Antibacterial Activity of Crude Extracts of Prasaprohyai Formula and Its Components against Pathogenic Bacteria

Chisanucha Sattaponpan BSc*,
Sumalee Kondo PhD**

** Student of Master Degree of Science Program in Medical Sciences (Nutraceutical), Faculty of Medicine, Thammasat University, Klongluang, Pathum Thani, Thailand*
*** Division of Molecular Genetics and Molecular Biology in Medicine, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Klongluang, Pathum Thani, Thailand*

Background: *Prasaprohyai formula is a Thai Traditional Medicine which has been used for reducing feverish in child. Fever is a symptom resulting from various infections and diseases. The major cause of fever is bacterial and viral infections. The Prasaprohyai formula and its components potentially have biological activities including antipyretic and antimicrobial activities. It is in a hope to develop the formula and its components for an alternative medicine of infectious diseases.*
Objective: *To study antibacterial activity of Prasaprohyai formula and its components against pathogenic bacteria.*
Material and Method: *Prasaprohyai formula and its components were extracted by different methods, A: maceration with 95% ethanol followed by evaporation (ET), B: ET followed by freeze drying (EF) and C: water distillation (VO). All extracts were tested against clinical isolates from Thammasat University Hospital, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922. Disk diffusion and broth dilution methods were performed.*
Results: *Crude extracts of ET had higher yield of extraction than other methods. The results showed that the crude extract from different methods of Syzygium aromaticum (Linn) Merr & Perry (Flower) was effective against all bacterial strains with the inhibition zone ranging from 9 to 19 mm. The VO extract of Prasaprohyai formula showed antibacterial activity against most of the pathogenic bacteria in the present study. The activity against Streptococcus pyogenes was found in the VO extract of some components. The ET extracts of Lepidium sativum Linn, Myristica fragrans Houtt (seed) and Myristica fragrans Houtt (aril) had no antibacterial activity against all microorganism. However, the EF extracts of this formula and some components were able to mostly inhibit Gram positive bacteria.*
Conclusion: *The results indicated that Prasaprohyai formula and its components were able to inhibit the growth of both Gram positive and Gram negative bacteria including multiresistant strains. The volatile oil extracts seemed to play an important role in antimicrobial activities. The development of Prasaprohyai formula for alternative medicine will be approached in future.*

Keywords: *Prasaprohyai formula and its components, Antibacterial activity, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Acinetobacter baumanii, Escherichia coli, Klebsiella pneumoniae and Streptococcus pyogenes*

J Med Assoc Thai 2011; 94 (Suppl. 7): S153-S161
Full text. e-Journal: *http://www.jmat.mat.or.th*

Infectious diseases due to microorganism such as bacteria, viruses, fungi and parasite caused several illnesses and death[1]. Inflammation and fever usually occurred after the infections resulting from body's immune response. The major cause of fever is bacterial and viral infections. Nowadays, infectious diseases caused by resistant strains have increased dramatically and resulted in loss of life and many costs of treatment. This problem leads to development of several new antibiotics to overcome antibiotic resistance. Many herbs of Thai traditional medicines have been studied for antimicrobial activity as for an alternative treatment. Prasaprohyai formula, one of the Thai traditional remedies, has been used as an antipyretic to reduce fever and cold in children. The major component of this formula is *Kaempferia galanga*

Correspondence to:
Kondo S, Division of Molecular Genetics and Molecular Biology in Medicine,Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rangsit campus, Klongluang, Pratumthani 12120, Thailand.
Phone: 0-2926-9756, Fax: 0-2926-9755
E-mail: ksumalee@alpha.tu.ac.th

Linn and 20 minor components listed in Table 1. There are many herbs in this formula involved in reducing fever and antimicrobial activity. The herbal plants containing in this formula established the antibacterial activity such as *Syzygium aromaticum* (Linn) Merr & Perry[2-5], *Cumimum cyminum* Linn[6-9] and *Foeniculum vulgare* Mill[10,11]. However, the crude extracts of the formula and its components have not been fully investigated. Therefore, this research study aims to examine the antibacterial activity of the Prasaprohyai formula and its components against pathogenic bacteria which cause respiratory tract infection. The obtained results will provide useful information for products development from Prasaprohyai formula and its components in an alternative treatment of infectious disease in future. The alternative Thai traditional formula will reduce the excess antibiotic usage and subsequently minimize the incidence of emerging resistant bacterial strains in future.

