Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Ethnopharmacology xxx (2005) xxx–xxx
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Ethnopharmacological communication

Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria

Mullika Traidej Chomnawang [a,*], Suvimol Surassmo [a], Veena S. Nukoolkarn [b], Wandee Gritsanapan [b]

[a] *Department of Microbiology, Faculty of Pharmacy, Mahidol University, 447 Sri Ayudthaya Road, Rachathevi, Bangkok 10400, Thailand*
[b] *Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri Ayudthaya Road, Rachathevi, Bangkok 10400, Thailand*

Received 16 December 2004; received in revised form 24 March 2005; accepted 13 April 2005

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.

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

* Corresponding author. Tel.: +662 644 8677 90x5507; fax: +662 644 8692.
E-mail address: scmtd@mahidol.ac.th (M.T. Chomnawang).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.04.038

of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. In the present study, 19 medicinal plants, which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against microorganisms frequently involved in acne inflammation, *Propionibacterium acnes* and *Staphylococcus epidermidis*.

2. Materials and methods

2.1. Preparation of plant extracts

The 19 plant materials used in this study were collected from various locations in Thailand. Authentication of the plant materials was done by comparison with plant specimens located at Bangkok Herbarium and the Botanical Section of the Botany and Weed Science Division, Department of Agriculture, Bangkok, Thailand. The specimens were deposited at Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

2.2. Microorganisms and media

The test organisms used in this study were as followed: *Propionibacterium acnes* (ATCC 6919) and *Staphylococcus epidermidis* (ATCC 14990). These bacteria were obtained from the American Type Culture Collection, USA and Thailand National Institutes of Health. All media were purchased from DIFCO (Detroit, MI). Mangostin was kindly provided by Associate Professor Wandee Gritsanapan (Department of Pharmacognosy, Mahidol University, Thailand).

2.3. Antimicrobial susceptibility testing

2.3.1. Disc diffusion method

This experiment was performed by the method of Hayes and Markovic (2002) with some modifications. *Propionibacterium acnes* was incubated in brain heart infusion medium (BHI) with 1% glucose for 72 h under anaerobic conditions and adjusted to yield approximately 1.0×10^8 CFU/ml. Aliquots of molten BHI with glucose agar were used as an agar base. A prepared inoculum was added to molten agar, mixed and poured over the surface of the agar base and left to solidity. A sterile paper disc was impregnated with test material and the disc was placed on the agar. Plates were then incubated at 37 °C for 72 h under anaerobic conditions.

Staphylococcus epidermidis was incubated in tryptic soy broth (TSB) for 24 h at 37 °C and adjusted to yield approximately 1.0×10^8 CFU/ml. The procedures were the same as mentioned above except the plates were incubated at 37 °C for 24 h under aerobic conditions. All disc diffusion tests were performed in three separate experiments and the antibacterial activity was expressed as the mean of inhibition diameters (mm).

2.3.2. Determination of minimum inhibitory and bactericidal concentrations

The minimal inhibitory concentration (MIC) values were determined by microdilution assay. This experiment was performed by the method of Sahin et al. (2003). The cultures were prepared at 24 h and 72 h broth cultures of *Staphylococcus epidermidis* and *Propionibacterium acnes*, respectively. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms and

Table 1
The MIC and MBC values of 19 medicinal plant extracts against *Propionibacterium acnes* and *Staphylococcus epidermidis*. The results are shown as average values from three separate experiments

Medicinal plants	Susceptibility of bacteria to medicinal plant extracts			
	Propionibacterium acnes		*Staphylococcus epidermidis*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Andrographis paniculata	0.625	>5	0.312	>5
Azadirachta indica	5	5	5	>5
Barleria lupulina	1.25	1.25	2.5	5
Carthamus tinctorius	>5	>5	>5	>5
Centella asiatica	5	>5	>5	>5
Clinacanthus nutans	>5	>5	>5	>5
Cymbopogon citratus	5	5	>5	>5
Eupatorium odoratum	0.625	1.25	0.625	>5
Garcinia mangostana	0.039	0.039	0.039	0.156
Hibiscus sabdariffa	2.5	5	0.625	5
Houttuynia cordata	0.039	2.5	1.25	>5
Lawsonia inermis	2.5	5	2.5	5
Lycopersicon esculentum	>5	>5	2.5	>5
Murdannia loriformis	>5	>5	1.25	>5
Psidium guajava	2.5	>5	2.5	>5
Senna alata	0.625	1.25	2.5	>5
Senna occidentalis	2.5	>5	>5	>5
Senna siamea	1.25	>5	>5	>5
Tagetes erecta	2.5	>5	5	>5

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 GF_{254} plates (Merck KGaA, Darmstadt, Germany) were developed with $CHCl_3$: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

