

# Antibacterial Activity of Extracts from a Thai Traditional Remedy Called Prasachandaeng and Its Plant Components

Alisa Sangphum BATM\*,  
Pannawat Chaiyawatthanananthn PhD\*\*\*\*\*, Arunporn Itharat PhD\*\*\*\*\*

\* Student of Master's Degree (Applied Thai Traditional Medicine), Faculty of Medicine,  
Thammasat University, Pathumthani, Thailand

\*\* Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

\*\*\* Center of Excellence on Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine,  
Thammasat University, Pathumthani, Thailand

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**Background:** Prasachandaeng remedy (PC) has long been used for relief of fever and internal heat. It is composed of 12 plants. It is in the National Herbal Drug List of Thailand, but there is no report of antibacterial activity of this remedy.

**Objective:** To investigate antibacterial activity of ethanolic and aqueous extracts of PC remedy and its plant components.

**Material and Method:** The extraction methods were the maceration in 95% ethanol and drying by evaporator, and decoction or boiling in water; filtering and drying by lyophilizer. In the primary studies, all extracts were tested for antibacterial activity by disc diffusion against two types of gram positive bacteria; *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus aureus* MRSA (DMST 20651), and three types of gram negative bacteria; *Escherichia coli* (ATCC 25922), *Shigella dysenteriae* (DMST 151161) and *Salmonella typhimurium* (DMST 22842). The active plant extracts which showed an inhibition zone were diluted to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values.

**Results:** The 95% ethanol extract of PC showed antibacterial activity against *S. aureus* MRSA (MIC = 0.312 mg/ml, MBC = 2.5 mg/ml). The inhibition zones of all plant extracts were in the range of 7 to 26 mm. The 95% ethanol and aqueous extracts of *Caesalpinia sappan* L. stems showed the largest inhibition zone against *S. aureus* MRSA 26 and 20 mm., respectively. The 95% ethanol extract of *Mammea siamensis* Kosterm. flowers exhibited the best gram positive bacteria activity against *S. aureus* MRSA with MIC and MBC values of 0.004 and 0.019 mg/ml., respectively. The 95% ethanol extract of *Caesalpinia sappan* L. stems exhibited the best inhibitory gram negative bacteria activity against *S. dysenteriae* with MIC and MBC values of 0.156 and 0.156 mg/ml.

**Conclusion:** The present study demonstrated that 95% ethanol extracts of PC had antibacterial. The 95% ethanol extract of *Mammea siamensis* Kosterm. flowers exhibited the best gram positive bacteria activity against *S. aureus* MRSA and the 95% ethanol extract of *Caesalpinia sappan* L. stems exhibited the best inhibitory gram negative bacteria activity against *S. dysenteriae*. The extracts of these two plants should be examined for active antimicrobial compound to be marker for quality control of PC.

**Keywords:** Prasachandaeng, Thai traditional medicine, Antibacterial activity

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Fever is a cardinal symptom of infectious or inflammatory diseases, which is characterized by an increase in body temperature induced by an elevation in thermoregulatory set point<sup>(1)</sup>. *Staphylococcus*

*aureus* causes superficial, deep-skin, and soft-tissue infections, endocarditis, bacteremia with metastatic abscess formation, a variety of toxin-mediated diseases including gastroenteritis, staphylococcal scalded-skin syndrome and toxic shock syndrome<sup>(2)</sup>. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes<sup>(3)</sup>. *Escherichia coli* is one of the most common pathogens in

**Correspondence to:**

Itharat A, Department of Applied Thai Traditional Medicine,  
Faculty of Medicine, Thammasat University, Klongnueng,  
Klongluang, Pathumthani 12120, Thailand.

