Antimicrobial activities of some local plants of Thailand against acne-producing bacteria

Prapassorn Bussaman[1,*], Paweena Rattanasena[1] and Piyarat Namsena[2]

Abstract

Natural antimicrobial agents have been recently in focus due to the emerging of drug-resistant bacteria. In this study, Thai local plants (*Curcuma longa* Linn., *Zingiber montanum* (Koenig) Link ex Dietr., *Andrographis paniculata* (Burm.f.) Wall. ex Nees., *Orthosiphon aristatus* (Blume) Miq., and *Boesenbergia rotunda* (L.) Mansf.) were examined for antimicrobial activities against acne-producing bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, and *Propionibacterium acnes*) using broth dilution technique. This study also explored the effects of using different solvents for extraction (distilled water (DW), ethanol (EtOH), dicholoromethane (DCM), and hexane (HEX)) and 1 month-storage of plant samples at 4°C. The results showed that ethanol was the most suitable extraction solvent for almost all the investigated plants to obtain the effective antimicrobial agents with more than 80% inhibitory activities against all four bacteria, especially *Streptococcus pyogenes* and *Staphylococcus aureus*. Moreover, DCM extract of *Boesenbergia rotunda* (L.) Mansf., HEX extracts of *Curcuma longa* Linn. and *Orthosiphon aristatus* (Blume) Miq. were also found to have strong antibacterial activities. Nonetheless, when the plant samples were stored at 4°C for 1 month, their antibacterial activities were shown to be moderately decreased. The ethanolic extracts were found to have lower than 60% inhibition and only against *Staphylococcus epidermidis* and *Streptococcus pyogenes*. Therefore, this study showed that Thai local plants possessed effective anti-acne properties, which however might be lost during the storage. The findings in this study can be useful for the future development of Thai local plants to be used in the products for acne treatment.

Keywords: antibacterial activity, acne vulgaris, Thai plant, solvent, storage

[1] Biological Control Research Unit, Department of Biotechnology, Faculty of Technology, Mahasarakham University, Maha Sarakham, 44150 Thailand

[2] Department of Biology, Faculty of Science and Technology, Rajabhat Mahasarakham University, Maha Sarakham, 44000 Thailand

* Corresponding author, e-mail: prapassorn.c@msu.ac.th

1. Introduction

Acne or acne vulgaris is one of the most common dermatological problems found among the populations. A large amount of money can be spent on managing the acne problems. Since the emerging of drug-resistant bacteria, many attempts have been made to search for the novel natural bioactive compounds effective for treatment of acne. In particular, plants are well known as rich sources of beneficial phytochemicals with pharmacological properties and there are reports of application of plant extracts for the treatment of acne. The bioactive compounds in plants, including flavonoid, alkaloid, essential oil, phenol and phenolic compound, tannin, xanthone and its derivative, and bisnaphthquione derivative, were shown to have anti-acne activities (Azimi, Fallah-Tafti, Khakshur, and Abdollahi, 2012). The key actions of plant bioactive compounds for the treatment of acne include anti-bacterial (Chomnawang, Surassmo, Nukoolkarn, and Gritsanapan, 2005), anti-inflammatory (Chomnawang, Surassmo, Nukoolkarn and Gritsanapan, 2007), anti-oxidant (Pothitirat, Chomnawang, Supabphol, and Gritsanapan, 2009) and anti-androgen activities (Azimi *et al*., 2012). In general, *Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus* and *Streptococcus pyogenes* are the major bacteria responsible for producing acne (Yamaguchi, Satoh-Yamaguchi and Ono, 2009).

Since Thailand has a great variety of plant species, and some of them remain unexplored for therapeutic purposes, this study therefore aims to evaluate the activities of the extracts derived from Thai local plants (*Curcuma longa* Linn., *Zingiber montanum* (Koenig) Link ex Dietr*., Andrographis paniculata* (Burm.f.) Wall. ex Nees., *Orthosiphon aristatus* (Blume) Miq., *Boesenbergia rotunda* (L.) Mansf.) against acne-producing bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, and *Propionibacterium acnes*) using broth dilution technique. The effects of using different solvents for extraction and 1 month-storage of plant samples at 4°C were also investigated.