Material and Method

Prasaprohyai formula consists of 21 components from 19 plant species including *Amomum krevanh* Pierre (Zingiberaceae), *Anethum graveolens* Linn (Umbelliferae), *Angelica sylvestris* Linn (Umbelliferae), *Artemisia annua* Linn (Compositae or Asteraceae), *Atractylodes lancea* (Thunb) DC (Compositae), *Conioselinum univitatum* Trucz (Umbelliferae), *Cumimum cyminum* Linn (Umbelliferae), *Dracaena loureiri* Gagnep (Dracaenaceae), *Foeniculum vulgare* Mill (Umbelliferea), *Kaempferia galanga* Linn (Zingiberaceae), *Lepidium sativum* Linn (Cruciferae), *Levisticum officinale* Koch (Umbelliferae), *Mammea siamensis* Kosterm (Guttiferae), *Mesua ferrea* Linn (Guttiferae), *Mimusops elengi* Linn (Sapotaceae), *Myristica fragrans* Houtt (Myristicaceae): fruit, aril and heartwood, *Negella savita* Linn (Ranunculaceae), *Nelumbo nucifera* Gaertn (Nymphaeaceae) and *Syzygium aromaticum* (Linn) Merr & Perry (Myrtaceae). The bacterial strains used in the present study were isolated from clinical specimens from Thammasat University Hospital including *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumanii*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as standard strains. Gentamicin (10 μg/ml) was included as drug control.

Table 1. Extracts of Prasaprohyai formula and its components by different methods

Herbs	Thai name	Part	% Yield		
			ET	EF	VO
Prasaprohyai formula	-	-	18.66	10.64	1.06
Amomum krevanh Pierre	Krawan	Fruit	2.42	1.79	0.25
Anethum graveolens Linn.	Thian ta takkatan	Fruit	4.34	3.67	0.40
Angelica sylvestris Linn.	Kot so	Root	5.14	4.54	0.01
Artemisia annua Linn.	Kot chula lampha	Whole plant	4.27	2.73	0.15
Atractylodes lancea (Thunb.) DC.	Kot kamao	Rhizome	16.89	13.85	0.90
Conioselinum univitatum Trucz.	Kot hua bua	Rhizome	12.19	10.30	0.25
Cumimum cyminum Linn.	Thian khao	Fruit	8.73	8.12	1.25
Dracaena loureiri Gagnep.	Chan daeng	Heartwood	17.87	14.30	0.02
Foeniculum vulgare Mill.	Thian khao plueak	Fruit	6.69	5.50	1.15
Kaempferia galanga Linn.	Proh hom	Rhizome	6.39	4.16	0.70
Lepidium sativum Linn.	Thian daeng	Seed	9.20	8.42	0.65
Levisticum officinale Koch.	Kot chiang	Root	15.05	13.12	0.55
Mammea siamensis Kosterm.	Saraphi	Flower	32.78	27.76	0.08
Mesua ferrea Linn.	Bunnak	Flower	23.17	20.19	0.10
Mimusops elengi Linn.	Phikul	Flower	8.82	8.41	0.40
Myristica fragrans Houtt.	Dok chan	Aril	18.97	16.24	4.50
Myristica fragrans Houtt.	Chan thet	Heartwood	7.07	6.04	0.70
Myristica fragrans Houtt.	Luk chan	Seed	13.67	12.18	1.50
Negella savita Linn.	Thian dam	Seed	32.29	28.93	0.25
Nelumbo nucifera Gaertn.	Kasorn bua luang	Pollen	10.59	9.31	0.15
Syzygium aromaticum (Linn.) Merr. & Perry	Kan phlu	Flower	31.24	23.41	4.65

Formula of Prasaprohyai and its components were extracted by different methods including maceration with 95% ethanol followed by evaporation (ET), ET followed by freeze dry (EF) to remove remaining ethanol and water distillation (VO). The yields of extracts were measured in percentage (% yield). Antibacterial susceptibility was performed by disc diffusion method[12] and modified microtitre plate-based antibacterial assay as described in previous report[13]. Both ET and EF extracts were prepared at concentration of 20 mg/disc, whereas VO extract prepared at concentration of 10 mg/disc. The tests were performed in triplicate. The extracts that produced inhibition zone $\geq$ 8 mm were selected for Minimum Inhibitory Concentration (MIC) determination by broth dilution method[14] and Minimum Bactericidal Concentration (MBC) was also performed.

Results

Prasaprohyai formula and its components extracted by different methods showed different yields of extraction (Table 1). Crude extracts of ET had higher yield than other methods. The percentage yields of ET, EF and VO extracts were 2.42% to 32.78%, 1.79% to 27.76% and 0.01% to 4.65%, respectively. Distillation method generated the lowest yields of *Angelica sylvestris* (0.01%) and *Dracaena loureiri* (0.02%).