Phone & Fax: +66-2-9269749

E-mail: iarunporn@yahoo.com

nosocomial and community-acquired infections in humans<sup>(4)</sup>. *Shigella* is the pathogen of shigellosis, a disease responsible for more than 500,000 deaths of children per year in developing countries<sup>(5)</sup>. *Salmonella* is the pathogen of salmonellosis, a major cause of enteric illness and typhoid fever, leading to many hospitalizations and a few rare deaths if no antibiotics are administered<sup>(6)</sup>. Mostly, antibiotics are prescribed for illness caused by bacteria, but they will not kill all disease-causing bacteria. Thus, many country in the world have used traditional medicine for infectious disease treatment by using cocktail ingredients. By the same way, Thai traditional medicine had the knowledge that can treat infectious disease which is cause of fever. Prasachandaeng remedy (PC) is an antipyretic drug in Thai traditional medicine and is in the National Herbal Drug list of Thailand. It is used to relieve fever and internal heat. In addition, it has been used to relieve thirst by mixing with jasmine water. This remedy consists of twelve plants. The list of plant components in PC remedy is shown in Table 1. There are reports of antibacterial activity of some component plants in PC. The 3-Benzylchroman derivative Brazilin from *Caesalpinia sappan* L. showed *in vitro* synergy of bactericidal activities against MRSA<sup>(7,8)</sup>. Leaf and fruit extracts from *Mesua ferrea* L. showed antibacterial activity against *S. aureus* with MIC/MBC values of 0.048 and 0.39 mg/ml<sup>(9)</sup>. However, there is no research report of antibacterial activity on Prasachandaeng remedy extract and extracts of some of its plant components. Therefore, the objective of this research was to determine antibacterial activity of Prasachandaeng remedy and its plant component extracts by different extraction methods against bacterial infection by two types of gram positive bacteria; *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus aureus* MRSA (DMST 20651), and three types of gram negative bacteria; *Escherichia coli* (ATCC 25922), *Shigella dysenteriae* (DMST 151161) and *Salmonella typhimurium* (DMST 22842).

## Material and Method

### Chemicals and reagents

The chemical compound and reagent for extract and assay were purchased in different sources such as 95% ethanol (CMJ Anchor company, Thailand), Distilled water (Milford, USA), Dimethylsulphoxide (RCI Labscan, Thailand), Mueller-Hinton agar (Difco, USA), Mueller-Hinton Broth (Difco, USA), Nutrient Agar (Difco, USA), Sabouraud Dextrose Agar (Difco, USA) and Resazurin (Sigma, USA).

### Plant materials and extraction

Plant materials were purchased from Charernsook Osot Pharmacy shop (Nakhon Pathom, Thailand) and shown in Table 1. Each plant ingredient was cleaned and dried by using a hot air oven at 45°C to 50°C. The formulated remedy was ground to be crude powder. The preparation was macerated in 95% ethanol and decocted in water which showed the method below. All crude extracts were stored at -20°C and diluted on the day of use.

### Maceration method

The crude powder of PC and each plant component of this remedy (500 g) were macerated in 95% ethanol for 3 days at room temperature and the maceration was repeated two times. Then, it was filtered and the filtrate was dried using a rotary evaporator. These extracts are the 95% ethanol extracts.

### Decoction method

The crude powder of PC and each plant ingredient of this remedy (500 g) were boiled in distilled water for 15 minutes and the decoction was repeated two times. Then, it was filtered and the filtrate was dried by lyophilizer. These extracts are aqueous extracts.

### Determination of antibacterial activity

#### Bacterium

Bacterial strains tested were two types of gram positive bacteria; *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus aureus* MRSA (DMST 20651), and three types of gram negative bacteria; *Escherichia coli* (ATCC 25922), *Shigella dysenteriae* (DMST 151161) and *Salmonella typhimurium* (DMST 22842). Bacteria were cultured in Nutrient Agar (NA) at 37°C for 24 hours.

#### Preparation of inocula

Separate colonies of each bacterium was cultured into Mueller-Hinton Broth (MHB) at 37°C for 2 hours. Then, suspension was adjusted turbidity to 0.5 McFarland standards by diluting with MHB.

#### Preparation of test disc

The extracts in 95% ethanol were dissolved in dimethylsulphoxide (DMSO) to a final concentration 500 mg/ml and the aqueous extracts were dissolved in distilled water (Milli-Q,  $\geq 18$  Mega Ohm) to a final concentration of 100 mg/ml. Then, 10  $\mu$ l of prepared extracts were used on 6 mm sterile paper discs.