2. Materials and Methods

2.1 Preparation of plant extracts

Five plant samples, including *Curcuma longa* Linn. (rhizomes), *Zingiber montanum* (Koenig) Link ex Dietr. (rhizomes)*, Andrographis paniculata* (Burm.f.) Wall. ex Nees. (leaves and stems), *Orthosiphon aristatus* (Blume) Miq. (leaves and stems), *Boesenbergia rotunda* (L.) Mansf. (rhizomes), were locally collected or purchased at Mahasarakham province, which is located at the centre of the northeast region of Thailand. Plant samples were air dried to have less than 10% moisture content, ground into fine powder and extracted immediately or stored at 4°C in refrigerator for 1 month before the extraction.

Plant powders were extracted using distilled water (DW), absolute ethanol (EtOH), dichloromethane (DCM) and hexane (HEX) as solvents at the ratio of 1:10 w/v for 12 h with agitation at 200 rpm at room temperature (28–30°C). The supernatants were collected, filtered through Whatman No.1 filter papers, and then centrifuged at 8000 rpm for 10 min. The resulting supernatants were concentrated using vacuum rotary evaporator at 45°C. The concentrated plant extracts were re-suspended with 2% Tween solution (diluted with sterile distilled water) to make a concentration of 12.5 mg/ml before being evaluated by broth dilution assay.

2.2 Bacterial preparation

The acne-inducing bacteria, including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, and *Propionibacterium acnes*, were maintained at 37°C on Muller Hinton agar (MHA), with the exception that *Propionibacterium acnes* was grown in anaerobic condition throughout the culture and experiments.

2.3 Broth dilution assay

The activities of plant extracts against four acne-inducing bacteria, including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, and *Propionibacterium acnes*, were evaluated by broth dilution assay (Adwan, Abu-Shanab, and Adwan, 2010) with some modifications. Bacteria were grown overnight at 37°C (except *Propionibacterium acnes* which was grown in anaerobic condition) and adjusted to have a concentration of approximately $1x10^5$ cfu/ml. The bacterial culture (1 ml) was mixed with Muller Hinton broth (MHB) (9 ml) and then added with each plant extracts (200 µl). The mixture was incubated at 37°C for overnight and then measured for its absorbance at 600 nm. The antibacterial activities of plant extracts were determined by the percentages of inhibition calculated using the following formula:

$$\% \text{ inhibition} = [A_{control}-A_{sample})/A_{control}] \times 100;$$

where $A_{control}$ = absorbance of culture without plant extract

and A_{sample} = absorbance of culture with plant extract.

2.4 Statistical analysis

The results were expressed as mean ± SD. Data were analyzed by SPSS version 16.0. One-way ANOVA and Duncan multiple range's test were used for data comparison at $p<0.05$.

3. Results and Discussion

3.1 Antimicrobial activities against acne-producing bacteria of freshly prepared plant extracts

The freshly prepared plant extracts were shown to have antimicrobial activities against acne-producing bacteria as determined by broth dilution assay (Table 1). The strong antibacterial activities were found in a majority of ethanolic extracts of all plants which possessed more than 80% of inhibitory activities, especially against *Streptococcus pyogenes* and *Staphylococcus aureus*. Moreover, effective antibacterial activities were also detected in DCM extract of *Boesenbergia rotunda* (L.) Mansf. and HEX extracts of *Curcuma longa* Linn. and *Orthosiphon aristatus* (Blume) Miq. Surprisingly, DW extracts showed relatively low antibacterial activities (less than 35% inhibition).

Table 1 Antimicrobial activities of freshly prepared Thai plant extracts against acne-producing bacteria

Plant	Solvent	% inhibition			
		Propionibacterium acnes	*Staphylococcus epidermidis*	*Streptococcus pyogenes*	*Staphylococcus aureus*
Curcuma longa Linn.	DW	31.85±1.78^{i}	1.56±0.15^{l}	28.29±2.00^{c}	9.95±1.24^{j}
Zingiber montanum (Koenig) Link ex Dietr.	DW	32.96±1.00^{hi}	13.22±0.23^{i}	10.85±0.89^{f}	12.04±2.44^{j}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	DW	27.83±2.25^{j}	19.48±0.45^{j}	21.74±2.65^{e}	13.33±2.04^{j}
Orthosiphon aristatus (Blume) Miq.	DW	10.85±0.80^{l}	25.04±0.89^{h}	14.60±6.89^{f}	10.58±1.60^{j}
Boesenbergia rotunda (L.) Mansf.	DW	15.16±0.29^{k}	18.26±1.87^{i}	15.12±1.97^{f}	18.77±1.17^{i}
Curcuma longa Linn.	EtOH	81.26±0.72^{c}	17.74±0.94^{i}	83.96±1.38^{b}	90.39±4.86^{b}
Zingiber montanum (Koenig) Link ex Dietr.	EtOH	66.48±1.44^{e}	5.91±2.32^{k}	83.44±0.59^{b}	90.02±2.53^{b}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	EtOH	68.33±1.72^{e}	85.00±4.58^{b}	84.14±0.25^{b}	90.33±0.87^{b}