The results of antibacterial activity showed that all extracts of Prasaprohyai formula and its components inhibited the growth of Gram positive bacteria more than Gram negative bacteria. The MIC and MBC of the extracts were at the same concentration. Most of the ET extracts from the formula and its components were able to inhibit *S. pyogenes* and all *S. aureus* strains including MRSA. In addition, the results indicated that *Syzygium aromaticum* of the formula could inhibit the growth of all tested strains ranged from 9 to 19 mm. Interestingly, the crude extracts by ET method of three components including *Lepidium sativum*, *Myristica fragrans* (seed) and *Myristica fragrans* (aril) had no activity against all microorganism. The MIC/MBC values of these ET extracts ranged from 0.31 to >10 mg/ml (Table 2).

The EF extracts of the formula and some components were able to inhibit most of the Gram positive bacteria. The EF extracts showed more activity against Gram negative bacteria than the ET extracts except *Atractylodes lancea, Negella savita*, Myristica fragrans (seed), Myristica fragrans (aril), *Dracaena loureiri, Mesua ferrea, Mammea siamensis, Mimusops elengi, Kaempferia galanga* and Prasaprohyai formula. The MIC/MBC values of these EF extracts ranged from 0.31 to > 10 mg/ml (Table 3).

The VO extracts showed the antibacterial activity against both Gram positive and Gram negative bacteria. In particular, the VO extracts of *Cumimum cyminum, Foeniculum vulgare, Amomum krevanh, Syzygium aromaticum, Nelumbo nucifera* and Prasaprohyai formula exhibited the antibacterial activity against all tested strains in the present study. Only the VO extract of *Lepidium sativum* had no activity against all the tested strains. The MIC/MBC value of these VO extracts range from 0.62 to >10 mg/ml (Table 4). In addition, the VO extract of some components of this formula exhibited the activity against *S. pyogenes*.

Discussion

The results indicated that the Prasaprohyai formula and its components were able to inhibit the growth of most tested strains including clinical isolates and standard strains. Especially, the crude extracts of *Syzygium aromaticum* from different extraction methods showed the ability to inhibit the growth of both Gram positive and Gram negative bacteria. The antibacterial activity of volatile oil and its major constituent, eugenol on the growth of *S. aureus, Enterococcus faecalis, E. coli* and *E. coli* O157:H7 has been previously reported[3]. In addition, clove oil was found to be effective against *K. pneumoniae, Pseudomonas aeruginosa, Salmonella* Typhi, *Shigella dysentriae, Vibrio cholerae, Bacillus subtilis* and *Proteus vulgaris*[15,16]. It was purposed previously that the bacterial cell structures became to cell death due to the major chemical compounds of herbal extracts[16]. Therefore, the authors suggested that the VO extract observed in the present study possibly consists of an active compound providing its antibacterial activity.

The ET and EF extracts of Prasaprohyai formula and its components were found more activity against Gram positive bacteria. It is possibly because Gram positive bacteria have only outer peptidoglycan layer which is not effective permeability barrier resulting in more susceptible to antimicrobial agent from the extracts. In contrast, Gram negative bacteria have outer membrane that was able to inhibit the attack of active compound in the extracts[8,17]. In addition, the ET and EF extract showed no different results of MIC/MBC values. The formula and its components of both extracts are possibly composed of the same chemical structure and functional group. Interestingly, the crude extracts by ET method of three components including *Lepidium sativum, Myristica fragrans* (seed) and *Myristica*

Table 2. Antibacterial activity of crude extract of Prasaprohyai formula and its components (ET)

