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บทความวิจัย

ฤทธิ์ต้านเชื้อแบคทีเรียของสมุนไพรไทยบางชนิดที่ใช้รักษาโรคผิวหนัง

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บทคัดย่อ

งานวิจัยนี้ศึกษาฤทธิ์ต้านเชื้อแบคทีเรียของสารสกัดด้วยเอทานอลของสมุนไพรไทย 10 ชนิด (ทองพันชั่ง พลูบัวบก พญายอ เสลดพังพอนตัวผู้ สำมะงา เหงือกปลาหมอ ฟ้าทะลายโจร กระเบา และชุมเห็ดเทศ) ต่อเชื้อ 11 สายพันธุ์ ด้วยวิธี disk diffusion และ broth dilution พบว่าสารสกัดจากสมุนไพรไทยทุกชนิดที่ศึกษามีฤทธิ์ยับยั้งเชื้อแบคทีเรียได้อย่างน้อย 2 สายพันธุ์ สารสกัดจากพลูและเสลดพังพอนตัวผู้มีฤทธิ์ยับยั้งเชื้อสูงสุดเมื่อเปรียบเทียบกับสมุนไพรไทยชนิดอื่น สามารถต้านเชื้อแบคทีเรียได้ทุกสายพันธุ์ที่ศึกษา จากการศึกษาพบว่าสารสกัดจากพลูมีศักยภาพสูงสุดในการยับยั้งเชื้อ Staphylococcus aureus ATCC 25923, methicillin-resistant S. aureus, methicillin-susceptible S. aureus, Staphylococcus epidermidis, Escherichia coli ATCC 25922, extended-spectrum beta-lactamases (ESBL)-producing E. coli, Klebsiella pneumoniae ATCC 700603 (ESBL-producing strain), carbapenem-resistant K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa ATCC 27853, และ P. aeruginosa โดยมีค่า inhibition zone ค่า minimal inhibitory concentration และค่า minimal bactericidal concentration อยู่ระหว่าง 13.7-32.0 มิลลิเมตร 0.5-8 มิลลิกรัมต่อมิลลิลิตร และ 2-8 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ แสดงให้เห็นว่าสารสกัดด้วยเอทานอลจากพลูมีฤทธิ์ต้านเชื้อแบคทีเรียที่หลากหลายและมีศักยภาพที่จะพัฒนาเป็นผลิตภัณฑ์ยาสมุนไพรไทยสำหรับรักษาโรคผิวหนังต่อไป

คำสำคัญ: สมุนไพร สารสกัดจากพืช ฤทธิ์ต้านเชื้อแบคทีเรีย โรคผิวหนัง

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Original Article

Antibacterial Activity of Some Thai Medicinal Plants Used in Traditional Treatment of Skin Disease

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Abstract

In this study, we evaluated the antibacterial activity of ethanolic extracts from 10 Thai medicinal plants (*Rhinacanthus nasutus*, *Piper betle*, *Centella asiatica*, *Clinacanthus nutans*, *Barleria lupulina*, *Volkameria inermis*, *Acanthus ebracteatus*, *Andrographis paniculata*, *Hydnocarpus anthelminthicus,* and *Cassia alata*) against 11 bacterial strains. The activity was evaluated using disk diffusion and broth dilution assays. All tested plants exhibited antibacterial activity against at least two of the tested bacteria. The extracts of *P. betle* and *B. lupulina* showed relatively high activity against all bacterial strains compared with that of the other plant extracts. The highest potential was observed in the extract of *P. betle* against *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus*, methicillin-susceptible *S. aureus*, *Staphylococcus epidermidis*, *Escherichia coli* ATCC 25922, extended-spectrum beta-lactamases (ESBL)-producing *E. coli*, *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing strain), carbapenem-resistant *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* ATCC 27853, and *P. aeruginosa* with zone of inhibition, minimal inhibitory concentration and minimal bactericidal concentration ranging from 13.7 to 32.0 mm, 0.5 to 8 mg/mL, and 2 to 8 mg/mL, respectively. This indicates that the ethanolic extract of *P. betle* exhibits various antibacterial activities and may be a good source for the development of Thai herbal products for skin disease treatment.