### **Antibacterial assay**

#### **Disc diffusion method**

The agar disc diffusion method was used to screen antibacterial activity of the extracts according to Lorian V., 1996<sup>(10)</sup>. Sterilized filter paper discs (6 mm in diameter) were contained with 10 µl of the extracts. The inoculum suspension turbidity to 0.5 McFarland standards was swabbed, excess fluid removed and the whole Muller-Hinton Agar (MHA) surface was swabbed equally in three directions with the sterile cotton swab and left on the plate for 3 to 5 minutes. Then, the dried paper discs were put on the MHA with the sterile forceps. Plates with the bacterium and test samples were incubated at 37°C for 18 to 24 hours. Finally, measurement of the inhibition zone (clear zone) around the disc showed the sensitivity and resistance of the microorganism to test antibacterial activity. The inhibition zone was evaluated by measuring the diameter. Positive control was Gentamicin (Conc. 1 µg).

#### **Minimal inhibitory concentration (MIC)**

The MIC values were determined by microtiter plate-based assay by Sarker et al., 2007<sup>(11)</sup>. The ethanolic extracts for testing were dissolved in DMSO. Aqueous extracts were dissolved in distilled water (Milli-Q, ≥18 Mega Ohm) and filtered with Millipore filter 0.22 µm (Merck Millipore, Tullagreen). Then, samples were prepared to 10 mg/ml. *S. aureus* (ATCC 25923), *S. aureus* MRSA (DMST 20651), *E. coli* (ATCC 25922), *S. dysenteriae* (DMST 151161) and *S. typhimurium* (DMST 22842) were prepared by culturing for 18 to 24 hours culture. The inoculum was adjusted for turbidity to 0.5 McFarland standard and diluted with sterile MHB at 1: 200 to give a final concentration of 5x10<sup>5</sup> CFU/ml. 50 µl of extract solution at the concentration of 5, 2.5, 1.25 or 0.625 mg/ml, was added to sterile 96 wells microtiter plates (Corning incorporated, USA). Then, 50 µl of the inoculum was added into the wells. The plates were covered with a sterile plate and plastic wrap. The substances of the wells were then mixed using a plate shaker and incubated at 37°C for 18 to 24 hours.

Later, 10 µl of 1 mg/ml resazurin solution (blue compound, 7-hydroxy-3H-phenoxazin-3-one 10-oxide) was added into each well. The plate was incubated at 37°C for 3 hours. MIC value is the lowest concentration of crude extract solution that can inhibit the growth of bacterium exhibiting by a color change. The blue color of resazurin shows that the extract has inhibited the growth of bacterium and when the color is purple or pink, the extract has not inhibited the bacterium. The assay was repeated in triplicate. The positive control,

negative control and viable control were included.

#### **Minimum bactericidal concentration (MBC)**

Bacterium from microtiter well plate was applied onto the MHA plates. Cell viability was determined by the growth of bacterium after incubating at 37°C for 18 to 24 hours. The MBC values were recorded as the lowest concentration of the extract that inhibits any visible bacteria colony growth on agar plate.

#### **Statistical analysis**

The results were completed in triplicate. Values of the different variable are shown as the mean ± standard deviation. Statistical analysis was calculated using Prism Software.

### **Results**

The yield of PC and its plant component extraction are shown in Table 1. They were screened for antibacterial activity by disc diffusion is shown in Table 2, MIC and MBC against two types of gram positive bacteria; *Staphylococcus aureus* and *Staphylococcus aureus* MRSA and three types of gram negative bacteria; *Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhimurium* are shown in Table 3. The results showed that 95% ethanol extract of PC exhibited antibacterial activity against *S. aureus* MRSA (MIC = 0.312 mg/ml, MBC = 2.5 mg/ml). The inhibition zones for all bacteria of all extracts were in the range of 7 to 26 mm. The 95% ethanolic and aqueous extracts of *Caesalpinia sappan* L. stems showed the largest inhibition zone against *S. aureus* MRSA 26 and 20 mm, respectively. The 95% ethanolic extract of *Mammea siamensis* Kosterm. flowers exhibited the best activity against gram positive against *S. aureus* MRSA with MIC and MBC values of 0.0048 and 0.0195 mg/ml, respectively. The 95% ethanolic extract of *Caesalpinia sappan* L. stems exhibited the best activity against gram negative bacteria *S. dysenteriae* with MIC and MBC values of 0.156 and 0.156 mg/ml. However, the PC extract had no activity against *E. coli* (ATCC 25922) and *S. typhimurium* (DMST 22842).

