

# Antibacterial Activity of Thai Herbal Plants and Development of Hand Washing Gel Product

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**Background:** Multiresistant bacteria have become a major cause of nosocomial infection and serious diseases worldwide. Antibiotic resistance due to overuse is a factor that causes treatment failure. Several herbal plants have been investigated for various useful properties such as antimicrobial, anti-inflammatory and antioxidant activities as an alternative to modern medicine.

**Objective:** To overcome bacterial multiresistance, development of products from plants with potential antibacterial properties was conducted.

**Material and Method:** Bacterial strains including *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, were clinical isolates collected from Thammasat University Hospital. Crude extracts were taken from the wooden part of *Caesalpinia sappan* Linn. and dried flower bud of *Syzygium aromaticum* (Linn.) Merr. & Perry (clove). The herbal plants extractions were performed by maceration and decoction. Antibacterial activity was carried out by broth dilution method. The potential antibacterial extracts were formulated for a hand washing gel. Stability of the product was subsequently tested.

**Results:** The results revealed that *C. sappan* Linn. exhibited better antibacterial activity against both Gram-positive and Gram-negative bacteria as compared to clove extract. The combination of two herbal extracts did not produce an apparent antibacterial synergistic effect. Minimum inhibitory concentration values of hand washing gel containing *C. sappan* Linn. extract alone ranged from 1.25 to 20 mg/ml.

**Conclusion:** This study identified *C. sappan* Linn. as a potential herbal plant for development of an alternative treatment of bacterial infection in the future.

**Keywords:** *Caesalpinia sappan* Linn., Multi-resistant bacteria, Antibacterial activity, Herbal product, Hand washing gel

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Bacterial infection is the most common infectious disease and causes a broad range of human illnesses resulting in high morbidity and mortality. It can spread from one host to another rapidly and is easily transmitted in medical facilities. Many bacteria, including *Pseudomonas aeruginosa*, *Klebsiella* spp., *Acinetobacter baumannii*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and enterococci, are responsible for nosocomial infections<sup>(1)</sup>. Since these

bacteria have become resistant to many antibiotics, alternative forms of treatment are gaining attention from both physicians and microbiologists. These alternative treatments, however, have yet to undergo vigorous research. Thai traditional remedies and herbs have been used in the treatment of various diseases in folk medicine formulas such as Prasaproyhai formula and Benchalokawichian formula<sup>(2)</sup>. In our previous reports, we explored the Prasaproyhai and Benchalokawichian formulas for antibacterial activity<sup>(3,4)</sup>. The authors noted that the individual components of the formula were capable of inhibiting both Gram-positive and Gram-negative bacteria. These studies provided informative clues of the possible herbal plants in Thailand regarding their potential antibacterial activities. This present study investigated the

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antimicrobial effects of herbal plant extracts against pathogenic bacteria and developed a hand washing gel containing herbal plants with antibacterial properties. Further implementation of Thai traditional herbs in medicine is promising.

### Material and Method

Crude extracts were taken from the wooden part of *Caesalpinia sappan* Linn. and dried flower bud of *Syzygium aromaticum* (Linn.) Merr. & Perry (clove). Maceration with 95% ethanol, followed by freeze drying to remove remaining ethanol and decoction method were performed for extraction.

Clinical isolates, including *S. aureus*, MRSA, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, as well as multiple resistant strains of the bacteria mentioned, were collected from Thammasat University Hospital.

The individual extracts of *C. sappan* Linn. and clove taken from both extraction methods were examined for antibacterial activity. In addition, combinations of both *C. sappan* Linn. and clove extracts, using the same extraction method, were tested. The antibacterial activity was performed by a modified microtitre plate-based antibacterial assay as described in previous reports<sup>(5)</sup>. Briefly, this assay was modified by adding resazurin after incubating at 35°C to 37°C for 16 to 18 hrs, and then with further incubation for 2 hrs. The inoculum was prepared equivalent to a 0.5 McFarland standard and further diluted 1: 200 to obtain 10<sup>6</sup> cfu/ml. Ampicillin and 1% Dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively. Viability of bacterial control was also included. The tests were performed in triplicate.

The most effective herbal extract was then formulated for a hand washing gel with and without preservatives (methylisothiazolinone and chlorphenesin). The final concentration of effective crude extract in the gel ranged from 0.16 to 10.00% w/w. The gel containing crude extract was tested for stability by heating the product at 45°C for 48 hr. and subsequently cooling at 4°C for 48 hr. The cycle of product heating and cooling was repeated 6 times<sup>(2)</sup>.