Keywords: medicinal plants, plant extracts, antibacterial activity, skin disease

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Introduction

Skin and soft tissue infections occur due to microbial invasion of the skin layers and underlying soft tissues. Patients exhibiting risk factors such as older age, diabetes mellitus, cirrhosis, and intravenous drug abuse can transform a typically mild infection into a rapidly severe threat to life. The most common bacterial species causing wound infections are *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis,* and *Acinetobacter baumannii*.[1-2] A study of a five-year (2015-2019) period, 12.6 % of the population in a primary care area in Thailand was affected by skin diseases. Infections of the skin and subcutaneous tissues (37.3%) and dermatitis (29.7%) were the two most common skin diseases.[3]

The inappropriate use of antibiotics in animal and human health, and their prolonged use in hospitals, have led to the development of antibiotic-resistant bacteria. This causes existing antibacterial drugs to become less effective or ineffective.[4-5] In developing countries, microbial infections are responsible for the annual deaths.[6] Therefore, novel and affordable drugs are required for the treatment of infectious diseases. Plant-derived chemicals are natural compounds that have attracted the attention of many researchers. Phytochemicals from medicinal plants have become a good choice for treating infectious diseases because of their natural origin and low adverse effects on patients. These compounds may help overcome the emergence of antibiotic-resistant bacteria.[7] Recently, many researchers have attempted to generate herbal medicines based on their properties. Many medicinal plants in Thailand have unique biochemical properties and have long been used in traditional Thai medicine. Medicinal plants, including *Rhinacanthus nasutus* (Linn.) Kurz, *Piper betle* (Linn.) *Centella asiatica* (Linn.) Urban, *Clinacanthus nutans* (Burm. f) Lindau, *Barleria lupulina* Lindl., *Volkameria inermis* (Linn.), *Acanthus ebracteatus* Vahl., *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Hydnocarpus anthelminthicus* Pieere ex Laness and *Cassia alata* (Linn.) Roxb. are used in primary health care for the treatment of skin diseases.[8-14] Their parts, such as leaves and flowers, are boiled for washing, or blended and mixed with alcohol to mask the wound. However, the efficiency of their bioactive compounds and their mechanisms of action against skin pathogens remain unclear. In this study, the antibacterial activities of ethanol extracts from 10 Thai medicinal plants used for the treatment of skin diseases were evaluated against 11 pathogenic bacteria. Their antimicrobial properties make them good candidates for further studies in novel drug recipes for the treatment of skin diseases.

Materials and methods

Plant samples and extraction

Ten medicinal plant species with rash-reducing activity were collected from Samut Prakan, Rayong, Kanchanaburi, and Chanthaburi Provinces (Table 1). The plants were identified by Wat Po Thai Traditional Medical School (Bangkok, Thailand) and Phenkhae Thai Traditional Medical School

(Pathum Thani, Thailand). The plants were washed with distilled water to remove debris and dust particles, air-dried, and then chopped into small pieces. Extraction was performed by adding 500 g of the plant sample to 500 mL of ethanol. The mixtures were blended thoroughly and incubated at 25°C for 7 days. Ethanol extracts were filtered and evaporated to dryness. The crude extracts were stored at -20°C until further use.

