Antibacterial Activities of Four Thai Medicinal Plants

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Medicinal plants have long been used and prescribed in Thailand for centuries. Some of them have been used for treating various diseases including infectious diseases. Pouzolzia pentandra Benn., Gelonium multiflorum A. Juss., Erycibe elliptilimba Merr.&Chun., Balanophora abbreviate Bl. are Thai medicinal plants from the Thai pharmacopoeia that have been prescribed for treating unknown fevers including some specific infectious diseases. This investigation demonstrated the effects of these Thai medicinal plants for their antibacterial activities by using the macrodilution assay. Based on the present study, the water methanol fraction (fraction 2) of Balanophora abbreviate Bl. showed the antibacterial activity at the MIC level of 250 g/ml but the activity was bacteriostatic in its effects. Therefore, the use of these medicinal plants in controlling fever and infectious diseases appears to be justified and further investigations may be required to obtain more information.

Keywords: *Antibacterial activities, Pouzolzia pentandra Benn, Gelonium multiflorum A Juss, Erycibe elliptilimba Merr & Chun, Balanophora abbreviate Bl*

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Natural products especially medicinal plants have long been prescribed in traditional medicine for centuries for treating various diseases such as malignancies and infections. Some of these specific medicinal plants and natural products have been found to be active against some specific types of infectious diseases, such as *Echium amoenum,* which is traditionally used in France and other countries for treating infectious diseases, flu and as an antifebrile[1]. Thailand is well known for various medicinal plants that have long been used for the treatment of diseases[2]. Though a lot of plants have been used in traditional medicine, little is known about the correlation in scientific reports and their specific activities.

One of the major health problems in Thailand is infectious disease. This is also a problem in many countries worldwide because of drug resistant pathogens[3,4]. However, nowadays, the prime cause of mortality rates in Thailand, excluding accidents, is malignant disease. Hence, infectious diseases still play an important role in health control programs. Moreover, plenty of medicinal plants are still commonly used in a wide range of clinical settings for infectious diseases without evaluation of their scientific activities. Therefore, this prompted the authors to evaluate the antibacterial activities of Thai medicinal plants that have long been prescribed in traditional medicine.

Four Thai medicinal plants from the Thai pharmacopoeia were used to evaluate the antibacterial activity. *Pouzolzia pentandra* Benn., *Gelonium multiflorum* A. Juss., *Erycibe elliptilimba* Merr. & Chun., *Balanophora abbreviate* Bl. are Thai medicinal plants in the family of Urticaceae, Euphobiaceae, Convolvulaceae, and Balanophoraceae, respectively. They have all long been prescribed in the Thai pharmacopoeia for treating various diseases. *Pouzolzia pentandra* Benn., Thai name "Kob-cha-nang-dang", has been used in dermatological, and urological diseases. *Gelonium*

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multiflorum A. Juss., called in Thai "Kun-tong-paya-baht", has been prescribed for treating unknown fevers, dermatological and malignant diseases. *Erycibe elliptilimba* Merr. & Chun., Thai name "Pra-Kun-chai-sri", is a medicinal plant that has long been used for treating unknown fevers including various malignancies. Finally, *Balanophora abbreviate* Bl., called " Hoh-ra-tao-su-nak", has been used for the treatment of dermatological problems, malignant diseases and asthma[5,6]. All of these medicinal plants are used as some of the components in Thai herbal remedies for the treatment of these infectious diseases.

Two different bacterial species were used to investigate the possible antibacterial activities from the chosen Thai medicinal plants. *Staphylococcus aureus* is one of the most common Gram positive bacteria that causes common infectious diseases such as soft tissue infection and community associated bloodstream infection and *Escherichia coli* which is a Gram negative bacillus causes serious infectious diseases such as urinary tract infection and bloodstream infection[7]. Both microorganisms were used to examine the antibacterial activities of Thai medicinal plants in the present report.

Material and Method

1. Plants and extraction process

Stems and leaves of *Pouzolzia pentandra* Benn., stems of *Erycibe elliptilimba* Merr. & Chun., stems of *Gelonium multiflorum* A. Juss, and stems and leaves of *Balanophora abbreviate* Bl. were purchased from the well known Thai Herb Pharmacy "Chao-Krom-Po". They were cut into small pieces and ground into powder. The powder was macerated three times with 4 liters of 95% ethanol for 7 days each. The extracts were concentrated under reduced pressure, resulting in crude extract

The ethanol extract was chromatographed on a Diaion HP-20 column (Mitsubishi Chemical Corp., Japan), an ion-exchange resins open column, which was dry packed using a glass column (inner diameter 4 cm, 60 cm long) and equilibrated with water (100 ml). The ethanol extract was dissolved in distilled water (100 ml.) and sonicated for 15 minutes in an ultrasonic bath to obtain the water-soluble fraction. The suspension was centrifuged at 20,000 rpm (about 42,000 g) for 30 minutes. The supernatant was applied onto the Diaion HP20 column and the eluent was collected (fraction 1). The precipitate was dissolved in methanol-water (1:1/100) and methanol (150 ml.) to get the fraction 2 and fraction 3, respectively. Each supernatant was repeatedly performed as described above. The precipitate was dissolved in ethyl acetate (fraction 4)[8]. The chemical composition of each fraction was monitored on thin layer chromatography.

