Antibacterial Activity of Extracts from Five Medicinal Plants and Their Formula against Bacteria That Cause Chronic Wound Infection

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Background: *Chronic wound is caused by various factors such as chemotherapy, gene damage, treatment with steroids, diabetes mellitus, renal failure, blood pressure, infection and nutritional factors. One of the most common causes is bacterial infection. Antibacterial activity of several herbal plants has been reported.Thai medicinal plants which possess biological activities are potential to develop an alternative treatment of bacterial infection.*
Objective: *To study efficiency of extracts from medicinal plants and their formula against bacteria that cause chronic wound infection.*
Material and Method: *Extraction of Thai medicinal plants including Curcuma longa Linn, Rhinacanthus nasutus Linn, Garcinia mangostana Linn, Caesalpinia sappan Linn and Centellia asiatica Linn was performed by maceration with 95% ethanol and decoction followed by freeze dry. Formulation was conducted by varying the ratio of each components. Antibacterial activity were determined disk diffusion and broth dilution against Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Acinetobacter baumanii, Escherichia coli and Klebsiella pneumoniae.*
Results: *Ethanolic extracts exhibited better antibacterial activity against tested strains than water extracts. Antibacterial activity of Caesalpinia sappan Linn. against S. aureus and MRSA showed the most effective with MIC value of 0.625 mg/ml. One of the five different formulas which contained two times proportion of C. sappan revealed that this formula was able to inhibit all tested strains with the MIC ranging between 0.156 mg/ml and 10 mg/ml.*
Conclusion: *C. sappan is the most effective herbal plant. The formula with two times proportion of C. sappan is potentially best formula for development of medicinal product of chronic wound infection. The potential active compound of C. sappan is suggested for further investigation of antimicrobial activity and other biological properties.*

Keywords: *Antibacterial activity, Curcuma longa Linn, Rhinacanthus nasutus Linn, Garcinia mangostana Linn, Caesalpinia sappan Linn, Centellia asiatica, Chronic wound infection*

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Chronic wound is commonly found in patients with underlying diseases such as diabetic, infection and vascular disease. The wound is difficult to be cured due to the resistant strains resulting from excessive use of antibiotics. Normal flora including *Staphylococcus epidermidis, Staphylococcus* coagulase negative, *Corynebacterium* spp and *Proprionibacterium acnes* were found in chronic wound resulted in serious wound infection[1]. Moreover, pathogenic bacteria such as *Staphylococcus aureus*, beta-hemolytic *Streptococcus* including *S. pyogenes* and *S. agalactiae, Escherichia coli, Proteus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Acinetobacter* spp, *Stenotrophomonas (Xanthomonas)* spp and anaerobic

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bacteria were also isolated from patients. *S. aureus* was frequently found in diabetes patients with chronic wound infection[1]. A long period usage of topical antibiotic treatment up to 6 months was recommended for wound infection[2]. The multiple infections and resistance to many antibiotics of chronic wound infection was subsequently taken place.

Thai traditional medicinal plants such as *Garcinia mangostana* Linn, *Curcuma longa* Linn, *Rhinacanthus nasutus* Linn, *Caesalpinia sappan* Linn and *Centellia asiatica* Linn containing alpha-mangostana[3], curcumin[4], rhinacanthin[5], brazilin[6] and asiatic acid[7] respectively showed various properties including wound healing, antibacterial activity[3,4,6] anti-fungi[5] and anti-inflammation[7]. The present study aims to determine antibacterial activity of five Thai herbal plants and further develop an effective formula of the herbal plants which have antibacterial activity for treatment of chronic wound infection in future.

Material and Method

Preparation of five formula with five different proportion of the herbal plants extracts (*Garcinia mangstana*: *Caesalpinia sappan*: *Curcuma longa*: *Rhinacanthus nasutus*: *Centellia asiatica*) were conducted as followed. Formula I (2:1:1:1:1), Formula II (1:2:1:1:1), Formula III (1:1:2:1:1), Formula IV (1:1:1:2:1) and Formula V (1:1:1:1:2). Gentamicin (10 µg/ml) was included for drug control. Bacterial strains were clinical isolates collected from patients' wound at Thammasat Hospital. The isolates were *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumanii*, *Escherichia coli*, *Klebsiella pneumonia* and *Psudomonas aeruginosa*. *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 9097 were included.

