Antimicrobial Activity of the Extracts from Benchalokawichian Remedy and Its Components

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Background: *Infectious diseases cause serious health problems worldwide due to multiresistant bacterial strains. Thai traditional formula such as Benchalokawichian remedy has been used to relieve fever, common cold and influenza. The remedy has been scientifically proved for antipyretic and antiseptic activities. However, the remedy and its components have not been fully studied for antimicrobial activity against pathogenic bacteria.*
Objective: *To determine antimicrobial activity of extracts from Benchalokawichian remedy and its components against clinical isolates by disk diffusion method.*
Material and Method: *The bacterial strains used in the present study were clinical isolates from Thammasat Hospital, Thailand. The ethanolic and water extracts of Benchalokawichian remedy and its components were screened for antimicrobial activity. The tests were performed in triplicate. The results were recorded by measuring diameter of growth inhibition zone. Means $\pm$ SD of the obtained results were calculated.*
Results: *The results of antimicrobial activity demonstrated that the ethanolic extracts of Benchalokawichian remedy and its components were effective against Candida albicans, Gram positive and Gram negative bacteria except some isolates. Tiliacora triandra and Clerodendrum petasites exhibited the most effective antimicrobial activity among other ethanolic extracts. The water extracts of Capparis micracantha, Tiliacora triandra and Harrisonia perforata were able to inhibit the tested strains. Both etahnolic and water extracts of Tiliacora triandra were the only one component of Benchalokawichian remedy that could inhibit the growth of C. albicans.*
Conclusion: *The present study provides basic knowledge of the antimicrobial activity of Benchalokawichian remedy and its components. Tiliacora triandra and Clerodendrum petasites were the most effective antimicrobial activity among other ethanolic extracts. They are potential candidates to produce medicinal formula for alternative medicine. Further study on minimum inhibitory concentration and minimum bactericidal concentration assay will be carried out in order to obtain more detailed insightful knowledge to develop medicinal products for treatment of bacterial infection and other infectious diseases.*

Keywords*: Antimicrobial activity, Benchalokawichian, Remedy, Pathogenic bacteria*

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Infectious diseases are caused by pathogenic microorganisms such as bacteria, viruses, fungi, protozoa or parasites. The Centers for Disease Control and Prevention (CDC) reported the consumption of 235 million doses of antibiotics in 2001[1]. Bacteria have increasingly developed resistance to these drugs[2]. The increasing number of infectious diseases of out-patients and in-patients caused by bacteria during 2005-2009 has been reported by Health service units, Ministry of Public Health, Thailand[3]. Infectious disease was secondary mortality rate in the world in 2008[4]. It caused serious health problems worldwide due to the antibiotic resistant strains. Guidelines for appropriate antibiotic use in order to reduce antibiotic resistance were launched in 2001 by the American College of Physicians (ACP) and the Centers for Disease Control and Prevention (CDC)[5]. However, the multiple drug

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resistance has been dramatically increased in Thailand. Thai traditional herbs have been widely consumed as food, drug and other purposes for a long time. It could be an alternative treatment for the infectious diseases. Many Thai traditional herbs have been studied intensively for scientific proofs of their potency and safety to use for treatments of many diseases[6-9].

Benchalokawichian remedy is one of Thai traditional formula used as an antipyretic drug, relieve fever, common cold and influenza. It has been registered as Herbal Medicinal Products AD 2006 from Thai Food and Drug Administration[10]. The remedy consists of five herbal roots from *Capparis micracantha* DC, *Tiliacora triandra* (Colebr) Diels, *Harrisonia perforata* (Blanco) Merr, *Clerodendrum petasites* S. Moore and *Ficus racemosa* L[11,12]. Their pharmacological effects such as antipyretic and antiseptic have been proved scientifically[13,14]. However, the antimicrobial activity of this remedy and each of its components has not been fully studied. Therefore, the determination of antimicrobial activity of the remedy and its components against pathogenic microorganisms was explored. It is hoped to be able to develop medicinal products and used as an alternative treatment of bacterial and fungal infections. As a consequence, it leads to reduce the excessive use of antibiotics and prevention of emerging resistant strains in future.