Test microorganisms

A panel of 11 well-documented pathogenic bacteria was used. The bacterial strains included Gram-positive *S. aureus* ATCC 29523, MRSA (clinical isolate), methicillin-susceptible *S. aureus* (MSSA) (clinical isolate), and *Staphylococcus epidermidis* (clinical isolate). Gram-negative strains included *Escherichia coli* ATCC 25922, extended-spectrum beta-lactamases (ESBL)-producing *E. coli* (clinical isolate), *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing strain), carbapenem-resistant *K. pneumoniae* (CRKP) (clinical isolate), *Acinetobacter baumannii* (clinical isolate), *P. aeruginosa* ATCC 27853, and *P. aeruginosa* (clinical isolate). All of the clinical strains were isolated from wound infections. The entire bacterial strains were maintained on tryptic soy agar slant at 4°C for further studies.

Table 1 Medicinal plants used in this study

Plants	Local name	Parts used
Rhinacanthus nasutus (Linn.) Kurz	*Thongphanchang*	Leaves
Piper betle (Linn.)	Phlu	Leaves
Centella asiatica (Linn.) Urban	Bua Bok	Aerial parts
Clinacanthus nutans (Burm.f.) Lindau	Phaya Yo	Leaves
Barleria lupulina Lindl.	Salet Phang Phon Tua Phu	Leaves
Volkameria inermis (Linn.)	*Samma* Nga	Leaves
Acanthus ebracteatus Vahl.	Ngueak Plaa Mo	Leaves
Andrographis paniculata (Burm.f.) Wall. ex Nees	Fa Thalai Chon	Leaves
Hydnocarpus anthelminthicus Pieere ex Laness	Kra Bao	Dry seeds
Cassia alata (Linn.) Roxb.	Chumhet Thet	Leaves

Screening the plant extracts for antibacterial activity by disk diffusion method

The method used in this study was modified from that described by Bereksi et al.[15] Briefly, the plant extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 512 mg/mL. Bacterial colonies on blood agar were picked and the concentration of 0.5 McFarland standard (10^8 CFU/mL) was adjusted using sterile normal saline solution. Bacterial suspensions were applied to Mueller-Hinton agar using sterile cotton swabs.[16] Sterile disks (6 mm in diameter) were impregnated with 10 µL of the crude extract solutions (5.12 mg/disk) and placed on the surface of the media. Two control disks containing DMSO and ciprofloxacin (5 µg/disk) were used as negative

and positive controls, respectively. The plates were incubated at 37°C for 18–24 h and the diameter of inhibition zones were measured in mm. The experiments were performed in triplicates. The activity index (AI) for each plant extract was calculated as[17]:

AI = Inhibition zone of the plant extract/inhibition zone of the antibiotic control

where, inhibition zone (in mm) includes the diameter of disc (6 mm).

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC and MBC values were determined for microorganisms that were sensitive to the crude extracts in the disk diffusion assay. To determine the MIC and MBC, tests were performed based on modified broth macro-dilution method of the Clinical and Laboratory Standards Institute (CLSI) guideline. [18-19] The bacterial inoculums were prepared from 0.5 McFarland standard suspensions, which were diluted to a final concentration of 10^6 CFU/mL. The crude extracts were dissolved in DMSO to the highest concentration (512 mg/mL), and serial two-fold dilutions were performed in a concentration range of 0.125–512 mg/mL using Mueller-Hinton broth (MHB). Five hundred microliters of each concentration of crude extracts were applied to 13×100 mm test tubes and 500 µL of the bacterial inoculum was added to give a final concentration of 5×10^5 CFU/mL. Test tubes containing only MHB and bacterial inocula were used as negative and positive controls, respectively. The inoculated tubes were incubated at 37°C for 18–24 h and the MIC was recorded. MIC was defined as the lowest concentration of the crude extract at which the microorganisms tested did not show visible growth, whereas MBC was defined as the minimum concentration with negative subcultures (no growth or 99.9% growth inhibition) on tryptic soy agar. The lower the MIC and MBC values, the higher the activity of the extract.

Results

Antibacterial activity

Disk diffusion assay

The antibacterial activity (assessed in terms of the inhibition zone and activity index) of the ethanolic extract of the plants was initially determined using the disk diffusion method against different bacteria. These bacterial strains were gram-positive and -negative species that are frequently encountered in skin and nosocomial infections. The diameters of the inhibition zones and the activity indices are shown in Tables 2 and 3, respectively.