Preparation of the plants extracts

All plant extracts were dissolved in dimethyl sulfoxide (DMSO, Sigma, St.Louis, USA) except for the water fraction (fraction 1) which was dissolved in water. For all experiments, final concentrations of the tested compound were prepared by diluting the stock with the broth cultures. All extracts were prepared and tested in triplicate.

2. Microorganisms

The antibacterial activities of all the plant extracts were determined against *Staphylococcus aureau* (ATCC 25923) and *Escherichia coli* (ATCC 25922). All cultures were stocks stored at -80°C. For the determination of antibacterial activities, all strains were grown in 10 ml. Mueller Hinton broth for 24 hours at 37°C.

3. Antibacterial activities

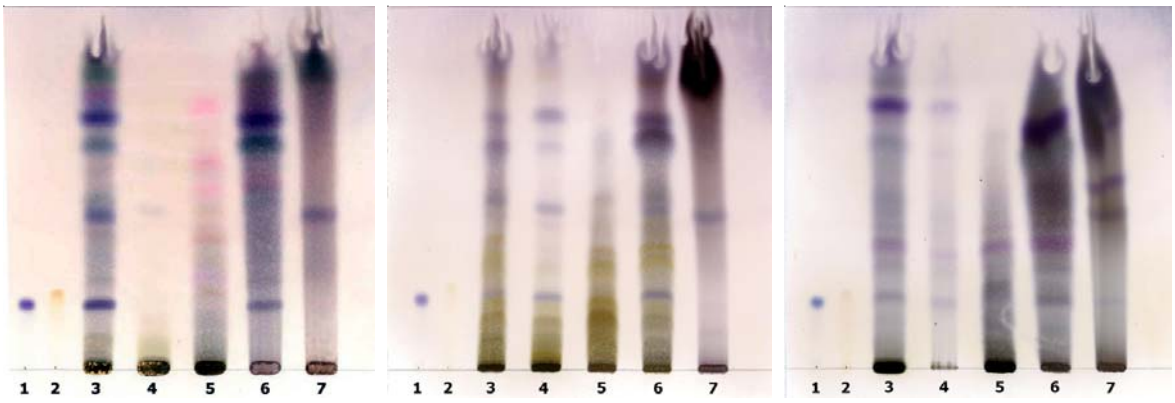
Macrodilution assay

The minimal inhibition concentration (MIC) values were studied for the microorganisms based on the broth dilution method as recommended by CLSI (Clinical and Laboratory Standard Institute; formerly NCCLS)[9].The plant extracts were dissolved to a final concentration by diluting the extracts with broth cultures and equal volumes of bacterial suspension at 10^6 CFU/ml was added to each dilution of the plant extracts. 1% DMSO was used as the control. All the plant extracts except fraction 1 dissolved in 1% dimethyl sulfoxide (DMSO) were first diluted to the highest concentration of 2000 g/ml to be tested, then serial two fold dilutions were made in a concentration which ranged from 3.9 to 2000 g/ml in Mueller Hinton broth. All mixtures were incubated at 37°C for 24 hours. MIC values of the plant extracts against bacterial strains was read as this minimum dilution that could inhibit bacterial growth after overnight incubation In the case of an MIC value lower than 2000 g/ml, the suspension was subcultured on Mueller Hinton agar to observe the growth of bacteria at 37°C for 24 hours.

Result

Thin layer chromatogram of the plant extracts

Chromatographic fractionation of the ethanolic extract was carried out using a Diaion HP-20 column as shown in Fig. 1 and 2. The gel is a highly



A B C

Fig. 1 Thin-layer chromatograms of Pouzolzia pentandra (A), Erycibe elliptilimba (B), and Gelonium multiflorum (C) ethanol (crude) extracts and fractions from Diaion HP-20

adsorbent : silica gel GF254 Alufolien, Merck, Germany
solvent system : Chloroform:Methanol (90:10)
detection : spray with anisaldehyde - sulfuric acid, heat at 100°C, 1-2 mins, in oven
reference : 1 = 1% Gla (Phytosteryl glucoside) in ethanol 3 l
2 = 1% Quercetin in ethanol 3 l
Sample* : 3 = ethanol extract 30 l
4 = water fraction 30 l
5 = water/methanol fraction 30 l
6 = methanol fraction 30 l
7 = ethyl acetate fraction 30 l
* sample concentration = 140 mg/ml

porous styrene-divinylbenzene copolymer carrying macropores and is suitable for the absorption of non-polar materials from polar solvent solutions. The fractionation of the ethanolic extract on a Diaion HP - 20 column provided 4 fractions using water (Fraction 1), water:methanol (Fraction 2) and methanol (Fraction 3) as eluents. Ethyl acetate was used at the last step for washing the column and yielded fraction 4.