Material and Method

Extraction

Roots of five plants in Benchalokawichian remedy were purchased from Chacheongsao province in Thailand. The roots of plants included *C. micracantha, T. triandra, H. perforata, C. petasites* and *F. racemosa* (Table 1). The roots were dried with hot air oven at 50°C for 12 hours and crushed to rough powders. Dried roots were divided into two parts: part one was boiled with water so called decoction and another was extracted by maceration using 95% ethanol. Water extracts were dissolved in sterile water and ethanolic extracts were dissolved in dimethylsulfonide (DMSO) before use. The yields of extracts were measured in percentage (% yield) listed in Table 2.

Strains

The microorganisms tested were isolated from patients and identified at Microbiology Laboratory, Thammasat University Hospital and AFRIMS (Armed Forces Research Institute of Medical Sciences). The isolates include Gram-positive bacteria: *Stahylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), *Streptococcus pyrogenes*; Gram-negative bacteria: *Escherichia coli* (ETEC, EAEC, EPEC and EIEC), *Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella* Typhimurium, *Shigella* spp and *Acinetobacter buamannii*. Gentamicin and Amphotericin B are used as positive control against *S. aureus* ATCC 25922, *Bacillus subtilis* ATCC 6633, *E. coli* ATCC 25922 and *C. albicans* ATCC 90028.

Disc diffusion assay[15]

Bacterial strains were grown in broth culture medium. The inoculums were adjusted to 0.5 McFarland Standard. Each inoculum was seeded on Mueller Hinton agar (MHA) plate. Sterile paper discs of 6 mm in size containing ethanolic extracts (20 mg/disk) and water extracts (4 mg/disk) per disc and control disc containing Gentamicin (10 μg) and Amphotericin B (10 μg) was placed on the surface of the seeded plates and incubated at 35 $\pm$ 37°C for 16-18 h or 48 h for *C. albicans*. Inhibition zone was measured. The extracts shown inhibition zone of $\geq$ 8 mm was considered preliminary effective for minimum inhibitory concentration (MIC). Disc containing DMSO was used as negative control. The inhibition zone was calculated by Mean $\pm$ SD.

Results

The extracts obtained by maceration and decoction methods showed % yield ranged from 1.37 to 2.74% and 3.12 to 9.14%, respectively. The highest yield of extraction was water extract from *T. triandra* (9.14 %) as shown in Table 2.

Most of ethanolic extracts exhibited their effective antimicrobial activities against tested strains. Only *C. micracantha* water extract showed antimicrobial activity against many isolates tested in this study. The ethanolic extracts of *C. petasites* and *T. triandra* were able to inhibit most of tested strains. Water extract of *C. petasites* was not able to inhibit the growth of Gram negative bacteria more than Gram positive bacteria. In contrast the water extract of *T. triandra* are effective against MRSA and *C. albicans*. Only ethanolic and water extracts of *T. triandra* was effective against *C. albicans* with the inhibition zone of 15.7 $\pm$ 1.2 mm and 10.8 $\pm$ 0.3 mm, respectively (Table 3).

The antibacterial activity against *K. pneumoniae* was found only in the ethanolic extract of Benchalokawichian remedy and both ethanolic and water extracts of *C. micracantha*. The ethoanolic extract of *C. micracantha* failed to inhibit all *E. coli* strains, *S.*

Table 1. List of medicinal plants for antimicrobial activity assay

Botanical name	Thai name	Family
Capparis micracantha DC.	Ching-Chi	Caparidaceae
Tiliacora triandra (Colebr.) Diels	Ya-Nang	Menispermaceae
Harrisonia perforata(Blanco) Merr.	Kon-Tha	Simaroubaceae
Clerodendrum petasites S.Moore	Tao-Yai-Mom	Verbenaceae
Ficus racemosa L.	Ma-Deo-Uthumporn	Moraceae

Table 2. Extraction of Benchalokawichian remedy and its components by maceration with 95% ethanol and decoction methods