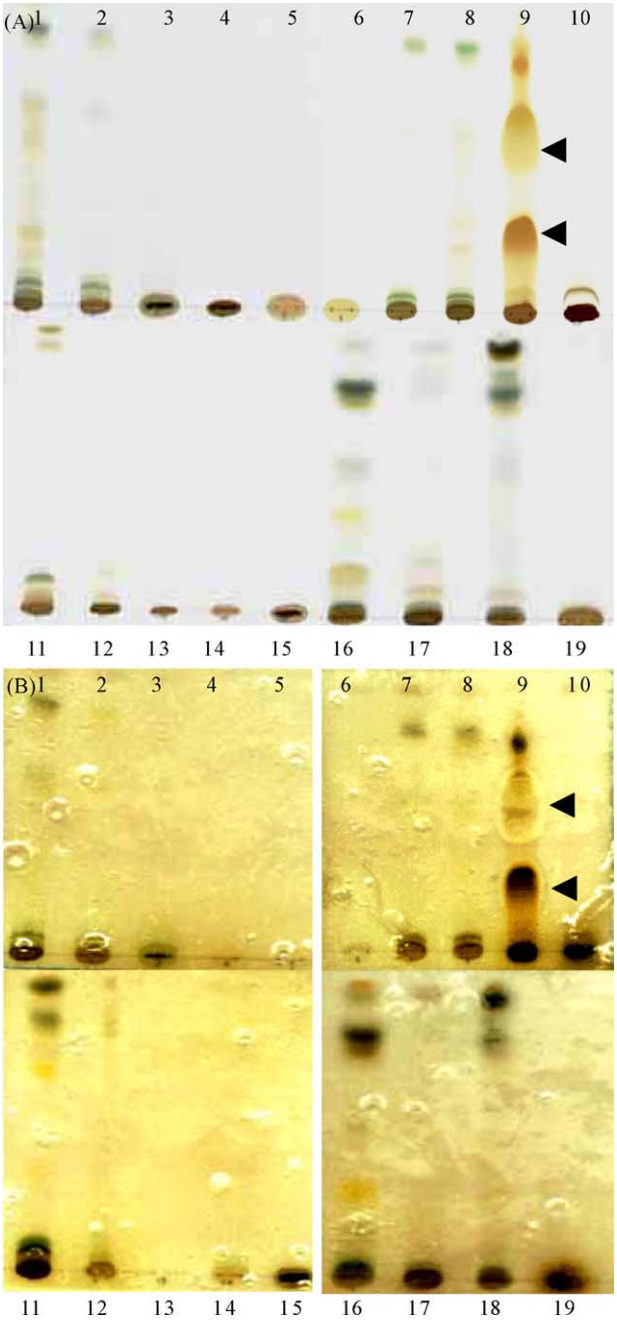

Fig. 1. TLC fingerprints (A) of crude extracts with chloroform: ethylacetate and bioautography (B) against *Propionibacterium acnes*: lane l, *Andrographis paniculata*; lane 2, *Azadirachta indica*; lane 3, *Barleria lupulina*; lane 4, *Carthamus tinctorius*; lane 5, *Centella asiatica*; lane 6, *Clinacanthus 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.

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}H NMR with the reported data (Mahabusarakam et al., 1987), the active compound was identified as mangostin.

Mangostin is a xanthone derivative produced by guttiferaeous 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.

Acknowledgments

This work was supported by the Thailand Research Fund, under the grant # MRG4680146. The authors wish to thank Professor Richard J. O'Callaghan and Dr. Mary E. Marquart from Department of Microbiology, Immunology, and Parasitology, Louisiana University Health Sciences Center, USA for critical readings. We would like to thank Julian Reed for manuscript preparation. We also thank staffs at Department of Microbiology and Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University for their assistance.

References

Burkhart, C.G., Burkhart, C.N., Lehmann, P.F., 1999. Acne: a review of immunologic and microbiologic factors. Journal of Postgraduate Medicine 75, 328–331.

Hamnerius, N., 1996. Acne-aetiology and pathogenesis. Treatment of Acne 32, 29–38.

Hayes, A.J., Markovic, B., 2002. Toxicity of Australian essential oil *Backhousia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity. Food and Chemical Toxicology 40, 535–543.

Leydon, J.J., 1997. Therapy for *Acne vulgaris*. The New England Journal of Medicine, 1156–1162.

Mahabusarakam, W., Wiriyachitra, P., Taylor, W.C., 1987. Chemical constituents of *Garcinia mangostana*. Journal of Natural Products 50, 474–478.

Munekazu, I., Hideki, T., Toshiyuki, T., Fujio, A., Yasuko, K., Ryoyu, S., Ken-Ichi, M., 1996. Antibacterial activity of xanthones from guttiferaeous plants against methicillin-resistant *Staphylococcus aureus*. Journal of Pharmacy and Pharmacology 48, 861–865.

Sahin, F., Karaman, I., Gulluce, M., Ogutcu, H., Sengul, M., Adiguzel, A., Ozturk, S., Kotan, R., 2003. Evaluation of antimicrobial activities of *Satureja hortensis* L. Journal of Ethnopharmacology 87, 61–65.

Swanson, I.K., 2003. Antibiotic resistance of *Propionibacterium acnes* in *Acnes vulgaris*. Dermatology Nursing 5, 359–361.