Herbals Individual component	Average Inhibition Zone (mm) and MIC/MBC (mg/ml)									
	SA (ATCC)	SA	MRSA	SP	EC (ATCC)	EC (ESBL)	AB	AB (MDR)	KP	KP (MDR)
Prasaprohyai formula	9/2.5	8	9/2.5	8/1.25	0	0	0	0	0	0
Amomum krevanh Pierre	9/10	0	8/10	9/5	8/> 10	0	9/>10	9/> 10	8/>10	0
Anethum graveolens Linn.	8/10	0	8/10	8/2.5	0	8/>10	0	8/> 10	0	0
Angelica sylvestris Linn.	9/10	9	22/5	8/10	9/> 10	8/>10	8/>10	8/> 10	0	8/>10
Artemisia annua Linn.	8/5	0	20/1.25	0	0	0	9/>10	0	0	0
Atractylodes lancea (Thunb.) DC.	0	0	9/10	0	0	0	0	0	0	0
Conioselinum univitatum Trucz.	9/10	9	8/> 10	8/1.25	0	0	0	0	0	0
Cumimum cyminum Linn.	10/5	9	20/1.25	8/2.5	0	0	0	8/>10	0	0
Dracaena loureiri Gagnep.	9/2.5	11	16/0.62	0	0	0	0	0	0	0
Foeniculum vulgare Mill.	0	0	16/10	0	0	0	0	0	0	0
Kaempferia galanga Linn.	8/5	0	0	0	0	0	0	0	0	0
Lepidium sativum Linn.	0	0	0	0	0	0	0	0	0	0
Levisticum officinale Koch.	11/5	11	8/10	8/2.5	0	0	0	0	0	0
Mammea siamensis Kosterm.	10/1.25	10	10/0.62	0	0	0	0	0	0	0
Mesua ferrea Linn.	9/0.62	0	8/0.31	0	0	0	0	0	0	0
Mimusops elengi Linn.	8/5	8	9/5	0	0	0	0	0	0	0
Myristica fragrans Houtt. (Aril)	0	0	0	0	0	0	0	0	0	0
Myristica fragrans Houtt. (Heartwood)	10/5	8	0	0	0	0	0	0	0	0
Myristica fragrans Houtt. (Seed)	0	0	0	0	0	0	0	0	0	0
Negella savita Linn.	13/5	10	19/1.25	9/2.5	0	0	0	0	0	0
Nelumbo nucifera Gaertn.	0	0	9/10	0	0	0	0	0	0	0
Syzygium aromaticum (Linn.) Merr. & Perry	16/1.25	16	17/2.5	11/2.5	14/5	13/5	19/2.5	19/2.5	9/5	9/5
No. of active components	15	10	16	8	3	3	4	5	2	2
(%)	(71.4)	(47.6)	(76.2)	(38.1)	(14.3)	(14.3)	(19)	(23.8)	(9.5)	(9.5)

* SA (ATCC) = *S. aureus* ATCC 25923, SA = *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, SP = *S. pyogenes*, EC (ATCC) = *Escherichia col*i (ATCC), EC (ESBL) = *E. coli* (Extended-Spectrum β-Lactamase), AB = *A. baumannii*, AB (MDR) = *A. baumannii* (Multidrug resistant strain), KP = *Klebsiella pneunoniae*, KP (MDR) = *K. pneunoniae* (Multidrug resistant strain)

Table 3. Antibacterial activity of crude extract of Prasaprohyai formula and its components (EF)

Herbals Individual component	Average of Inhibition Zone (mm) and MIC/MBC (mg/ml)									
	SA (ATCC)	SA	MRSA	SP	EC (ATCC)	EC (ESBL)	AB	AB (MDR)	KP	KP (MDR)
Prasaprohyai formula	9/0.62	0	9/0.31	0	0	0	0	0	0	0
Amomum krevanh Pierre	8/10	8	12/5	0	9/>10	9/>10	9/>10	10/>10	0	8/>10
Anethum graveolens Linn.	8/10	8	9/>10	8/10	0	9/>10	0	0	0	0
Angelica sylvestris Linn.	11/10	10	22/5	9/10	9/>10	10/>10	10/>10	10/>10	8/10	8/>10
Artemisia annua Linn.	8/5	8	19/1.25	8/1.25	8/>10	12/>10	9/10	9/10	0	0
Atractylodes lancea (Thunb.) DC.	0	0	9/>10	0	0	0	0	0	0	0
Conioselinum univitatum Trucz.	9/10	11	10/10	0	8/>10	9/>10	0	0	0	0
Cumimum cyminum Linn.	14/5	12	25/0.62	9/5	8/>10	11/>10	9/10	10/>10	8/>10	0
Dracaena loureiri Gagnep.	10/2.5	11	16/2.5	0	0	0	0	0	0	0
Foeniculum vulgare Mill.	9/5	9	15/10	8/5	8/>10	10/>10	10/10	9/>10	8/>10	0
Kaempferia galanga Linn.	0	0	0	0	0	0	0	0	0	0
Lepidium sativum Linn.	0	0	0	0	0	0	0	8/>10	0	0
Levisticum officinale Koch.	10/5	11	10/10	8/2.5	8/>10	9/>10	8/>10	9/>10	0	8/>10
Mammea siamensis Kosterm.	9/0.62	10	9/0.31	0	0	0	0	0	0	0
Mesua ferrea Linn.	9/0.62	0	8/0.62	0	0	0	0	0	0	0
Mimusops elengi Linn.	8/5	0	0	0	0	0	0	0	0	0
Myristica fragrans Houtt. (Aril)	0	0	0	0	0	0	0	0	0	0
Myristica fragrans Houtt. (Heartwood)	10/5	9	10/10	0	9/>10	10/>10	8/5	10/5	8/10	0
Myristica fragrans Houtt. (Seed)	0	0	0	0	0	0	0	0	0	0
Negella savita Linn.	8/>10	10	12/5	8/2.5	0	0	0	0	0	0
Nelumbo nucifera Gaertn.	0	0	12/10	0	10/>10	8/>10	10/>10	12/5	9/>10	8/>10
Syzygium aromaticum (Linn.) Merr. & Perry	15/1.25	15	17/2.5	11/1.25	14/5	13/2.5	18/1.25	19/2.5	8/5	9/5
No. of active components	15	13	16	8	10	11	9	10	6	5
(%)	(71.4)	(61.9)	(76.2)	(38.1)	(47.6)	(52.4)	(42.9)	(47.6)	(28.6)	(23.8)