Fraction 1 (water eluate) chiefly contained inorganic salts and sugars that constituted the major amount.

Fraction 2 comprised a large amount of sugar as well as several phenolic substances.

Fraction 3 contained some phenolic components, polar triterpenes, phytosteryl glycosides, and aglycones.

Fraction 4 mainly contained chlorophyll and less polar components.

Growth inhibition activities of plant extracts against the microorganisms

For evaluation of antibacterial activities, all plant extracts except fraction 4 were used to examine the effects since the amount all of fraction 4 was not enough for evaluation. The results of the antibacterial activities of all the extracts and bacterial strains are demonstrated as minimal inhibitory concentration (MIC) as shown in Table 1. The MIC is defined as the lowest concentration that visibly inhibits growth of the microorganism after overnight incubation. The activities of all the extracts did not exhibit any inhibition of the microorganisms but the growth of *Staphylococcus aureus* was affected by the water:methanol fraction of *Balanophora abbreviate*, which displayed the MIC value at 250 g/ml. However, this activity of the extract appeared to be only bacteriostatic and not a bactericidal effect since the growth of these bacteria could still be detected after subculture.

Discussion

The antibacterial activities of all plant extracts against *Staphylococcus aureus* and *Escherichia coli* examined in the present study were assessed by the MIC value as illustrated in Table 1. For the study of these plant extracts, the number of bacterial strains was reduced in accordance with their known function as a human pathogenic. One species of both Gram negative and Gram positive bacterium was chosen. This was

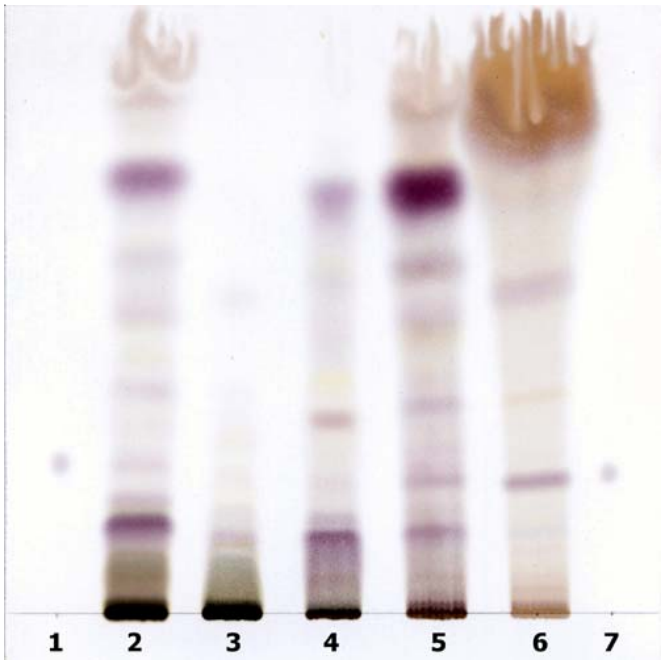


Fig. 2 Thin-layer chromatogram of Balanophora abbreviate ethanol (crude) extract and fractions from Diaion HP-20

adsorbent : silica gel GF254 Alufolien, Merck, Germany
solvent system : Chloroform:Methanol (90:10)
detection : spray with 10% sulfuric acid, heat at 100°C, 1-2 mins, in oven
reference : 1 and 7 = 1% Gla (Phytosteryl glucoside) in ethanol 3 l
Sample* : 2 = ethanol extract 30 l
3 = water fraction 30 l
4 = water/methanol fraction 30 l
5 = methanol fraction 30 l
6 = ethyl acetate fraction 30 l
* sample concentration = 200 mg/ml

assumed sufficient for the preliminary antimicrobial screening of the plant extracts.

Staphylococcus aureus and *Escherichia coli* were used in this antimicrobial testing because of their medical significance in causing common problems of bacterial infections found in the community[7]. Based on the present results, all plant extracts except the water: methanol fraction of *Balanophora abbreviate* did not show any inhibitory activity against these microorganisms. Findings in the present study supported the observations of some other researchers about the medicinal plants named *Andrographis paniculata* (Burma) Wall. ex Ness and *Phyllanthus amarus* in their antibacterial and antiviral properties respectively[10-12]. In general, plant extracts did not produce as marked an inhibition as many of the pure compounds because plant extracts usually contain flavonoids in glycosidic form[13].