Table 2 Antibacterial activity (inhibition zone, mm) of 10 Thai medicinal plant extracts

Plant extracts/ Antibiotic control	Average of inhibition zone (mm) ±SD *										
	Gram-positive bacteria				Gram-negative bacteria						
	Staphylococcus aureus ATCC 25923	Methicillin-resistant *S. aureus* (MRSA)	Methicillin-susceptible *S. aureus* (MSSA)	*Staphylococcus epidermidis*	*Escherichia coli* ATCC 25922	*Escherichia coli* (ESBL)	*Klebsiella pneumoniae* ATCC 700603 (ESBL)	*Klebsiella pneumoniae* (CRKP)	*Acinetobacter baumannii*	*Pseudomonas aeruginosa* ATCC 27853	*Pseudomonas aeruginosa*
R. nasutus	10.7 ±1.0	13.0 ±0.9	12.3 ±1.5	13.0 ±0.9	-	-	-	-	8.3 ±0.5	7.0 ±0.9	7.0 ±0.0
P. betle	23.3 ±0.5	22.0 ±0.9	19.7 ±0.6	32.0 ±2.4	16.0 ±1.0	16.7 ±1.0	18.0 ±0.9	14.0 ±0.0	24.7 ±0.5	15.3 ±0.5	13.7 ±1.4
C. asiatica	7.0 ±0.0	7.0 ±0.0	-	-	-	-	-	-	7.3 ±0.5	-	-
C. nutans	8.0 ±2.7	-	-	-	-	-	-	-	7.3 ±1.5	-	-
B. lupulina	22.0 ±1.0	22.0 ±1.0	13.3 ±1.7	28.7 ±2.3	12.0 ±2.0	12.0 ±1.7	12.0 ±1.0	10.3 ±0.6	17.0 ±0.0	14.3 ±0.6	13.3 ±0.6
V. inermis	16.7 ±1.2	13.0 ±1.0	8.3 ±0.6	18.3 ±2.1	-	-	-	-	10.0 ±1.0	-	-
A. ebracteatus	11.5 ±1.2	11.0 ±0.0	-	13.7 ±1.4	-	-	6.3 ±0.5	-	9.0 ±1.6	-	7.0 ±0.0
A. paniculata	13.0 ±1.0	13.0 ±1.0	10.0 ±1.0	15.0 ±2.7	-	-	-	-	7.7 ±1.5	-	-
H. anthelminthicus	-	9.3 ±0.6	-	-	-	-	-	-	-	7.3 ±0.6	-
C. alata	10.7 ±2.5	10.7 ±0.6	8.7 ±0.6	-	-	-	-	-	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin 5 µg/disc (Control range) **	25.3 ±0.5 (22-30)	-	30.0 ±1.0	33.0 ±0.9	34.0 ±1.4 (29-38)	6.8 ±0.7	29.7 ±0.5	12.0 ±1.0	-	29.0 ±0.0 (25-33)	32.3 ±1.4

*Results from 3-independent experiments, Zone of inhibition including 6 mm disk diameter

Plant extracts/ Antibiotic control	Average of inhibition zone (mm) ±SD *										
	Gram-positive bacteria				Gram-negative bacteria						
	Staphylococcus aureus ATCC 25923	Methicillin-resistant *S. aureus* (MRSA)	Methicillin-susceptible *S. aureus* (MSSA)	*Staphylococcus epidermidis*	*Escherichia coli* ATCC 25922	*Escherichia coli* (ESBL)	*Klebsiella pneumoniae* ATCC 700603 (ESBL)	*Klebsiella pneumoniae* (CRKP)	*Acinetobacter baumannii*	*Pseudomonas aeruginosa* ATCC 27853	*Pseudomonas aeruginosa*