Sample	% yield of extract	
	Ethanolic extract	Water extract
Benchalokawichian remedy	2.04	5.49
C. micracantha	1.81	7.31
T. triandra	2.16	9.14
H. perforata	2.67	3.12
C. petasites	2.74	4.17
F. racemosa	1.37	3.94

sonnei, *P. aeruginosa* and *C. albicans*. The water extract of *C. micracantha* inhibited most pathogenic isolates except *S. flexneri*, *P. aeruginosa*, *S. aureus* ATCC 25923, MRSA, *B. subtilis* and *C. albicans*. The ethanolic extracts of *H. perforata* and *F. racemosa* inhibited the growth of few strains (Table 3). The susceptibility of gentamicin against bacteria showed that all except *S. dysenteriae*, *S. flexneri*, *A. baumannii*, *P. aeruginosa*, *K. pneumonia*, MRSA and *S. sonnei* were susceptible.

Discussion

The obtained results indicated that % yield of water extracts was higher than ethanolic extracts. However, ethanolic extracts had better antimicrobial activity against most tested strains than the water extracts except water extract of *C. micracantha*. The bioactive components in these plants has probably less polarity. The ethanolic extract of *C. micracantha* showed that it had less activity than the water extract with less concentration. It is suggested that the water extract of *C. micracantha* has a potential bioactive compound resulting in better activity against bacteria due to higher polarity in the water extract.

The water extracts of Benchalokawichian remedy, *C. petasites* and *F. racemosa* could inhibit none of the tested strains in the present study. It is possibly due to the low concentration of water extracts used per disk. However, the activity was detected in most of the ethanolic extracts of the remedy and each component.

The Benchalokawichian remedy showed no activity against all *E. coli* strains, *S. sonnei*, *S.* Typhimurium and *C. albicans* compared to individual component such as *T. triandra* and *C. petasites* which had most effective activity. It revealed that the activity was reduced resulting from the combination of the five herbal plants for Benchalokawichian remedy. The development of medicinal products has to be concerned for the proportion of each component in order to produce the products with the most effective activity.

The extract of *H. perforata* was previously reported that it showed anti-pyretic, anti-diarrhea and dysentery[16]. The obtained result in the present study indicated that the ethanolic extract of this component inhibited not only *S. dysenteriae* but also all tested Gram positive bacteria. In addition, the water extract could inhibit *A. baumanii* which commonly causes hospital-acquired infection.

F. racemosa extract showed some antimicrobial activity in the present study. However, the extract of *F. racemosa* from stem bark was previously reported that it possessed potential antioxidant[17]. Although the method of extraction and part of the plant were different from the present study, the previous report provided an important clue that this component

Table 3. Antimicrobial activities of ethanolic and water extracts of Benchalokawichian remedy and its components