### Results

The ethanolic extracts of *C. sappan* Linn. and clove exhibited effective antibacterial activity against both Gram-positive and Gram-negative bacteria in this study. Minimum inhibitory concentration (MIC) values of *C. sappan* Linn. and clove against all tested strains ranged from 0.62 to 2.5 mg/ml and 2.5 to 5 mg/ml,

respectively (Table 1). In addition, the obtained result of antibacterial activity of hand washing gel containing mixture of both extracts revealed no synergistic effect on inhibition of the growth of bacterial strains (Data not shown). MIC values of the preservative alone was between 1.25 to 10 mg/ml whereas the product of herbal extracts containing preservative demonstrated lower MIC values from less than 0.16 to 0.62 mg/ml. This indicated that the preservative is likely to enhance the antibacterial activity of extract-containing hand washing gel. However, in the hand washing gel containing *C. sappan* Linn. the ethanolic extract retained its antibacterial activity after testing the stability by heating and cooling for 24 days (Table 2), except gel with preservative (data not shown). The results of MICs values of hand washing gel containing ethanolic extract of *C. sappan* Linn. against tested strains after stability testing indicated the gel was relatively stable, providing some increments of only 1 to 2 dilutions compared to the MICs values of the hand washing gel formula with no preservatives. The product formulation was therefore prepared with the range of MICs values (0.16 to 10.00% w/w) which provided the efficacy of its antibacterial activity (Table 1 and Table 2).

### Discussion

Ethanolic crude extracts of *C. sappan* Linn. and clove were more efficient in bacterial growth inhibition than the water crude extracts in our previous report<sup>(6)</sup>. Traditionally Thai folk doctors rely on a remedial form of decoction as a method of formulating treatment. In contrast, the effective individual components of Benchalokawichian and Prasaproyhai formulas containing antibacterial property were aqueous crude extract and essential oil extracts<sup>(3,4,7)</sup>. It is therefore noted that methods of extraction to be used must be considered for individual herbal plants for further biological assays and product development.

Antibacterial activity of the gel base containing preservative was observed, while the *C. sappan* Linn. extract containing the preservative displayed an obvious enhancement of antibacterial activity. In contrast, the activity of the preservative alone in gel base declined after the stability test was undertaken. This indicated that there was no significant effect of the preservative on the activity of the herbal extract contained in the hand washing gel.

The combination of *C. sappan* Linn. and clove extracts denoted no synergistic effects of antimicrobial activity of both plant extracts. The ethanolic extract of *C. sappan* Linn. was therefore designated as the most

**Table 1.** Antibacterial activity of hand washing gel containing mixture of ethnolic extracts of *C. sappan* Linn. and *S. aromaticum* (Linn.) Merr. & Perry. (clove) using broth dilution method

Product	Minimal Inhibitory Concentration (MIC) (mg/ml)				
	SA	MRSA	EC	AB	PA
Gel base (no preservative)	>10	>10	>10	>10	>10
Gel base + preservative	>10	>10	10	5	5
Gel formula (no preservative)	1.25	1.25	10	10	>10
Gel formula + preservative	<0.16	<0.16	0.62	0.31	0.62
<i>C. sappan</i> extract	0.62	0.62	2.50	1.25	2.50
<i>S. aromaticum</i> extract	2.5	5	5	2.50	5
Preservatives	5	10	1.25	1.25	1.25

SA = *S. aureus*; MRSA = Methicillin-resistant *S. aureus*; Multiresistant bacterial strains; EC = *E. coli*; AB = *A. baumannii*; PA = *P. aeruginosa*

**Table 2.** Minimal inhibitory concentrations of hand washing gel containing ethanolic extract of *C. sappan* Linn. against tested bacterial strains after stability testing

Day	MIC (mg/ml)				
	<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>
0	1.25	1.25	5	5	10
4	1.25	1.25	10	5	10
8	2.5	2.5	20	10	20
12	2.5	2.5	20	10	20
16	1.25	1.25	10	5	10
20	1.25	1.25	10	5	10
24	2.5	1.25	10	10	10

SA = *S. aureus*; MRSA = Methicillin-resistant *S. aureus*; Multiresistant bacterial strains; EC = *E. coli*; AB = *A. baumannii*; PA = *P. aeruginosa*

effective herbal plant to be used for developing herbal products.

*C. sappan* Linn. hold most significant potential for antibacterial activity against multiresistant bacterial strains. In addition, the obtained results of stability testing for antibacterial activity revealed that the product of hand washing gel formula containing *C. sappan* Linn. extract harbors its effectiveness of antibacterial activity for at least two year shelf-life. The stability of natural products is affected by several factors such as storage condition, container, packaging interaction, insect attacks, microorganism including mold, moisture content, content variation, drug interaction and environment factors including temperature, light, air and humidity<sup>(8,9)</sup>. Hence, herbal products must be evaluated for quality, efficacy and shelf-life.