* SA (ATCC) = *S. aureus* ATCC 25923, SA = *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, SP = *S. pyogenes*, EC (ATCC) = *Escherichia coli* ATCC 25922, EC (ESBL) = *E. coli* (Extended-Spectrum β-Lactamase), AB = *A. baumannii*, AB (MDR) = *A. baumannii* (Multidrug resistant strain), KP = *Klebsiella pneunoniae*, KP (MDR) = *K. pneunoniae* (Multidrug resistant strain)

Table 4. Antibacterial activity of crude extract of Prasaprohyai formula and its components (VO)

Herbals Individual component	Average of Inhibition Zone (mm) / MIC/MBC (mg/ml)									
	SA (ATCC)	SA	MRSA	SP	EC (ATCC)	EC (ESBL)	AB	AB (MDR)	KP	KP (MDR)
Prasaprohyai formula	8/5	8	8/5	8/5	9/>10	8/>10	12/5	10/5	7/>10	7/>10
Amomum krevanh Pierre	8/5	8	7/5	8/1.25	9/10	8/>10	11/10	10/5	8/>10	8/>10
Anethum graveolens Linn.	0	0	0	0	8/>10	9/>10	13/5	9/5	7/>10	7/>10
Angelica sylvestris Linn.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Artemisia annua Linn.	0	0	0	0	0	0	8/>10	7/>10	0	0
Atractylodes lancea (Thunb.) DC.	7/2.5	7	7/2.5	7/1.25	0	0	0	0	0	0
Conioselinum univitatum Trucz.	8/1.25	7	7/5	7/1.25	0	7/>10	7/>10	7/>10	0	0
Cumimum cyminum Linn.	9/10	9	9/>10	8/10	11/10	11/10	15/5	11/>10	9/>10	10/>10
Dracaena loureiri Gagnep.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Foeniculum vulgare Mill.	7/5	7	10/5	7/2.5	8/>10	9/>10	13/5	9/>10	7/>10	7/>10
Kaempferia galanga Linn.	7/10	0	0	0	7/>10	0	7/10	7/>10	0	0
Lepidium sativum Linn.	0	0	0	0	0	0	0	0	0	0
Levisticum officinale Koch.	8/10	7	8/10	7/5	8/>10	8/>10	8/5	8/>10	7/>10	0
Mammea siamensis Kosterm.	7/2.5	0	0	7/2.5	0	0	0	0	0	0
Mesua ferrea Linn.	7/>10	0	7/5	8/5	0	0	0	0	0	0
Mimusops elengi Linn.	7/>10	0	7/>10	7/>10	9/>10	7/>10	9/>10	8/>10	7/>10	7/5
Myristica fragrans Houtt. (Aril)	0	7	0	8/10	10/>10	10/>10	14/>10	12/>10	9/>10	10/>10
Myristica fragrans Houtt. (Heartwood)	7/>10	7	7/0.62	7/0.62	0	0	0	0	0	0
Myristica fragrans Houtt. (Seed)	7/5	8	0	7/5	11/10	11/>10	15/10	12/10	11/>10	10/>10
Negella savita Linn.	9/>10	9	14/10	0	8/>10	8/>10	9/>10	8/>10	7/>10	7/>10
Nelumbo nucifera Gaertn.	10/10	9	9/5	10/5	10/>10	9/>10	14/10	14/10	8/>10	8/>10
Syzygium aromaticum (Linn.) Merr. & Perry	12/2.5	12	11/5	13/1.25	13/5	13/2.5	18/2.5	20/2.5	9/5	9/5
No. of active components (%)	15 (71.4)	12 (57.1)	12 (57.1)	14 (66.7)	12 (57.1)	12 (57.1)	14 (66.7)	14 (66.7)	11 (52.4)	10 (47.6)

* SA (ATCC) = *S. aureus* ATCC 25923, SA = *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, SP = *S. pyogenes*, EC (ATCC) = *Escherichia coli* ATCC 25922, EC (ESBL) = *E. coli* (Extended-Spectrum β-Lactamase), AB = *A. baumannii*, AB (MDR) = *A. baumannii* (Multidrug resistant strain), KP = *Klebsiella pneunoniae*, KP (MDR) = *K. pneunoniae* (Multidrug resistant strain)

** NA = Not available

fragrans (aril) had no activity against all the tested strains. This obtained result revealed that the method of extraction influences the discovery of possible active compounds harboring antimicrobial activity of each component of Prasaprohyai.