Table 1 Antimicrobial activities of freshly prepared Thai plant extracts against acne-producing bacteria (continue)

Plant	Solvent	% inhibition			
		Propionibacterium acnes	*Staphylococcus epidermidis*	*Streptococcus pyogenes*	*Staphylococcus aureus*
Orthosiphon aristatus (Blume) Miq.	EtOH	54.94±2.13^{g}	80.21±0.37^{c}	84.48±0.50^{b}	89.39±3.50^{b}
Boesenbergia rotunda (L.) Mansf.	EtOH	86.41±1.30^{b}	69.92±0.63^{e}	82.85±3.01^{b}	91.66±2.88^{a}
Curcuma longa Linn.	DCM	68.74±0.14^{e}	6.44±1.73^{k}	13.24±2.42^{f}	28.75±2.84^{g}
Zingiber montanum (Koenig) Link ex Dietr.	DCM	60.44±0.40^{f}	12.99±0.60^{j}	13.73±3.22^{f}	25.75±1.37^{gh}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	DCM	62.70±0.25^{f}	4.53±0.64^{k}	21.66±1.15^{e}	29.70±1.67^{g}
Orthosiphon aristatus (Blume) Miq.	DCM	77.05±1.31^{d}	7.33±2.00^{k}	26.43±2.75^{cd}	22.51±3.97^{hi}
Boesenbergia rotunda (L.) Mansf.	DCM	81.15±2.40^{c}	54.24±1.07^{g}	11.34±1.66^{f}	81.93±1.78^{c}
Curcuma longa Linn.	HEX	60.42±0.37^{f}	77.29±2.03^{d}	24.18±1.41^{cde}	53.58±3.95^{e}
Zingiber montanum (Koenig) Link ex Dietr.	HEX	67.44±2.22^{e}	67.62±2.50^{e}	22.55±2.36^{de}	65.16±5.03^{d}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	HEX	35.62±3.55^{h}	10.43±0.08^{j}	2.81±2.38^{gh}	40.53±1.28^{f}
Orthosiphon aristatus (Blume) Miq.	HEX	79.40±3.93^{cd}	27.48±1.28^{h}	2.79±2.78^{gh}	66.41±2.75^{d}
Boesenbergia rotunda (L.) Mansf.	HEX	65.81±2.75^{e}	63.11±3.25^{f}	6.08±4.01^{g}	57.14±2.91^{e}
Streptomycin 100 Units/ml (Positive control)	-	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Distilled water (Negative control)	-	0.00±0.00^{l}	0.00±0.00^{l}	0.00±0.00^{l}	0.00±0.00^{l}

Note: DW = distilled water; EtOH = absolute ethanol; DCM = dichloromethane; HEX = hexane.

Same letters, $^{a, b, c,\ldots}$, in the same column indicated no significant differences between the data at $p<0.05$.

3.2 Antimicrobial activities against acne-producing bacteria of the extracts derived from plant samples stored at 4°C for 1 month

After plant samples were stored at 4°C in refrigerator for 1 month, they were extracted and tested again for their antimicrobial activities against acne-producing bacteria. The results showed that plant samples' antibacterial properties were found to be moderately decreased after storage (Table 2). Although the ethanolic extracts of all plants were still found to possess antibacterial activities, their activities were shown to be rather low (less than 60% inhibition) and only against *Staphylococcus epidermidis* and *Streptococcus pyogenes*. In contrast, DCM extracts of *Curcuma longa* Linn. and *Boesenbergia rotunda* (L.) Mansf. and HEX extract of *Curcuma longa* Linn. were shown to have significant antimicrobial activities, especially against *Propionibacterium acnes* and *Staphylococcus aureus*.

