lane 9.

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Eupatorium odoratum, showed outstanding antimicrobial properties against *Propionibacterium acnes* based on the disc diffusion assay, each had a MIC value of 0.625 mg/ml and a MBC of 1.25 mg/ml for *Propionibacterium acnes*.

The plant extracts were further analyzed by TLC. The assay for bioautography demonstrated strong inhibition zones of *Garcinia mangostana* extract against the growth of *Propionibacterium acnes* (Fig. 1). The clear zones were located in separate places on the TLC plate, suggesting that more than one compound possessed an antimicrobial effect. There were no inhibition zones presented above the bands of the other plant extracts covered with *Propionibacterium acnes*. This implied that the strongest effect of the *Garcinia mangostana* extract was against *Propionibacterium acnes*. In addition, the crude extract of *Garcinia mangostana* was subjected to silica gel column chromatography and TLC using a chloroform:ethylacetate:methanol (8:1:1) solvent. Mangostin, a main compound from *Garcinia mangostana*, was also tested and showed the R_f value close to those of bands in fractions that retained antimicrobial activity (data not shown). In addition, the active antimicrobial fraction was subjected to silica gel column using hexane and diethyl acetate (1:2) as eluting solvent, yielded the active compound in 29.9 mg. Based on the spectral evidence and by comparison of $^1\text{H NMR}$ with the reported data (Mahabubusarakam et al., 1987), the active compound was identified as mangostin.

Mangostin is a xanthone derivative produced by guttiferous plants. Xanthone and its derivatives have activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (Munekazu et al., 1996), but the mechanism of action is still unknown. It is possible that mangostin may act in the same mechanism to inhibit *Propionibacterium acnes* and *Staphylococcus epidermidis*. Therefore, the active component of the *Garcinia mangostana* extract could be of interest for further development as an alternative treatment for acne.