**CLSI quality control range (31th ed. CLSI supplement M100 p: 171) -, no inhibition zone

Table 3 Activity index (AI) of 10 Thai medicinal plant extracts

Plant extracts	Activity index (AI) *										
	Gram-positive bacteria				Gram-negative bacteria						
	Staphylococcus aureus ATCC 25923	Methicillin-resistant *S. aureus* (MRSA) **	Methicillin-susceptible *S. aureus* (MSSA)	*Staphylococcus epidermidis*	*Escherichia coli* ATCC 25922	*Escherichia coli* (ESBL)	*Klebsiella pneumoniae* ATCC 700603 (ESBL)	*Klebsiella pneumoniae* (CRKP)	*Acinetobacter baumannii* **	*Pseudomonas aeruginosa* ATCC 27853	*Pseudomonas aeruginosa*
R. nasutus	0.42	2.17	0.41	0.39	-	-	-	-	1.39	0.24	0.22
P. betle	0.92	3.67	0.66	0.97	0.47	2.44	0.61	1.17	4.11	0.53	0.42
C. asiatica	0.28	1.17	-	-	-	-	-	-	1.22	-	-
C. nutans	0.32	-	-	-	-	-	-	-	1.22	-	-
B. lupulina	0.87	3.67	0.44	0.87	0.33	1.76	0.41	0.86	2.83	0.49	0.41
V. inermis	0.66	2.17	0.28	0.56	-	-	-	-	1.67	-	-
A. ebracteatus	0.45	1.83	-	0.41	-	-	0.21	-	1.50	-	0.22
A. paniculata	0.51	2.17	0.33	0.45	-	-	-	-	1.28	-	-
H. anthelminthicus	-	1.56	-	-	-	-	-	-	-	0.25	-
C. alata	0.42	1.78	0.29	-	-	-	-	-	-	-	-

*AI is expressed as inhibition zone of the plant extract / inhibition zone of the antibiotic control

Plant extracts	Activity index (AI) *								
	Gram-positive bacteria				Gram-negative bacteria				
	Staphylococcus aureus ATCC 25923	Methicillin-resistant *S. aureus* (MRSA) **	Methicillin-susceptible *S. aureus* (MSSA)	*Staphylococcus epidermidis*	*Escherichia coli* ATCC 25922	*Escherichia coli* (ESBL)	*Klebsiella pneumoniae* ATCC 700603 (ESBL)	*Klebsiella pneumoniae* (CRKP)	*Acinetobacter baumannii* **	*Pseudomonas aeruginosa* ATCC 27853	*Pseudomonas aeruginosa*

** No inhibition zone produced by antibiotic control (indicating 6.0 mm of disk) -, no inhibitory activity

The results revealed that the 10 medicinal plants evaluated exhibited antibacterial activity against at least two of the tested bacteria. Notably, *P. betle* and *B. lupulina* showed antibacterial activity against all tested bacterial strains with zones of inhibition ranging from 13.7 to 32.0 mm and 10.3 to 28.7 mm, respectively. The extract of *P. betle* presented the highest activity against *S. epidermidis* with an inhibition zone diameter of 32.0 mm and the lowest activity against *P. aeruginosa* (13.7 mm). The extract of *B. lupulina* showed the highest activity against *S. epidermidis* (28.7 mm) and lowest activity against *K. pneumoniae* (CRKP) (10.3 mm). The other eight plant extracts, including *R. nasutus*, *C. asiatica*, *C. nutans*, *V. inermis*, *A. ebracteatus*, *A. paniculata*, *H. anthelminthicus,* and *C. alata* exhibited varying degrees of antibacterial activity against the two to seven bacterial strains tested. *R. nasutus* was the most effective plant extract. This indicated a zone of inhibition against the seven bacterial strains tested. However, the largest inhibition zone was recorded for the extract of *V. inermis* against *S. epidermidis* with an inhibition zone diameter of 18.3 mm. It can be noted that these plants had a low activity with diameter ranging between 6.3 and 10.0 mm or no activity against gram-negative bacterial strains.