Table 2 Antimicrobial activities of Thai plant extracts against acne-producing bacteria after the plant samples being stored at 4°C for 1 month

Plant	Solvent	% inhibition			
		Propionibacterium acnes	*Staphylococcus epidermidis*	*Streptococcus pyogenes*	*Staphylococcus aureus*
Curcuma longa Linn.	DW	8.93±0.10^{hi}	13.72±1.03^{klm}	1.21±0.20^{no}	9.78±0.31^{kl}
Zingiber montanum (Koenig) Link ex Dietr.	DW	7.69±0.27^{ij}	12.25±1.34^{lm}	19.30±0.70^{k}	9.65±2.33^{kl}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	DW	14.37±1.23^{f}	14.50±1.33^{kl}	3.22±0.32^{n}	7.87±1.25^{l}
Orthosiphon aristatus (Blume) Miq.	DW	10.72±0.53^{gh}	11.85±0.56^{m}	13.62±0.53^{l}	11.67±1.60^{jl}
Boesenbergia rotunda (L.) Mansf.	DW	15.72±1.33^{f}	2.24±0.06^{n}	5.62±0.88^{m}	11.74±0.81^{jk}
Curcuma longa Linn.	EtOH	34.53±2.90^{d}	29.62±0.53^{g}	48.44±0.80^{d}	33.61±1.33^{f}
Zingiber montanum (Koenig) Link ex Dietr.	EtOH	2.85±0.50^{l}	48.78±0.31^{b}	58.28±0.40^{b}	17.78±1.81^{i}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	EtOH	4.75±0.35^{kl}	15.25±1.76^{k}	54.25±0.35^{c}	32.70±1.17^{f}
Orthosiphon aristatus (Blume) Miq.	EtOH	12.11±0.64^{g}	40.12±0.17^{d}	24.39±1.33^{j}	12.61±1.34^{j}
Boesenbergia rotunda (L.) Mansf.	EtOH	10.44±0.63^{gh}	42.29±1.47^{c}	35.01±2.50^{h}	38.33±0.94^{e}

Table 2 Antimicrobial activities of Thai plant extracts against acne-producing bacteria after the plant samples being stored at 4°C for 1 month (continue)

Plant	Solvent	% inhibition			
		Propionibacterium acnes	*Staphylococcus epidermidis*	*Streptococcus pyogenes*	*Staphylococcus aureus*
Curcuma longa Linn.	DCM	80.78±1.72^{b}	35.50±0.71^{e}	44.62±2.30^{e}	52.27±1.84^{d}
Zingiber montanum (Koenig) Link ex Dietr.	DCM	9.56±0.80^{hi}	32.72±1.02^{f}	42.37±0.20^{f}	62.83±1.20^{b}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	DCM	10.56±0.62^{gh}	26.25±0.35^{h}	37.28±1.81^{g}	19.56±1.41^{i}
Orthosiphon aristatus (Blume) Miq.	DCM	15.17±0.71^{f}	32.88±1.86^{f}	37.74±0.21^{g}	28.32±0.46^{g}
Boesenbergia rotunda (L.) Mansf.	DCM	81.46±1.11^{b}	32.78±1.72^{f}	36.78±0.31^{gh}	57.78±1.10^{c}
Curcuma longa Linn.	HEX	72.62±0.88^{c}	28.72±1.10^{g}	17.38±0.25^{k}	38.93±0.09^{e}
Zingiber montanum (Koenig) Link ex Dietr.	HEX	5.55±1.2^{jk}	22.67±0.94^{i}	3.36±0.05^{n}	13.28±1.63^{j}
Andrographis paniculata (Burm.f.) Wall. ex Nees.	HEX	3.85±0.50^{kl}	33.72±1.03^{ef}	1.35±0.71^{no}	5.33±0.50^{m}
Orthosiphon aristatus (Blume) Miq.	HEX	20.28±0.40^{e}	11.62±0.87^{m}	6.60±0.85^{m}	17.67±1.25^{i}
Boesenbergia rotunda (L.) Mansf.	HEX	34.28±1.82^{d}	20.28±1.02^{j}	32.38±1.25^{i}	25.11±2.20^{h}
Streptomycin 100 Units/ml (Positive control)	-	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100±0.00^{a}
Distilled water (Negative control)	-	0.00±0.00^{m}	0.00±0.00^{o}	0.00±0.00^{o}	0.00±0.00^{n}

Note: DW = distilled water; EtOH = absolute ethanol; DCM = dichloromethane; HEX = hexane.