## Conclusion

This study identified *C. sappan* Linn. as a potential herbal plant for development of an effective treatment of bacterial infection in the future. As multidrug resistant bacteria have been dramatically increasing in hospitals, researchers and physicians will continue finding alternative and safe treatments.

## What is already known on this topic?

The extracts of individual components of other Thai formulas such as Prasaproyhai and Benchalokawichian showed antimicrobial activities, reported in our previous studies<sup>(3,4)</sup>. Other activities such as antifungal<sup>(7)</sup>, anti-inflammatory<sup>(10)</sup>, and antioxidant properties<sup>(11,12)</sup> of other components of the formula were also cited. Hence, more alternative herbal extracts from the individual components of

the formulas in this study are worth exploring and combining for a new medicinal product development.

#### What this study adds?

This study revealed that the formula containing *C. sappan* has an effective antibacterial activity. It is promising that *C. sappan* Linn. possesses an as of yet unidentified active compound harboring this antibacterial property. Further detailed information of Thai herbal plants' properties must be explored to create products with the ability to effectively treat antibiotic-resistant bacterial infections.

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#### Potential conflicts of interest

None.

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## ฤทธิ์ต้านเชื้อแบคทีเรียของสมุนไพรไทยและการพัฒนาผลิตภัณฑ์เจลล้างมือ

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**ภูมิหลัง:** เชื้อแบคทีเรียคือยาเป็นสาเหตุการติดเชื้อในโรงพยาบาลและก่อโรครุนแรงทั่วโลก การดื้อยาเกิดจากการใช้ยาปฏิชีวนะอย่างเกินความจำเป็น ซึ่งเป็นปัจจัยที่ส่งผลให้การรักษาล้มเหลว มีการศึกษาคุณสมบัติต่างๆ ของสมุนไพรหลายชนิด พบว่ามีฤทธิ์ต้านเชื้อจุลินทรีย์ ฤทธิ์ต้านการอักเสบ ฤทธิ์ต้านอนุมูลอิสระ ซึ่งจะเป็นทางเลือกในการรักษาโรคต่างๆ

**วัตถุประสงค์:** เพื่อศึกษาพัฒนาผลิตภัณฑ์จากสมุนไพรที่มีคุณสมบัติต้านเชื้อจุลินทรีย์โดยเฉพาะเชื้อแบคทีเรียคือยา

**วัสดุและวิธีการ:** เชื้อทดสอบได้แก่ *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus (MRSA)*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* และ *Acinetobacter baumannii* เป็นเชื้อที่แยกจากตัวอย่างผู้ป่วยในโรงพยาบาล เฉลิมพระเกียรติ สารสกัดสมุนไพรสกัดจากส่วนเนื้อไม้ของฝาง (*Caesalpinia sappan* Linn.) ส่วนดอกแห้งของกานพลู (*Syzygium aromaticum* (Linn.) Merr. & Perry (clove)) โดยวิธีหมักแอลกอฮอล์และวิธีการต้ม การทดสอบฤทธิ์ต้านเชื้อจุลินทรีย์ทำโดยวิธี *broth dilution* สารสกัดที่ทดสอบแล้ว มีคุณสมบัติต้านเชื้อจุลินทรีย์ได้นำมาทำการพัฒนาผลิตภัณฑ์เจลล้างมือ โดยมีการทดสอบความคงตัวของเจลล้างมือ

**ผลการศึกษา:** ผลการศึกษาพบว่าสารสกัดฝางมีฤทธิ์ต้านเชื้อแบคทีเรียทั้งกลุ่มแกรมบวกและแกรมลบที่ดีกว่าสารสกัดกานพลู การใช้สารสกัดทั้งสองอย่างร่วมกันพบว่าไม่มีการเสริมฤทธิ์การต้านเชื้อแบคทีเรีย ค่าความเข้มข้นต่ำสุดของสารสกัดฝางในเจลล้างมือที่สามารถยับยั้งเชื้อแบคทีเรียที่ทดสอบ มีค่าในช่วงความเข้มข้นระหว่าง 1.25 ถึง 20 มก./มล.

**สรุป:** การศึกษานี้แสดงให้เห็นว่าฝางเป็นพืชสมุนไพรที่มีศักยภาพที่จะนำไปพัฒนาผลิตภัณฑ์ที่เป็นทางเลือก ในการรักษาการติดเชื้อแบคทีเรียในอนาคต

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