The growth of *Staphylococcus aureus*, not *Escherichia coli*, was partially inhibited by the water: methanol fraction of *Balanophora abbreviate*. This observation confirmed the evidence in previous studies reported that methanol is a better solvent for extraction of antimicrobial substances from medicinal plants than water, ethanol, and hexane[14-16].

The effect of this special plant extract may be due to some compounds other than flovonoids such as its phenolic content[17]. The present result also suggested that *Balanophora abbreviate* extract possesses some compounds with bacteriostatic property, since incubation of the herb treated broth for one week still showed the growth of bacteria. However, the maximum dilution in this study was only 2000 g/ml and may not have been enough for killing microorganism *in vivo*. However, this is the first study providing more information that the water:methanol extract of *Balanophora*

Table 1. In vitro antibacterial activities of four Thai medicinal plants against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922

Plant name	Fraction	MIC (mg/L)	
		S. aureus (25923)	*E. coli* (25922)
Pouzolzia pentandra	F1	>2000	>2000
(ขอบชะนางแดง)	F2	>2000	>2000
	F3	>2000	>2000
	Fc	>2000	>2000
Gelonium multiflorum	F1	>2000	>2000
(ขันทองพยาบาท)	F2	>2000	>2000
	F3	>2000	>2000
	Fc	>2000	>2000
Erycibe elliptilimba	F1	>2000	>2000
(พระขรรค์ไชยศรี)	F2	>2000	>2000
	F3	>2000	>2000
	Fc	>2000	>2000
Balanophora abbreviate	F1	>2000	>2000
(โหราเทาสุนัข)	F2	=250	>2000
	F3	>2000	>2000
	Fc	>1000	>2000

Note: F1 = water fraction
F2 = water:methanol fraction
F3 = methanol fraction
Fc = crude extract

abbreviate possesses some antibacterial activity against *Staphylococcus aureus*. Therefore, this plant could possibly be the new therapeutic agent for treating infectious diseases in humans.

Conclusion

Numerous herbs and plants have been reported to be the potential sources of antimicrobial agents but not many have been studied with respect to levels and range of activities. Because of this, it is essential to use herbal medicine cautiously. However, the results obtained in the present report exhibited the correlation between scientific observations and the recommendations of traditional medicine. Further studies on the isolation and characterization of the active compounds in this plant extract may be required to provide a new antibacterial agent in the clinical setting in the future.

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การประเมินฤทธิ์ต้านแบคทีเรียของสมุนไพรไทย 4 ชนิด

สุวรรณา ตระกูลสมบูรณ์, ธนวรรณ กุมมาลือ, วีณา จิรัจฉริยากูล

ประเทศไทยเป็นประเทศที่มีการนำพืชสมุนไพรมาใช้อย่างแพร่หลายนานกว่าศตวรรษพืชสมุนไพรเหล่านี้ถูกนำมาใช้เพื่อรักษาโรคต่าง ๆ มากมาย อาทิเช่น โรคติดเชื้อ เป็นต้น พืชสมุนไพรไทย 4 ชนิด ได้แก่ ขอบชะนางแดง ขันทองพยาบาท พระขรรค์ไชยศรี โหราเท้าสุนัข เป็นพืชสมุนไพรไทยที่ระบุไว้ในตำรายาแพทย์แผนไทยว่ามีสรรพคุณในการรักษาโรคติดเชื้อบางชนิดรวมถึงรักษาไข้ที่ไม่ทราบสาเหตุได้ด้วย คณะผู้ทำการวิจัยได้ตรวจหาฤทธิ์ต้านแบคทีเรียของพืชสมุนไพรไทยทั้ง 4 ชนิดนี้โดยวิธี macrodilution assay ผลการวิจัยปรากฏว่าสารสกัดน้ำ: เมธานอลของสมุนไพรไทยโหราเท้าสุนัขมีฤทธิ์ต้านแบคทีเรียโดยมีระดับ MIC เท่ากับ 250 ไมโครกรัมต่อมิลลิลิตร แต่เป็นฤทธิ์ต้านการเจริญเติบโตของแบคทีเรีย ผลการวิจัยในครั้งนี้บ่งชี้ว่าการที่จะนำสมุนไพรไทยมาใช้ในการรักษาโรคติดเชื้อควรจะต้องมีการพิจารณาและอาจต้องทำการศึกษาเพิ่มเติมเพื่อให้ได้ข้อมูลมากขึ้น