Among the antibiotic-resistant strains, the extracts of all plants, except *C. nutans*, showed antibacterial activity against MRSA, whereas ciprofloxacin, which was used as a positive control, had no inhibition zone. Notably, the extracts of *P. betle* and *B. lupulina* exhibited antibacterial activity against MRSA, *E. coli* (ESBL), *K. pneumoniae* ATCC 700603 (ESBL), and *K. pneumoniae* (CRKP) with diameters of inhibition zones greater than that of ciprofloxacin, in some cases, as an antibiotic control.

The highest AI was noted in the extract of *P. betle* against all bacterial strains tested, *S. aureus* ATCC 29523 (AI = 0.92), MRSA (AI = 3.67), MSSA (AI = 0.66), *S. epidermidis* (AI = 0.97), *E. coli* ATCC 25922 (AI = 0.47), *E. coli* (ESBL) (AI = 2.44), *K. pneumoniae* ATCC 700603 (ESBL) (AI = 0.61), *K. pneumoniae* (CRKP) (AI = 1.17), *Acinetobacter baumannii* (AI = 4.11), *P. aeruginosa* ATCC 27853 (AI =

0.53), and *P. aeruginosa* (AI = 0.42) followed by the extract of *B. lupulina* (AI = 0.87, 3.67, 0.44, 0.87, 0.33, 1.76, 0.41, 0.86, 2.83, 0.49, and 0.41) against the same. Out of the eleven bacteria tested, MRSA and *A. baumannii* were sensitive to most plant extracts in this study, whereas ciprofloxacin, as the antibiotic control, had no inhibition zone. *A. baumannii*, a pathogen associated with hospital-acquired infections worldwide and resistant to many antimicrobial agents, was markedly inhibited by the ethanolic extracts of all plants except *H. anthelminthicus* and *C. alata*. The inhibition zone produced by the extracts ranged from 7.0–22.0 mm (AI = 1.17–3.67) for MRSA and 7.3–24.7 mm (AI = 1.22–4.11) for *A. baumannii*. The AI values of these plants, compared to that of ciprofloxacin, were > 1, indicating better antibacterial activity.

MIC and MBC value of plant extracts

The MIC and MBC were measured to determine the effectiveness of the plant extracts on the bacterial strains that were sensitive to the extracts in the disk diffusion assay (Table 4).

Table 4 Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 10 Thai medicinal plant extracts

Plant extracts	MIC/MBC (mg/L)										
	Gram-positive bacteria				Gram-negative bacteria						
	Staphylococcus aureus ATCC 25923	Methicillin-resistant *S. aureus* (MRSA)	Methicillin-susceptible *S. aureus* (MSSA)	*Staphylococcus epidermidis*	*Escherichia coli* ATCC 25922	*Escherichia coli* (ESBL)	*Klebsiella pneumoniae* ATCC 700603 (ESBL)	*Klebsiella pneumoniae* (CRKP)	*Acinetobacter baumannii*	*Pseudomonas aeruginosa* ATCC 27853	*Pseudomonas aeruginosa*
R. nasutus	1/32	1/32	1/64	0.25/32	-	-	-	-	8/32	32/64	32/32
P. betle	1/2	1/4	1/2	0.5/4	4/4	4/4	4/4	4/4	2/4	8/8	2/4
C. asiatica	8/256	32/256	-	-	-	-	-	-	16/32	-	-
C. nutans	64/64	-	-	-	-	-	-	-	32/>256	-	-
B. lupulina	4/4	4/8	64/256	4/8	8/8	8/64	16/16	64/256	8/16	8/>256	8/8
V. inermis	4/4	16/16	4/16	2/8	-	-	-	-	16/16	-	-