The VO extract of *Cumimum cyminum*, *Syzygium aromaticum*, *Nelumbo nucifera* and Prasaprohyai formula provided the most effectiveness against all tested strains in the present study. The volatile oil extract of *Cumimum cyminum* also exhibited the antibacterial activity against *K. pneumoniae, E. coli* and *S. aureus* in previous reports[18,19]. Moreover, Sittiwet reported that the volatile oil extract of *Nelumbo nucifera* had antibacterial activity against *E. coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028[20]. The obtained results indicated that volatile oil extracts is likely to play an important role in antimicrobial activities as previous reports. The VO extract of major component of Prasaprohyai formula, *Kaempferia galanga* was able to inhibit *A. baumanii*, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Tewtrakul et al[21] showed that the volatile oil extract inhibited both bacteria and fungi. Previous reports demonstrated antibacterial and other biological activities such as anti-allergic[22], anti-inflammatory[23-25], antioxidant[21,25,26] and immunity[27,28] found in most of the components in Prasaprohyai formula. *K. galangal* was the major part of the formula. However, *K. galangal* exhibited less antibacterial activity than other components. This indicated that the formula of Prasaprohyai was most likely to have other components harboring the activity resulting in synergistic effect of antibacterial activity.

The activity of the Prasaprohyai formula was firstly observed in the present study. Interestingly, most of the VO extracts of Prasaprohyai formula and its components except *Atractylodes lancea, Lepidium sativum, Myristica fragrans* and *Mesua ferrea* has activity against Gram positive and Gram negative bacteria. However, further studies are necessary to explore more detailed information in order to discover the marker of active compounds. Subsequently, development of any medicinal products for infectious diseases will be conducted efficiently in future.

Conclusion

The obtained results provided an insightful detailed information of the Thai traditional medicine, Prasaprohyai formula and its components in the aspect of antibacterial activity. The formula of Prasaprohyai and its components exhibited the antibacterial activity leading to potential developing of medicinal products for bacterial infection and other infectious diseases. The development of its formula or other combinations of the components for alternative treatment of infectious diseases will be further approached. The benefit of the Thai traditional formula will produce more drugs of choices for bacterial infection and prevent the incidence of emerging resistant strains of bacteria due to the excess antibiotic usage.

Acknowledgement

This research is financially supported by research fund from Faculty of Medicine, Thammasat University, Thailand. Sincere thanks to Assoc. Prof. Dr. Arunporn Itarat for her initial guidance. Special thanks go to Ms. Narissara Mungkornkaew for collecting isolates from Thammasat University Hospital, Thailand. I deeply appreciated Faculty of Medicine, Thammasat University, Thailand for supporting laboratory facilities to complete this successful work.

Potential conflicts of interest

None.

References

1. World Health Organization. The global burden of disease: 2004 Update. Geneva: WHO; 2008.
2. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, et al. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. Phytother Res 2007; 21: 501-6.
3. Sanla-Ead N, Jangchud A, Chonhenchob V, Suppakul P. Antimicrobial activity of cinnamon, clove and galangal essential oils and their principal constituents and possible application in active packaging. The Proceedings of 15th IAPRI World Conference on Packaging (WorldPak2006): Technical session; October 2-5, 2006. Tokyo, Japan: 214-8.
4. Nanasombat S, Lohasupthawee P. Antibacterial activity of crude ethanolic extracts and essential oils of spices against *Salmonellae* and other Enterobacteria. KMITL Sci Tech J 2005; 5: 527-38.
5. Stonsaovapak S, Chareonthamawat P, Boonyaratanakornkit M. Inhibitory effects of selected Thai spices and medicinal plants on *Escherichia coli* O157:H7 and *Yersinia enterocolitica*. Kasetsart J (Nat Sci) 2000; 34: 510-7.
6. Bourgou S, Pichette A, Marzouk B, Legault J.