The herbal plants and their combined formula were extracted by two different methods including maceration with 95% ethanol and decoction followed by rotary evaporation and freeze dry. Antibacterial susceptibility was performed by disk diffusion method[8]. The bacterial inoculums were adjusted to 0.5 McFarland standard and spread onto sterile Mueller Hinton Agar (MHA) plate. Disk containing 20 µl of extract was placed on MHA seeded plate and incubated at 35-37°C for 16-18 hours. Diameters of inhibition zones were then measured. Data analysis was performed by mean of inhibition zone. The samples with inhibition zone $\geq$ 8 mm were selected for minimum inhibitory concentration determination using broth dilution method with some modifications[9]. Briefly, the inoculum was diluted (1:200) and added into each well containing serial two-fold dilutions of each extract. The tests were performed in triplicate. Gentamycin (10 µg/ml) was used as positive control. Negative control and viable cell control were included.

Results

The herbal plant extracts using maceration and decoction generated % yields ranged from 1.03 to 18.1% and 0.81 to 11.87%, respectively (Table 1). The ethanolic of *G. mangostana*, *C. sappan* and *C. longa* were able to inhibit all three *S. aureus* strains with the MIC values ranging from 0.156 to 5 mg/ml (Table 4).

The ethanolic extract of *C. sappan* showed the most effective activity against both Gram positive and Gram negative bacteria tested in the present study (Table 3). The MIC values were ranged from 0.156 to 10 mg/ml (Table 4). The isolates of MRSA and ESBL *E. coli* were inhibited by the ethanolic extract of *C. sappan* and the formula II with the MIC values of 0.625 and 10 mg/ml, respectively.

The extracts of *R. nasutus* and *C. asiatic*a were screened for disk diffusion. The result showed no activity against all strains. The extracts of *G. mangostana*, *C. sappan*, *C. longa* and five different formulas were able to inhibit the growth of all *S. aureus*

Table 1. Extraction of five herbal plants by maceration and decoction methods

Herbal plants	Common Name	Part	% yield	
			Maceration	Decoction
Garcinia mangostana Linn	Mangosteen	pericarp	9.59	8.45
Caesalpinia sappan Linn	Sappan	heart wood	7.47	4.25
Curcuma longa Linn	Turmeric	root	18.10	11.87
Rhinacanthus nasutus Linn	Rhinacanthus	leaf	1.03	0.81
*Centellia asiatic*a Linn	Pennywort	leaf	4.13	6.95

strains with the range of MIC values from 0.156 to 5 mg/ml (Table 4). However, all formulas except formula II showed high MIC against Gram negative bacteria ranging from 2.5 mg/ml to more than 10 mg/ml. The

Table 2. Antibacterial activities of five different formula extracts using maceration technique

Herbal extracts	Average inhibition zone (mm)									
	SA (ATCC)	SA	MRSA	EC (ATCC)	EC	EC (ESBL)	AB	PA (ATCC)	PA	KP
Formula I	21.22	22.00	24.00	8.00	7.78	8.00	10.11	8.00	0	0
Formula II	24.89	24.67	26.00	9.33	8.56	8.89	11.89	8.00	9.00	0
Formula III	22.67	22.00	24.00	8.00	8.00	7.78	9.67	8.33	0	0
Formula IV	23.33	22.00	24.00	8.00	8.00	7.67	9.00	8.33	0	0
Formula V	23.56	24.00	22.00	8.00	8.00	7.67	9.44	8.00	0	0

* SA (ATCC) = *S. aureus* ATCC 25923, SA = *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, EC (ATCC) = *E. coli* ATCC 25922, EC = *E. coli*, EC (ESBL) = *E. coli* (Extended-Spectrum β-Lactamase), AB = *A. baumannii*, PA (ATCC) = *P. aeruginosa* ATCC 9097, PA = *P. aeruginosa*, KP = *K. pneunoniae*

Table 3. Antibacterial activities of five different formula extracts using decoction technique