Microorganism	Inhibition Zone in mm (Mean ± SD)													
	Ethanolic extracts[a]						Water extracts[b]				Control			
	BL	CM	T T	HP	CP	FR	BLw	CMw	TTw	HPw	CPw	FRw	Gentamicin	Amphotericin B
E . coli (ATCC 25922)	0	0	12.0 ± 1.0	0	9.8 ± 0.3	0	0	9.3 ± 1.1	0	0	0	0	19.6 ± 0.8	ND
ETEC	0	0	10.7 ± 0.6	0	9.0 ± 0.0	0	0	9.8 ± 0.3	0	0	0	0	17.8 ± 0.3	ND
EAEC	0	0	11.0 ± 1.0	0	10.3 ± 0.6	0	0	9.0 ± 0.0	0	0	0	0	16.2 ± 1.0	ND
EPEC	0	0	11.3 ± 0.4	0	9.7 ± 0.6	0	0	10.5 ± 0.5	0	0	0	0	21.0 ± 1.0	ND
EIEC	0	0	11.5 ± 0.5	0	9.7 ± 0.6	0	0	10.2 ± 0.3	0	0	0	0	15.8 ± 1.0	ND
S. boydii	7.4 ± 0.6	8.0 ± 1.0	10.3 ± 0.6	0	10.7 ± 1.7	0	0	13.0 ± 2.6	0	0	0	0	16.6 ± 0.6	ND
S. dysenteriae	8.0 ± 1.0	8.8 ± 0.3	8.5 ± 0.5	7.2 ± 0.8	11.0 ± 1.0	0	0	11.0 ± 1.0	0	7.8 ± 0.3	0	0	13.3 ± 1.1	ND
S. flexneri	7.8 ± 0.3	8.7 ± 1.2	12.0 ± 0.0	0	10.7 ± 1.7	0	0	0	0	0	0	0	12.5 ± 0.5	ND
S. sonnei	0	0	10.3 ± 0.6	0	9.0 ± 1.0	0	0	9.3 ± 0.6	0	0	0	0	ND	ND
*S.*Typhimurium	0	8.3 ± 0.6	11.3 ± 0.6	0	9.3 ± 0.6	9.0 ± 0.3	0	11.3 ± 0.6	0	0	0	0	17.3 ± 2.1	ND
A. buamannei	7.7 ± 0.8	6.8 ± 0.4	11.5 ± 0.5	6.2 ± 0.3	11.3 ± 0.6	8.5 ± 1.3	0	10.3 ± 1.5	0	11.0 ± 1.0	0	0	0	ND
P. aeruginosa	7.5 ± 0.5	0	0	0	6.3 ± 0.2	0	0	0	0	0	0	0	0	ND
K.pneumoniae	7.8 ± 0.3	9.0 ± 1.0	0	0	R	0	0	10.2 ± 0.3	0	0	0	0	0	ND
S. aureus (ATCC 25923)	8.7 ± 1.5	7.2 ± 0.7	9.3 ± 2.3	7.8 ± 1.0	9.7 ± 2.1	0	0	0	6.7 ± 0.3	0	0	0	19.3 ± 1.2	ND
MRSA	8.0 ± 1.0	7.1 ± 0.1	6.8 ± 0.3	7.4 ± 0.5	8.7 ± 0.6	0	0	0	7.3 ± 0.6	0	0	0	0	ND
S. pyogenes	13.0 ± 1.4	10.3 ± 1.2	16.3 ± 0.6	12.0 ± 2.6	11.3 ± 1.5	11.7 ± 2.1	0	14.7 ± 0.6	0	8.0 ± 0.0	0	0	16.0 ± 1.2	ND
B. subtilis (ATCC 6633)	10.3 ± 0.6	9.7 ± 0.6	15.3 ± 1.5	7.2 ± 0.8	11.3 ± 1.5	8.2 ± 0.8	0	0	0	11.7 ± 1.5	0	0	25 ± 0.0	ND
C. albicans (ATCC 10231)	0	0	15.7 ± 1.2	0	0	0	0	0	10.8 ± 0.3	0	0	0	ND	21.0 ± 1.0

[a] Ethanolic extracts; BL = Benchalokawichian remedy, CM =*C. micracantha,*, TT= *T. triandra*, HP =*H. perforata*, CP =*C. petasites*, FR = *F. racemosa*
[b] Water extracts; BLw = Benchalokawichian remedy, CMw =*C. micracantha,*, TTw= *T. triandra*, HPw =*H. perforata*, CPw =*C. petasites*, FRw = *F. racemosa*
ND = Not done

harbors not only antimicrobial activity but also another important role of bioactivity. It is recommended to further investigate for antimicrobial activity by using different techniques of extraction to gain more detailed information for future medicinal products.

The extracts of Benchalokawichian remedy and its components were suggested to be aware of using the remedy with nitrite containing food due to indirect mutagenicity[18]. This previous report provided useful information for safe consumption of the remedy. Hence, future development of medicinal products from the component must be concerned for safety before use.

Conclusion

This determination provides basic knowledge leading to develop an effective treatment of infectious diseases especially resistant bacterial strains. Determination of minimal inhibitory concentration and minimal microbicidal concentration will be further studied in order to obtain more detailed insightful knowledge. In addition, fractionation of herbal plants will be performed to gain pure compound and used for development of medicinal formula in future. It is in a hope to develop new formula with different proportion of the components for an alternative treatment of bacterial infections.

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Potential conflicts of interest

None.