Plant extracts	MIC/MBC (mg/L)										
	Gram-positive bacteria				Gram-negative bacteria						
	Staphylococcus aureus ATCC 25923	Methicillin-resistant *S. aureus* (MRSA)	Methicillin-susceptible *S. aureus* (MSSA)	*Staphylococcus epidermidis*	*Escherichia coli* ATCC 25922	*Escherichia coli* (ESBL)	*Klebsiella pneumoniae* ATCC 700603 (ESBL)	*Klebsiella pneumoniae* (CRKP)	*Acinetobacter baumannii*	*Pseudomonas aeruginosa* ATCC 27853	*Pseudomonas aeruginosa*
A. ebracteatus	4/32	4/16	-	2/8	-	-	32/32	-	8/32	-	32/64
A. paniculata	2/64	2/256	16/64	2/32	-	-	-	-	16/128	-	-
H. anthelminthicus	-	64/128	-	-	-	-	-	-	-	128/128	-
C. alata	256/256	256/256	256/256	-	-	-	-	-	-		-

-, Not assessed

The MIC and MBC values of the plant extracts varied against different bacterial strains. As previously observed with a qualitative test, disk diffusion, the extract of *P. betle* exhibited the highest effective activity against all tested bacterial strains with MICs of 0.5 to 8 mg/mL and MBCs of 2 to 8 mg/mL. The extract of *B. lupulina* also showed antibacterial activity against all the tested bacteria, with MICs of 4 to 64 mg/mL and MBCs of 4 to >256 mg/mL. Other plant extracts were effective against two to seven bacterial strains tested with MICs and MBCs of 0.25 to 256 mg/mL and 4 to >256 mg/mL, respectively. In some cases, the MBC values of the extracts against the tested bacterial strains were higher than their MIC values, whereas in others, the MIC and MBC values were the same.

Conclusion and Discussion

In this study, we evaluated the antibacterial activity of ethanolic extracts from 10 Thai medicinal plants (*R. nasutus*, *P. betle*, *C. asiatica*, *C. nutans*, *B. lupulina*, *V. inermis*, *A. ebracteatus*, *A. paniculata*, *H. anthelminthicus,* and *C. alata*) against 11 bacterial strains (*S. aureus* ATCC 25923, methicillin-resistant *S. aureus*, methicillin-susceptible *S. aureus*, *S. epidermidis*, *E. coli* ATCC 25922, extended-spectrum beta-lactamases (ESBL)-producing *E. coli*, *K. pneumoniae* ATCC 700603 (ESBL-producing strain), carbapenem-resistant *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* ATCC 27853, and *P. aeruginosa*). Comparing the results of the extracts from the 10 plants, the extracts of *P. betle* and

B. lupulina exhibited highest antibacterial activity against all tested bacterial strains. For *P. betle*, the gram-positive bacteria were more sensitive (inhibition zone of 19.7 to 32.0 mm) to the extract than gram-negative bacteria (inhibition zone of 13.7 to 24.7 mm). The difference in sensitivity may be due to variations in cell wall structures. Gram-negative bacteria contain complex outer membranes, including lipopolysaccharides and various efflux pump systems, whereas the cell walls of gram-positive bacteria contain only a peptidoglycan layer.[20] In addition, the results showed that the extract of *P. betle* was active against many strains of drug-resistant bacteria, including MRSA, ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae* and carbapenem-resistant *K. pneumoniae* (CRKP). *P. betle* leaves contain many chemical components, including eugenol, betal-phenol, chavicol and other phenolic compounds.[21] These components, especially eugenol and hydroxychavicol, exhibit strong antifungal and antibacterial activities.[22] For *B. lupulina*, the extract demonstrated inhibitory activity against all tested bacteria, including drug-resistant strains, and displayed maximum activity against *S. epidermidis*. In a previous study, the ethanol extract of *B. lupulina* leaves inhibited the growth of *S. aureus*, *E. coli*, *K. pneumoniae*, *Salmonella* Typhi, and *P. aeruginosa*. The maximum growth inhibition activity was observed against *P. aeruginosa*.[23]