### **Discussion**

The results of this study showed that the ethanolic extract of Prasachandaeng (PC) remedy can inhibit the growth of gram positive but not inhibit gram negative. Especially, this extract showed high inhibitory activity against *S. aureus* MRSA with MIC

**Table 1.** The ethnobotanical information and percentage of yield of Prasachandaeng remedy and its plant components

Scientific name	Family	Thai name	Part Used	Thai traditional used <sup>(2,13)</sup>	% Yield	
					95% ethanol	Aqueous
<i>Bouea macrophylla</i> Griff.	ANACARDIACEAE	MaPrangWan	Root	Relieve common cold, detoxify	2.29	1.32
<i>Caesalpinia sappan</i> L.	FABACEAE	FangSaeng	Stem	Blood tonic, Promote lymphatic system	12.42	1.95
<i>Citrus aurantifolia</i> Swing.	RUTACEAE	MaNow	Root	Relieve common cold, detoxify, Anti-inflammatory	1.69	3.52
<i>Dracaena loureiri</i> Gagnep.	AGAVACEAE	ChanDaeng	Stem	Relieve common cold, Blood tonic, Promote hepatic system	22.76	5
<i>Helicia terminalis</i> Kurz.	PROTEACEAE	MutengKon	Root	Relieve common cold, Tonic coolant, detoxify	2.73	3.77
<i>Jasminum sambac</i> L.	OLEACEAE	MaLi	Flower	Relieve common cold, Cardio tonic	8.17	25.78
<i>Kaempferia galanga</i> L.	ZINGIBERACEAE	ProhHorm	Rhizome	Urticaria, Relieve cough	5.25	9.83
<i>Ligusticum chuanxiong</i> Hort.	APIACEAE	KotHuaBua	Rhizome	Carminative, Relieve common cold	11.87	27.29
<i>Mammea siamensis</i> Kosterm.	CLUSIACEAE	SaRaPee	Flower	Tonic, Cardio tonic, Blood tonic	17.13	14.35
<i>Mesua ferrea</i> L.	CALOPHYLLACEAE	BoonNgan	Flower	Tonic, Cardio tonic, Blood tonic	18.61	12.19
<i>Myristica fragrans</i> Houtt.	MYRISTICACEAE	ChanTet	Stem	Tonic, Cardio tonic, Carminative	1.24	0.85
<i>Nelumbo nucifera</i> Gaertn.	NELUMBONACEAE	BuaLuang	Pollen	Tonic, Cardio tonic	8.77	15.71
Prasachandaeng remedy	-	-	-	Relieve common cold, Relieve internal heat	17.29	3.35

**Table 2.** Antibacterial activity of Prasachandaeng remedy and its plant components by disc diffusion (n = 3)

Herbal	Extraction	Inhibition zone (mm)					
		<i>S. aureus</i> (ATCC 25923)	<i>S. aureus</i> MRSA (DMST 20651)	<i>E. coli</i> (ATCC 25922)	<i>S. dysenteriae</i> (DMST 151161)	<i>S. typhimurium</i> (DMST 22842)	
<i>Bouea macrophylla</i> Griff.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Caesalpinia sappan</i> L.	ET	21.67±1.53	26.00±1.00	11.67±1.15	21.00±0.00	14.33±1.15	
	AQ	18.00±1.00	20.00±1.00	7.00±0.00	13.00±0.00	9.00±0.00	
<i>Citrus aurantifolia</i> Swing.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Dracaena loureiri</i> Gagnep.	ET	9.67±0.58	10.67±0.58	NI	7.67±0.58	NI	
	AQ	NI	8.67±0.58	NI	NI	NI	
<i>Helicia terminalis</i> Kurz.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Jasminum sambac</i> L.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Kaempferia galanga</i> L.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Ligusticum chuanxiong</i> Hort.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Mammea siamensis</i> Kosterm.	ET	8.33±0.58	10.67±1.15	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Mesua ferrea</i> L.	ET	7.33±0.58	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Myrsine fragrans</i> Houtt.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Nelumbo nucifera</i> Gaertn.	ET	NI	NI	NI	NI	NI	
	AQ	7.33±0.58	7.33±0.58	NI	NI	NI	
Prasachandaeng remedy	ET	10.00±1.00	11.67±0.58	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
Positive control: Gentamicin	ET	22	NI	20	20	20	
	AQ	26	NI	18	20	20	
Ampicilin	ET	21	NI	30	30	33	
	AQ	25	NI	NI	NI	NI	