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ฤทธิ์ต้านจุลชีพของสารสกัดจากตำรับเบญจโลกวิเชียรและสมุนไพรเดี่ยวในตำรับ

ศุภลักษณ์ เหนืออิสระ, สุมาลี คอนโด, อรุณพร อิฐรัตน์

***ภูมิหลัง**: โรคติดเชื้อเป็นสาเหตุสำคัญของปัญหาสุขภาพทั่วโลกจากสาเหตุการดื้อยาของเชื้อต่าง ๆ ตำรับยาไทยเช่น ตำรับเบญจโลกวิเชียรเป็นตำรับยาที่มีการใช้ลดอาการไข้ต่าง ๆ และแก้หวัด ตำรับยานี้เคยมีการนำมาศึกษาทดลองในการใช้ลดไข้ แก้ไข้ติดเชื้อ แต่กระนั้นตำรับยาเบญจโลกวิเชียรและสมุนไพรเดี่ยวในตำรับยังไม่เคยมีการศึกษาฤทธิ์ต้านเชื้อจุลชีพก่อโรคอย่างจริงจัง*

***วัตถุประสงค์**: ศึกษาฤทธิ์ต้านเชื้อจุลชีพก่อโรคของสารสกัดของตำรับยาเบญจโลกวิเชียรและสมุนไพรเดี่ยวในตำรับ*

***วัสดุและวิธีการ**: เชื้อแบคทีเรียที่ใช้ในการศึกษาครั้งนี้มาจาการคัดแยกเชื้อจากผู้ป่วยในโรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ ประเทศไทย สารสกัดที่ใช้ในการทดสอบฤทธิ์ต้านเชื้อจุลชีพ ได้แก่ สารสกัดด้วยเอทานอลและการสกัดด้วยน้ำ การทดสอบจะต้องมีการทำซ้ำทั้งหมดเป็น 3 ซ้ำ ผลการทดสอบบันทึกในรูปแบบการวัดค่าเส้นผ่าศูนย์กลาง การต้านเชื้อจุลชีพในรูปของค่าเฉลี่ยเลขคณิตและค่าความเบี่ยงเบนมาตรฐานที่ถูกคำนวณออกมา*

***ผลการศึกษา**: ผลการทดสอบเบื้องต้นของการต้านเชื้อจุลชีพของสารสกัดตำรับเบญจโลกวิเชียรและสมุนไพรเดี่ยวในตำรับ พบว่าสารสกัดมีความสามารถในการต้าน Candina albicans แบคทีเรียแกรมบวกและแกรมลบยกเว้นบางสายพันธุ์ ในส่วนของสารสกัดด้วยเอทานอลของรากย่านาง (Tiliacora triandra) และเท้ายายม่อม (Clerodendrum petasites) มีฤทธิ์ต้านเชื้อจุลชีพได้ครอบคลุมมากที่สุด นอกจากนี้สารสกัดด้วยน้ำของรากชิงชี่ (Capparis micracantha) ย่านาง และคนทา (Harrisonia perforata) สามารถยับยั้งเชื้อจุลชีพได้ สารสกัดของราก ย่านาง ทั้งสกัดด้วยเอทานอลและด้วยน้ำเป็นเพียงชนิดเดียวเท่านั้นจากตำรับเบญจโลกวิเชียรที่มีผลในการต้านเชื้อ C. albicans*

***สรุป**: การศึกษาทั้งหมดในครั้งนี้เป็นความรู้เบื้องต้นฤทธิ์ต้านเชื้อจุลชีพของตำรับเบญจโลกวิเชียรและสมุนไพรเดี่ยวในตำรับ สารสกัดด้วยเอทานอลของรากย่านาง และเท้ายายม่อมเป็นสารสกัดที่มีฤทธิ์ต้านเชื้อจุลชีพได้ครอบคลุมมากที่สุด สารสกัดย่านาง และเท้ายายม่อม จะเป็นสารสกัดสำคัญในการพัฒนาตำรับยาเพื่อการรักษาโรคติดเชื้อจากการแพทย์ทางเลือก และการศึกษาความเข้มข้นต่ำสุดที่สามารถต้านเชื้อจุลชีพ และฆ่าเชื้อจุลชีพจะทำการศึกษาเพิ่มเติมได้ เพื่อประโยชน์ในการพัฒนาไปเป็นผลิตภัณฑ์ในการรักษาการติดเชื้อแบคทีเรียและโรคติดเชื้ออื่นๆ ต่อไป*