Although some extracts exhibited a good antibacterial activity towards different tested bacterial isolates, many plant extracts exhibited a limited antibacterial activity against the test bacterial isolates as judged by their MIC values.[24] In traditional medicine, it is widely accepted that plant extracts having MIC values below 8 mg/ml are considered to possess some antimicrobial activity, while extracts with MIC values below 1 mg/mL exhibit remarkable antimicrobial activity.[25-26] Based on the above criteria, six plants (including *R. nasutus*, *P. betle*, *B. lupulina*, *V. inermis*, *A. ebracteatus* and *A. paniculata*) had antibacterial activities as MIC values below 8 mg/mL for at least one of the tested bacteria. The extract of *P. betle* exhibited the highest effective activity against all tested bacterial strains with MICs of 0.5 to 8 mg/mL and showed remarkable antibacterial activity (MIC of 0.5 mg/mL) against *S. epidermidis*. The extract of *B. lupulina* also showed antibacterial activity against all the tested bacteria, with MICs of 4 to 64 mg/mL. It was considered as having potential antimicrobial activities against *S. aureus*, MRSA and *S. epidermidis* with MIC of 4 mg/ml.

According to the MBC/MIC ratio, antibacterial extracts are categorized into two classes: bacteriostatic (MBC/MIC ratio >4) and bactericidal (MBC/MIC ratio $\leq$ 4).[27] Following this classification, the extract of *P. betle* with MBC/MIC ratio ranging from 1 to 4 were bactericidal for all tested bacteria except *S. epidermidis* (bacteriostatic with MBC/MIC ratio of 8). Whereas the extract of *B. lupulina* were bactericidal for *S. aureus*, MRSA and *S. epidermidis* with MBC/MIC ratio of 2.

The fluoroquinolone (FQ) antibiotic ciprofloxacin is a broad-spectrum antibiotic effective against gram-positive and -negative bacteria. It has been used to treat a range of diseases, including skin and soft tissue infections. However, there are increasing reports of ciprofloxacin resistance in

MRSA and *A. baumannii*. The resistance could develop by efflux pumps or mutations in DNA gyrase genes (gyrA).[28-29] High prevalence of the ciprofloxacin resistance (more than 90%) was also reported among MRSA and *A. baumannii* isolates from hospitals in some regions of Thailand.[30-31] The results revealed that extract from *P. betle* demonstrated the highest efficacy against MRSA and *A. baumannii* with MIC values of 1 and 2 mg/mL, respectively. While the MIC value of 4 mg/ml obtained for *B. lupulina* extract against MRSA.

Multidrug resistant pathogens have increased the number of infectious diseases and human deaths worldwide. It is crucial to identify new antimicrobial agents for the treatment of infections. Natural compounds from medicinal plants must be discovered to overcome antibiotic resistance. As in other countries, the empirical use of medicinal plants continues to be favored in traditional Thai medicine. In this study, the antibacterial activities of the ethanolic extracts of 10 Thai medicinal plants used in the treatment of skin diseases were assessed. The results showed that the extracts exhibited potential activity against the tested bacterial strains. The findings of this study provide evidence supporting the traditional use of these plants. The extracts of *P. betle* and *B. lupulina* exhibited antibacterial activity against all tested bacteria, including the resistant strains. *P. betle* was the most active and had strong activity against both gram-positive and gram-negative bacteria, which was confirmed by the diameter of the inhibition zones and AI compared to ciprofloxacin as the standard control, MIC, and MBC values. Both *P. betle* and *B. lupulina* may be good sources for treating skin diseases. Furthermore, they could be a reservoir of molecules to fight against antibiotic resistance bacterial. Nevertheless, the cytotoxicity and mechanisms of action of these extracts should be considered in future studies.

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