NI = No inhibition, ET = Ethanol extract, AQ = Aqueous extract

**Table 3.** Antibacterial activity of Prasachandaeng remedy and its plant components in minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. (n = 3)

Herbal	Extraction	MIC / MBC (mg/ml), Positive control (µg/ml)					
		<i>S. aureus</i> (ATCC 25923)	<i>S. aureus</i> MRSA (DMST 20651)	<i>E. coli</i> (ATCC 25922)	<i>S. dysenteriae</i> (DMST 151161)	<i>S. typhimurium</i> (DMST 22842)	
<i>Bouea macrophylla</i> Griff.	ET	5.00/5.00	NI	NI	NI	NI	
	AQ	NI	2.50/2.50	NI	NI	NI	
<i>Caesalpinia sappan</i> L.	ET	0.156/0.156	0.078/0.625	1.25/1.25	0.15/1.15	0.625/0.625	
	AQ	0.078/1.25	0.078/0.078	1.25/2.50	0.15/0.312	0.625/1.25	
<i>Citrus aurantifolia</i> Swing.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Dracaena loureiri</i> Gagnep.	ET	5.00/5.00	1.25/1.25	NI	5.00/5.00	NI	
	AQ	5.00/>5.00	1.25/1.25	NI	2.5/2.5	NI	
<i>Helicia terminalis</i> Kurz.	ET	1.25/1.25	0.625/1.25	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Jasminum sambac</i> L.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Kaempferia galanga</i> L.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Ligusticum chuanxiong</i> Hort.	ET	5.00/5.00	2.50/2.50	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Mammea siamensis</i> Kosterm.	ET	0.009/0.078	0.004/0.019	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Mesua ferrea</i> L.	ET	0.625/2.50	0.156/0.625	NI	NI	NI	
	AQ	5.00/>5.00	2.50/2.50	NI	NI	NI	
<i>Myristica fragrans</i> Houtt.	ET	NI	NI	NI	NI	NI	
	AQ	NI	NI	NI	NI	NI	
<i>Nelumbo nucifera</i> Gaertn.	ET	NI	NI	NI	NI	NI	
	AQ	5.00/>5.00	1.25/1.25	NI	NI	NI	
Prasachandaeng remedy	ET	5.00/5.00	0.312/2.50	NI	5.00/5.00	NI	
	AQ	5.00/>5.00	1.25/1.25	NI	5.00/5.00	NI	
Positive control: Gentamicin		0.195/0.195	>100	1.562/1.562	0.781/0.781	>100	