Bioactivities of black cumin essential oil and its main terpenes from Tunisia. South African Journal of Botany 2010; 76: 210-6.
7. Ferdous AJ, Islam SN, Ahsan M, Hasan CM, Ahmed ZU. In vitro antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug-resistant isolates of *Shigella* spp. and isolates of *Vibrio cholerae* and *Escherichia coli*. Phytother Res 1992; 6: 137-40.
8. Salman MT, Khan RA, Indu S. Antimicrobial activity of *Nigella sativa* Linn. seed oil against multi-drug resistant bacteria from clinical isolates. Natural Product Radiance 2008; 7: 10-4.
9. Zuridah H, Fairuz ARM, Zakri AHZ, Rahim MNA. In vitro antibacterial activity of *Nigella savita* against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* and *Bacillus cerues*. Asian J Plant Sci 2008; 7: 331-3.
10. Costa MM, Bounatirou S, Miguel MG, Faleiro ML, Figueiredo AC, Barroso JG, et al. Essential oils from *Anethum graveolens, Levisticum officinale* and *Piminella anisum* hairy root cultures: composition, antibacterial and antioxidant activities. Planta Med 2006;72 [Contact: Costa, MM; Univ Lisbon, Fac Ciencias Lisboa, DBV, Ctr Biotecnol Vegetal, P-1749016 Lisbon, Portugal].
11. Dadalioglu I, Evrendilek GA. Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas* L.), and fennel (*Foeniculum vulgare*) on common foodborne pathogens. J Agric Food Chem 2004; 52: 8255-60.
12. Lorian V. Antibiotics in laboratory medicine. 4th ed. Baltimore, MD: Williams & Wilkins; 1996.
13. Kondo S, Sattaponpan C, Phongpaichit S, Srijan A, Itharat A. Antibacterial activity of Thai medicinal plants Pikutbenjakul. J Med Assoc Thai 2010; 93 (Suppl 7): S131-5.
14. 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.
15. Saeed S, Tariq P. In vitro antibacterial activity of clove against Gram negative bacteria. Pak J Bot 2008; 40: 2157-60.
16. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med 2006; 6: 39.
17. Tegos G, Stermitz FR, Lomovskaya O, Lewis K. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrob Agents Chemother 2002; 46: 3133-41.
18. Derakhshan S, Sattari M, Bigdeli M. Effect of subinhibitory concentrations of cumin (*Cuminum cyminum* L.) seed essential oil and alcoholic extract on the morphology, capsule expression and urease activity of *Klebsiella pneumoniae*. Int J Antimicrob Agents 2008; 32: 432-6.
19. Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem 2007; 102: 898-904.
20. Sittiwet C. Antimicrobial activity of essential oil from *Nelumbo nucifera* Gaertn. pollen. Int J Pharmacol 2009; 5: 98-100.
21. Tewtrakul S, Yuenyongsawad S, Kummee S, Atsawajarruwan L. Chemical components and biological activities of volatile oil of *Kaempferia galanga* Linn. Songklanakarin J Sci Technol 2005; 27(Suppl 2): 503-7.
22. Tewtrakul S, Subhadhirasakul S. Anti-allergic activity of some selected plants in the Zingiberaceae family. J Ethnopharmacol 2007; 109: 535-8.
23. Aroonrerk N, Kamkaen N. Anti-inflammatory activity of *Quercus infectoria, Glycyrrhiza uralensis, Kaempferia galanga* and *Coptis chinensis*, the main components of thai herbal remedies for aphthous ulcer. J Health Res 2009; 23: 17-22.
24. Sulaiman MR, Zakaria ZA, Daud IA, Ng FN, Ng YC, Hidayat MT. Antinociceptive and anti-inflammatory activities of the aqueous extract of *Kaempferia galanga* leaves in animal models. J Nat Med 2008; 62: 221-7.
25. Makchuchit S, Itharat A, Tewtrakul S. Antioxidant and nitric oxide inhibition activities of Thai medicinal plants. J Med Assoc Thai 2010; 93 Suppl 7: S227-S235.
26. Mekseepralard C, Kamkaen N, Wilkinson JM. Antimicrobial and antioxidant activities of traditional Thai herbal remedies for aphthous ulcers. Phytother Res 2010; 24: 1514-9.
27. Halder S, Mehta AK, Mediratta PK, Sharma KK. Essential oil of clove (*Eugenia caryophyllata*) augments the humoral immune response but decreases cell mediated immunity. Phytother Res 2011 Feb 1. doi: 10.1002/ptr.3412.

28. Liu CP, Tsai WJ, Shen CC, Lin YL, Liao JF, Chen CF, et al. Inhibition of (S)-armepavine from *Nelumbo nucifera* on autoimmune disease of MRL/MpJ-lpr/lpr mice. Eur J Pharmacol 2006; 531: 270-9.