Herbal Extracts	Average inhibition zone (mm)									
	SA (ATCC)	SA	MRSA	EC (ATCC)	EC	EC (ESBL)	AB	PA (ATCC)	PA	KP
Formula I	16.11	13.00	16.22	0	0	0	0	0	0	0
Formula II	19.33	16.11	26.00	0	0	0	7.00	0	0	0
Formula III	16.56	13.67	17.78	0	0	0	0	0	0	0
Formula IV	17.67	13.78	18.11	0	0	0	0	0	0	0
Formula V	17.67	14.44	17.67	0	0	0	0	0	0	0

* SA (ATCC) = *S. aureus* ATCC 25923, SA = *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, EC (ATCC) = *E. coli* ATCC 25922, EC = *E. coli*, EC (ESBL) = *E. coli* (Extended-Spectrum β-Lactamase), AB = *A. baumannii*, PA (ATCC) = *P. aeruginosa* ATCC 9097, PA = *P. aeruginosa*, KP = *K. pneunoniae*

Table 4. Antibacterial activities of herbal crude extracts and five different formula extracts using maceration technique

Herbal Extracts	Minimal Inhibitory Concentration(mg/ml)									
	SA (ATCC)	SA	MRSA	EC (ATCC)	EC	EC (ESBL)	AB	PA (ATCC)	PA	KP
Garcinia mangstana Linn.	5	1.25	1.25	> 10	> 10	> 10	> 10	> 10	> 10	> 10
Caesalpinia sappan Linn.	0.156	0.625	0.625	2.5	5	5	5	5	5	10
Curcuma longa Linn.	2.5	5	5	> 10	> 10	> 10	> 10	> 10	> 10	> 10
Formula I	0.312	1.250	1.250	10	10	> 10	10	> 10	10	> 10
Formula II	0.156	0.625	0.625	10	10	10	5	5	5	10
Formula III	0.312	1.250	1.250	10	10	> 10	10	> 10	10	> 10
Formula IV	0.312	1.250	1.250	10	> 10	> 10	10	> 10	10	> 10
Formula V	0.312	1.250	1.250	10	10	> 10	10	> 10	10	> 10

* SA (ATCC) = *S. aureus* ATCC 25923, SA = *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, EC (ATCC) = *E. coli* ATCC 25922, EC = *E. coli*, EC (ESBL) = *E. coli* (Extended-Spectrum b-Lactamase), AB = *A. baumannii*, , PA (ATCC) = *P. aeruginosa* ATCC 9097, PA = *P. aeruginosa*, KP = *K. pneunoniae*

MIC of gentamicin (10 μg/ml) against *S. aureus* ATCC 25923 was 0.31 μg/ml.

Discussion

The ethanolic extract of *C. longa* was able to inhibit the growth of all *S. aureus* strains but not Gram negative bacteria. The failure of inhibition of *E. coli* and *P. aeruginosa* was also reported previously[10]. In contrast, some other reports demonstrated the antibacterial activity of the ethanolic extract of *C. longa* against *S. aureus*[10,11], *E. coli, K. pneumoniae* and *P. aeroginosa*[12]. In addition, clear liquid soap containing *C. longa* was demonstrated the effects of antioxidant and antimicrobial activities for wound infection of HIV patients[10]. Anti-inflammatory activities of curcumins I-III from *C. longa* were also reported[13]. *C. longa* and *C. asiatic*a extracted by decoction in the present study had no activity to inhibit the bacteria growth (data not shown). The ethanolic extract of *C. longa* showed the MIC values of 2.5 to 5 mg/ml. Interestingly, *C. longa* extracted by maceration with 95% alcohol previously demonstrated significantly different MIC value against *S. aureus* from our study[10]. It is suggested that the ethanol is possibly remained in the extract resulting in more activity against the bacterial strains.

The extracts of *C. asiatica* and *R. nasutus* had no antibacterial activity against all tested stains. However, the extract of *C. asiatica* was incorporated into the formula not only as for antibacterial activity but also for anti-inflammatory effect as reported previously[14]. The extract of *R. nasutus*, a local plant in Malaysia were potentially effective against *Candida albicans* and *Trichophyton mentagorphytes*. In addition, the antibacterial activity was also observed and showed the same results as the present study[5].

The extract of *G. mangostana* showed the activity on inflammatory mediator in RAW264.7 macrophage cells[15]. The activity against Gram positive bacteria of *G. mangostana* extracts produced by both decoction and maceration methods was observed in the present study. The properties of both anti-inflammatory and antibacterial activities of five herbal extracts are promising efficient for treatment of chronic wound infection.