NI = No inhibition, ET = Ethanol extract, AQ = Aqueous extract

0.312 mg/ml. MIC of gentamycin as positive control is more than 0.1mg/ml. However, the aqueous extract of this remedy had no antibacterial activity against all strain microbe. This result can be used to support using the ethanolic extract of PC remedy to against microbe as resistance strain. For plant component of PC which showed the best activity as *Mammea siamensis* flowers and *Caesalpinia sappan* wood. The 95% ethanol extract of *Mammea siamensis* flowers has ever been reported the ability to inhibit the growth of gram positive bacteria, it showed antibacterial activity against *S. aureus* and *S. aureus* MRSA with MIC values as 1.250 and 0.625 mg/ml<sup>(14)</sup>, respectively. However, in this study, the 95% ethanol extract of *Mammea siamensis* Kosterm. flowers showed higher antibacterial activity than the previously reported against *S. aureus* and *S. aureus* MRSA with MIC values of 0.009 and 0.004 mg/ml, respectively. The different result may be due to collecting time, land of growing plant which have the effect on ingredients and biological activity. The 95% ethanol and aqueous extracts of *Caesalpinia sappan* L. stems showed the ability to inhibit the growth of both gram positive and negative bacteria. The previously work reports found that the chloroform, *n*-butanol, methanol, and aqueous extracts of the *Caesalpinia sappan* showed an antimicrobial activity against standard methicillin-sensitive *Staphylococcus aureus* (MSSA) as well as MRSA<sup>(15)</sup>. The antimicrobial results of *Caesalpinia sappan* in this study related with the previous results because both ethanolic and aqueous extracts showed against all positive and negative bacteria. The aqueous extract of *Caesalpinia sappan* showed higher antibacterial against *S. aureus* and *S. aureus* MRSA than ethanolic extract (MIC = 78, 78, 156, and 78 µg/ml respectively). *Caesalpinia sappan* extracts also showed high antibacterial against gram negative bacteria cause of diarrhea. These results support using *Caesalpinia sappan* in form of maceration in ethanol to be tonic and postpartum supplement. For *Dracaena loureiri* Gagnep. stems which is an ingredient in PC remedy and up to 50% of remedy exhibit low antibacterial efficacy. Therefore, PC has also low antibacterial efficacy against gram positive and negative bacteria. Furthermore, some extracts showed no inhibition zone, and MIC/MBC values showed less activity (MIC >1 mg/ml). This may be due to diffusion of extracts from paper disc perhaps depend on concentration of each extract. This report is the first report on antibacterial activity of PC. Even though, PC had less antibacterial activity but

it should be continuously investigate for anti-inflammatory activity in which inflammation is cause of fever. However, the 95% ethanol extract of *Mammea siamensis* Kosterm. flowers and *Caesalpinia sappan* L. stems should be further investigated and developed for treatment of bacterial infection, and PC should be further tested for anti-inflammation property. All component plant which have antibacterial properties except *Citrus aurantifolia* Swing. roots, *Kaempferia galanga* L. rhizome, *Myristica fragrans* Houtt. stems and *Jasminum sambac* L. flowers should be continuously investigated for active antibacterial compounds. However, *Kaempferia galanga* L. rhizome showed acute and chronic anti-inflammation in rats in previously report so it relates to the activity of this remedy<sup>(16)</sup>. *Jasminum sambac* L. flower showed vasodilation effect which may help heat dissipation in patients with fever<sup>(17)</sup>. Although, there are some plants which show antibacterial activity and PC shows less antibacterial activity, but the effect of PC should be continuously study on the other cause of fever such as inflammation or on the other bacterial strain which cause of fever.

### Conclusion

The present study demonstrated that 95% ethanol extracts of PC, *Mammea siamensis* Kosterm. flowers and *Caesalpinia sappan* L. stems had antibacterial activity. The active components which showed antibacterial activity are *Dracaena loureiri* Gagnep. stems, *Helicia terminalis* Kurz. roots, *Bouea macrophylla* Griff. roots, *Ligusticum chuanxiong* Hort. rhizomes, *Caesalpinia sappan* L. stems, *Nelumbo nucifera* Gaertn. pollen, *Mesua ferrea* L. flowers and *Mammea siamensis* Kosterm. flowers, but *Citrus aurantifolia* Swing. roots, *Kaempferia galanga* L. rhizome, *Myristica fragrans* Houtt. stems and *Jasminum sambac* L. flowers had no anti-bacterial activity. However, these results can support using PC remedy to relieve fever cause by *S. aureus*, *S. aureus* MRSA infection.

### What is already known on this topic?

Prasachandaeng is used in Thai Traditional Medicine for relief of fever and internal heat. The ethanolic extracts of some plants of this remedy such as *Caesalpinia sappan* L., *Mesua ferrea* L. and *Mammea siamensis* Kosterm. showed antibacterial activity against *S. aureus* MRSA. Interestingly,

Prasachandaeng remedy is the first report showed MIC and MBC values of aqueous extract and ethanolic extract against gram positive and negative bacteria.

#### What this study adds?

The present study showed the antibacterial activity of ethanolic extract of Prasachandaeng remedy against *S. aureus*, *S. aureus* MRSA and *S. dysenteriae* that cause fever infection but the aqueous extract had low effective values. In addition, the ethanolic extract of *Mammea siamensis* Kosterm. showed the highest activity. This report will support the Prasachandaeng remedy to relieve fever cause by *S. aureus*, *S. aureus* MRSA infection.