Same letters, $^{a, b, c,\ldots}$, in the same column indicated no significant differences between the data at $p<0.05$.

In this study, ethanol was found to be the effective solvent for extracting the antimicrobial agents from freshly prepared plant samples (Table 1). Ethanol has been previously used for extraction of many other plants to obtain the extracts with high antimicrobial activities against acne-inducing bacteria (Rattanasena, 2012; Saising and Voravuthikunchai, 2012; Sharma, Kishore, Hussein and Lall, 2014; Wang, Yang, Qin, Shan and Ren, 2013). There was a possibility that ethanol might not only extract the antimicrobial agents, it might also effectively extract the antioxidants from plant samples. Antioxidant activity

is one of the key actions for inhibiting acne-producing bacteria (Azimi *et al*., 2012; Pothitirat *et al*., 2009). The importance of antioxidant activities for suppressing acne-producing bacteria might explain the reduction of antimicrobial activities in plant samples that were stored at 4°C for 1 month (Table 2). During the storage, the antioxidants in plant samples used in this study might be lost and therefore their extracts were shown to have rather low antimicrobial activities against acne-producing bacteria. Therefore, the search for effective plant extracts to be applied in anti-acne products should focus not only in the methods for extracting and preserving plants' antimicrobial agents, but also their antioxidant contents. This observation remained to be determined in the future experiments.

4. Conclusion

This study showed that the ethanolic extracts of some Thai local plants might have great potentials to be developed as anti-acne products due to their high levels of antimicrobial activities against acne-producing bacteria. The findings in this study also suggested that the appropriate methods for preservation of plants' antimicrobial and antioxidant agents should be carefully focused. This knowledge can be beneficial for future application of plant extracts as natural antimicrobial agents in both pharmaceutical and cosmetic industries.

Acknowledgements

This study was financially supported by Mahasarakham University. Thanks the Department of Biotechnology, Faculty of Technology for partial funding and laboratory facilities.

References

Adwan, G., Abu-Shanab, B. and Adwan, K. 2010. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug–resistant *Pseudomonas aeruginosa* strains. Asian Pacific Journal of Tropical Medicine. 3: 266–269.

Azimi, H., Fallah-Tafti, M., Khakshur, A.A. and Abdollahi, M. 2012. A review of phytotherapy of acne vulgaris: Perspective of new pharmacological treatments. Fitoterapia. 83: 1306–1317.

Chomnawang, M.T., Surassmo, S., Nukoolkarn, V.S. and Gritsanapan, W. 2005. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. Journal of Ethnopharmacology. 101: 330–333.

Chomnawang, M.T., Surassmo, S., Nukoolkarn, V.S. and Gritsanapan, W. 2007. Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. Fitoterapia. 78: 401–408.

Pothitirat, W., Chomnawang, M.T., Supabphol, R. and Gritsanapan, W. 2009. Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. Fitoterapia. 80: 442–447.

Rattanasena, P. 2012. Antioxidant and antibacterial activities of vegetables and fruits commonly consumed in Thailand. Pakistan Journal of Biological Sciences. 15: 877–882.

Saising, J. and Voravuthikunchai, S.P. 2012. Anti *Propionibacterium acnes* activity of rhodomyrtone, an effective compound from *Rhodomyrtus tomentosa* (Aiton) Hassk. leaves. Anaerobe. 18: 400–404.

Sharma, R., Kishore, N., Hussein, A. and Lall, N. 2014. The potential of *Leucosidea sericea* against *Propionibacterium acnes*. Phytochemistry Letters. 7: 124–129.

Wang, L., Yang, X., Qin, P., Shan, F. and Ren, G. 2013. Flavonoid composition, antibacterial and antioxidant properties of tartary buckwheat bran extract. Industrial Crops and Products, 49: 312–317.

Yamaguchi, N., Satoh-Yamaguchi, K. and Ono, M. 2009. *In vitro* evaluation of antibacterial, anticollagenase, and antioxidant activities of hop components (*Humulus lupulus*) addressing acne vulgaris. Phytomedicine. 16: 369–376.