The variation of components in five different formulas indicated that *C. sappan* extract plays an important role in antibacterial activity against the tested strains. Although *C. sappan* extract showed the best MIC against most of the tested bacteria, formula II combine with other extracts containing other biological activities is more promising for further development to possibly enhance wound healing process.

Conclusion

Most of the extracts using maceration method produced higher yield of extracts than decoction method. *C. sappan* is the most effective herbal plant in the present study. It potentially contains an active compound for antimicrobial activity. In addition, the other components of the formula provide other biological activities including anti-inflammatory, antioxidant and antifungal activities as previous reports. The herbal plant extracts with the most efficient antimicrobial activity and other related properties for chronic wound infection are potentially developed for an alternative medicine in future.

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Potential conflicts of interest

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ฤทธิ์ต้านแบคทีเรียก่อโรคติดเชื้อในแผลเรื้อรังของสารสกัดสมุนไพรและสูตรสมุนไพร

พรเทพ เต็มรังษี, สุมาลี คอนโด, อรุณพร อิฐรัตน์

ภูมิหลัง: *แผลเรื้อรังมีสาเหตุมาจากหลายปัจจัย ได้แก่ เคมีบำบัด จีนถูกทำลายการรักษาด้วยสเตียรอยด์ ภาวะเบาหวาน ไตล้มเหลว ความดันโลหิต ปัจจัยทางด้านโภชนาการ และการติดเชื้อแบคทีเรีย มีรายงานวิจัยพบว่าสมุนไพรหลายชนิดมีฤทธิ์ในการต้านเชื้อแบคทีเรีย ดังนั้นการพัฒนายาจากสมุนไพรใช้ในการต้านเชื้อแบคทีเรียจะเป็นยาทางเลือกในการนำมาใช้รักษาผู้ป่วยที่มีแผลเรื้อรังต่อไป*

วัตถุประสงค์: *เพื่อศึกษาประสิทธิภาพของสารสกัดสมุนไพรและสูตรสมุนไพรสกัดในการต้านเชื้อแบคทีเรียที่เป็นสาเหตุของการติดเชื้อในแผลเรื้อรัง*

วัสดุและวิธีการ: *ทำการสกัดสมุนไพรขมิ้นชัน (Curcuma longa Linn.) ทองพันชั่ง (Rhinacanthus nasutus Linn.) มังคุด (Garcinia mangostana Linn.) ฝาง (Caesalpinia sappan Linn.) และบัวบก (Centellia asiatica Linn.) ด้วยการหมักด้วยแอลกอฮอล์ 95% และการต้มสกัดด้วยน้ำ จากนั้นนำไปทำแห้งด้วยเครื่อง Freeze dryer นำสมุนไพรที่สกัดได้มาผสมในอัตราส่วนที่ต่างกันได้เป็นสูตรสมุนไพร ทดสอบฤทธิ์ต้านเชื้อด้วยวิธี disk diffusion และวิธี broth dilution โดยทดสอบกับเชื้อ taphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Acinetobacter baumanii, Escherichia coli, Klebsiella pneumoniae และ Psudomonas aeruginosa*

ผลการศึกษา: *สารสกัดโดยวิธีหมักด้วยแอลกอฮอล์ 95% มีฤทธิ์ต้านเชื้อได้ดีกว่าวิธีสกัดด้วยน้ำ สมุนไพรที่มีฤทธิ์ดีที่สุดในการยับยั้งเชื้อคือฝาง ได้ค่ายับยั้งต่ำสุดที่ 0.625 mg/ml ต่อเชื้อ S. aureus และ MRSA สูตรสมุนไพรที่มีฝาง 2 ส่วน มีฤทธิ์ที่ดีที่สุดในการต้านเชื้อทั้งหมดที่ใช้ในการทดสอบ*

สรุป: *ฝางเป็นพืชสมุนไพรที่มีสารออกฤทธิ์ในการต้านเชื้อแบคทีเรียที่มีประสิทธิภาพดีที่สุดในสูตรที่มีฝาง 2 ส่วน เป็นสูตรที่ดีที่สุดที่จะนำไปพัฒนาเป็นผลิตภัณฑ์ยารักษาแผลติดเชื้อชนิดเรื้อรังต่อไป*