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#### Potential conflict of interest

None.

#### References

1. Fraga D, Zanoni CI, Zampronio AR, Parada CA, Rae GA, Souza GE. Endocannabinoids, through opioids and prostaglandins, contribute to fever induced by key pyrogenic mediators. *Brain Behav Immun* 2016; 51: 204-11.
2. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. *J Clin Microbiol* 1998; 36: 618-23.
3. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci U S A* 2002; 99: 7687-92.
4. Li Y, Zheng B, Li Y, Zhu S, Xue F, Liu J. Antimicrobial susceptibility and molecular mechanisms of fosfomycin resistance in clinical *Escherichia coli* isolates in mainland China. *PLoS One* 2015; 10: e0135269.
5. Willer EM, Lima RL, Giugliano LG. *In vitro* adhesion and invasion inhibition of *Shigella dysenteriae*, *Shigella flexneri* and *Shigella sonnei* clinical strains by human milk proteins. *BMC Microbiol* 2004; 4: 18.
6. Hadjinicolaou AV, Demetriou VL, Emmanuel MA, Kakoyiannis CK, Kostrikis LG. Molecular beacon-based real-time PCR detection of primary isolates of *Salmonella Typhimurium* and *Salmonella Enteritidis* in environmental and clinical samples. *BMC Microbiol* 2009; 9: 97.
7. Zuo GY, Han ZQ, Hao XY, Han J, Li ZS, Wang GC. Synergy of aminoglycoside antibiotics by 3-Benzylchroman derivatives from the Chinese drug *Caesalpinia sappan* against clinical methicillin-resistant *Staphylococcus aureus* (MRSA). *Phytomedicine* 2014; 21: 936-41.
8. Nirmal NP, Panichayupakaranant P. Antioxidant, antibacterial, and anti-inflammatory activities of standardized brazilin-rich *Caesalpinia sappan* extract. *Pharm Biol* 2015; 53: 1339-43.
9. Aruldass CA, Marimuthu MM, Ramanathan S, Mansor SM, Murugaiyah V. Effects of *Mesua ferrea* leaf and fruit extracts on growth and morphology of *Staphylococcus aureus*. *Microsc Microanal* 2013; 19: 254-60.
10. Lorian V. Antibiotics in laboratory medicine. 4th ed. Baltimore: Williams & Wilkins; 1996.
11. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods* 2007; 42: 321-4.
12. Phichianshunthorn C, Chawalit M, Jeerawong W. Explanation the text of Osot Pra Narai. Bangkok: Amarin Printing and Publishing; 2005.
13. Wutthithammawet W. Encyclopedia and principle of Thai traditional pharmacy. Bangkok: Odeon Store; 1997.
14. Sattaponpan C. Antibacterial activity of crude extracts of Prasaproyhai formula and its components against pathogenic bacteria [thesis]. Pathumthani, Thailand: Thammasat University; 2011.
15. Kim KJ, Yu HH, Jeong SI, Cha JD, Kim SM, You YO. Inhibitory effects of *Caesalpinia sappan* on growth and invasion of methicillin-resistant *Staphylococcus aureus*. *J Ethnopharmacol* 2004; 91: 81-7.
16. Jagadish PC, Latha KP, Mudgal J, Nampurath GK. Extraction, characterization and evaluation of *Kaempferia galanga* L. (Zingiberaceae) rhizome extracts against acute and chronic inflammation in rats. *J Ethnopharmacol* 2016; 194: 434-9.



17. Kunhachan P, Banchonglikitkul C, Kajsongkram T, Khayungarnawee A, Leelamanit W. Chemical composition, toxicity and vasodilatation effect of

the flowers extract of *Jasminum sambac* (L.) Ait. "G. Duke of Tuscany". Evid Based Complement Alternat Med 2012; 2012: 471312.