ฤทธิ์ต้านแบคทีเรียก่อโรคของสารสกัดตำรับประสะเปราะใหญ่ และสารสกัดเดี่ยว

ชิษณุชา สัตพนพันธุ์, สุมาลี คอนโด

ภูมิหลัง: *ประสะเปราะใหญ่เป็นหนึ่งในตำรับยาของการแพทย์แผนไทยที่ใช้ในการแก้พิษไข้ในเด็ก ซึ่งไข้เป็นอาการแสดงอย่างหนึ่งของโรคติดเชื้อต่างๆ ที่เกิดจากการติดเชื้อแบคทีเรียและไวรัส นอกจากนี้ประสะเปราะใหญ่และสมุนไพรเดี่ยวที่เป็นส่วนประกอบในตำรับยังมีศักยภาพในการออกฤทธิ์ทางชีวภาพรวมถึงฤทธิ์ในการแก้ไข้ และฤทธิ์ในการต้านจุลินทรีย์ ซึ่งสามารถที่จะพัฒนาเป็นยาทางเลือกเพื่อรักษาโรคติดเชื้อต่อไป*

วัตถุประสงค์: *เพื่อศึกษาฤทธิ์ต้านเชื้อแบคทีเรียก่อโรคในระบบทางเดินหายใจของสารสกัดตำรับประสะเปราะใหญ่ และสารสกัดเดี่ยว ของตำรับประสะเปราะใหญ่*

วัสดุและวิธีการ: *สกัดตำรับประสะเปราะใหญ่และสารสกัดเดี่ยวด้วยวิธีที่แตกต่างกัน 3 วิธี คือ สกัดด้วยเอทานอลแล้วทำแห้งด้วยเครื่องปั่นเหวี่ยงภายใต้ระบบสุญญากาศ (ET), สารสกัด ET ที่ทำให้แห้งด้วยเครื่องทำแห้งแบบแช่แข็ง (EF) และกลั่นด้วยน้ำ (VO) ทดสอบกับเชื้อแบคทีเรียที่แยกได้จากผู้ป่วยของโรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ รวมทั้ง Staphylococcus aureus ATCC 25923 และ Escherichia coli ATCC 25922 ทำการทดสอบฤทธิ์ต้านเชื้อโดยวิธี disc diffusion และคัดเลือกสารสกัด ET และ EF ที่ให้ค่า inhibition zone ≥ 8 มิลลิเมตร และสารสกัด VO ที่มีค่า inhibition zone ≥ 7 มิลลิเมตรมาทำการทดสอบเพื่อหาค่า MIC และ MBC โดยทำการทดสอบ 3 ซ้ำ และมีตัวควบคุม (positive, negative และ viable cell control)*

ผลการศึกษา: *สารสกัด ET มีร้อยละของผลผลิตมากกว่าสารสกัดที่สกัดด้วยวิธีอื่นๆ ผลจากการศึกษาพบว่าสารสกัด ET ของดอกกานพลู (Syzygium aromaticum (Linn.) Merr. & Perry) มีประสิทธิภาพในการต้านเชื้อแบคทีเรียทั้งหมดที่ใช้ทดสอบ โดยมีค่า inhibition zone อยู่ในช่วง 9 ถึง 19 มิลลิเมตร ซึ่งสารสกัดน้ำมันหอมระเหยของตำรับประสะเปราะใหญ่มีฤทธิ์ต้านเชื้อแบคทีเรียส่วนมากที่ใช้ในการทดสอบครั้งนี้ ฤทธิ์ต้านเชื้อ S. pyogenes พบในส่วนน้ำมันหอมระเหยของสารสกัดเดี่ยวของพืชสมุนไพรบางชนิดในตำรับ ส่วนสารสกัด ET ของสมุนไพรเทียนแดง (Lepidium sativum Linn.) ลูกจันทน์ (Myristica fragrans Houtt, seed) และดอกจันทน์ (Myristica fragrans Houtt. aril) ไม่มีฤทธิ์ในการต้านเชื้อที่ทดสอบทั้งหมด และสารสกัด EF ของทั้งตำรับและสารสกัดเดี่ยวส่วนมากสามารถยับยั้งเชื้อแบคทีเรียแกรมบวกได้*

สรุป: *จากผลการทดลองแสดงให้เห็นว่าตำรับประสะเปราะใหญ่และสารสกัดเดี่ยวสามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียทั้งแกรมบวกและแกรมลบ รวมถึงเชื้อที่ดื้อต่อยาปฏิชีวนะหลายๆ ชนิดด้วย สารสกัดน้ำมันหอมระเหยของตำรับและสมุนไพรเดี่ยวน่าจะมีบทบาทสำคัญในการต้านเชื้อ ดังนั้น ตำรับยานี้อาจนำมาพัฒนาเป็นยารักษาโรคติดเชื้อต่อไปในอนาคตเพื่อลดการใช้ยาปฏิชีวนะที่มีแนวโน้มการดื้อยามากขึ้น*