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### การศึกษาฤทธิ์ต้านเชื้อแบคทีเรียของตำรับยาประสะจันทน์แดงและสมุนไพรวัดเดียวในตำรับ

อลิษา แสงพุ่ม, พรรณณวิชัย ไชยวัฒน์นนท์, อรุณพร อธิรัตน์

**ภูมิหลัง:** ตำรับยาประสะจันทน์แดงเป็นตำรับยาไทยที่ไชบรรเทาอาการไข้ตัวร้อน แกร่อนใน ที่ประกอบไปด้วยสมุนไพรรวม 12 ชนิด อยู่ในบัญชียาหลักแห่งชาติ ซึ่งไม่เคยมีรายงานการศึกษาถึงฤทธิ์ต้านเชื้อแบคทีเรียมาก่อน

**วัตถุประสงค์:** เพื่อศึกษาฤทธิ์ต้านเชื้อแบคทีเรียของสารสกัดจากตำรับยาประสะจันทน์แดงและสมุนไพรวัดเดียวในตำรับด้วยวิธีการสกัดที่ต่างกัน

**วัสดุและวิธีการ:** สกัดสารสกัดด้วยวิธีการหมักเอทานอล 95% และทำแห้งด้วยเครื่องปั่นเหวี่ยงภายใต้ระบบสุญญากาศ สกัดสารด้วยกรรมวิธีคั้นและทำแห้งด้วยเครื่องทำแห้งแบบแช่แข็ง ขึ้นแรกนำสารสกัดไปทดสอบฤทธิ์ ต้านเชื้อแบคทีเรียด้วยวิธี disc diffusion โดยทดสอบกับเชื้อแบคทีเรียแกรมบวก 2 ชนิด; *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus aureus* MRSA (DMST 20651) และแกรมลบ 3 ชนิด; *Escherichia coli* (ATCC 25922), *Shigella dysenteriae* (DMST 151161) and *Salmonella typhimurium* (DMST 22842) นำสารสกัดสมุนไพรมีไซโนไซมาทดสอบ เพื่อหาค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญเติบโตของเชื้อได้ (MIC) และความเข้มข้นต่ำสุดที่สามารถฆ่าเชื้อได้ (MBC)

**ผลการศึกษา:** สารสกัดด้วยเอทานอล 95% ของตำรับยาประสะจันทน์แดงมีฤทธิ์ในการต้านเชื้อแบคทีเรีย *S. aureus* MRSA (MIC = 0.312 มิลลิกรัม/มิลลิลิตร, MBC = 2.5 มิลลิกรัม/มิลลิลิตร) สารสกัดด้วยเอทานอล 95% และสารสกัดด้วยน้ำของแก่นฝางเสนมีขนาดไซโนไซมาที่สกัดคือเชื้อ *S. aureus* MRSA ไซโนไซมาขนาด 26 และ 20 มิลลิเมตรตามลำดับสำหรับผลของสารสกัดจากพืชสมุนไพรวัดเดียวในตำรับ พบว่าทั้งหมดอยู่ในช่วง 7 ถึง 26 มิลลิเมตร สารสกัดด้วยเอทานอล 95% ของดอกสารภีต่อเชื้อแกรมบวก *S. aureus* MRSA มีค่า MIC และ MBC ต่ำที่สุดคือ 0.004 และ 0.019 มิลลิกรัม/มิลลิลิตร สารสกัดด้วยเอทานอล 95% ของแก่นฝางเสนต่อเชื้อแกรมลบ *S. dysenteriae* มีค่า MIC และ MBC ต่ำที่สุดคือ 0.156 และ 0.156 มิลลิกรัม/มิลลิลิตร

**สรุป:** จากผลการศึกษาทำให้ทราบว่าสารสกัดจากตำรับยาประสะจันทน์แดงมีฤทธิ์ต้านเชื้อแบคทีเรีย และสารสกัดด้วยเอทานอล 95% ของดอกสารภีและแก่นฝางเสนในตำรับยาประสะจันทน์แดง มีฤทธิ์ในการต้านเชื้อแบคทีเรีย ซึ่งสามารถใช้เป็นข้อมูลสนับสนุนการนำตำรับนี้ในการรักษาอาการไข้ที่เกิดจากการติดเชื้อแบคทีเรีย *S. aureus* และ *S. aureus* MRSA ต่อไป

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