Antimicrobial Activity of Thai Medicinal Preparation of Khampramong Temple Used for Cancer Treatment and Its Plant Components

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Background: *Thai medicinal preparation of Khampramong Temple has been used for cancer treatment more than ten years ago. It is composed of eleven herbs. Many anticancer drugs exhibited antimicrobial activity as antitumor antibiotics such as the anthracycline group [daunorubicin] and quinone group [mitomycin C].*
Objective: *To determine antimicrobial activity of Thai medicinal plants used to treat cancer patients of Khampramong Temple by disc diffusion and agar dilution methods.*
Material and Method: *The extraction procedure was maceration method using 95% ethanol and drying by evaporator. In the preliminary study, all extracts were evaluated for antimicrobial activity by disc diffusion method against two strains of Gram positive bacteria (Staphylococus aureus and Bacillus subtilis), one Gram negative bacteria (Escherichia coli) and one fungus (Candida albicans). The active plant extracts were diluted to determine the minimum inhibitory concentration (MIC) by agar dilution method.*
Results*: The preparation showed antimicrobial activity against S. aureus, B. subtilis and E. coli (MIC = 1.25, 0.625 and 5 mg/ml, respectively) but no inhibition against Candida albicans. Most extracts showed activity against B. subtilis and Rhinacanthus nasutus extract showed the highest antimicrobial activity (MIC = 0.156 mg/ml). Hydnophytum formicarum Jack, Tectona grandis L.f. and Salacia chinensis L. exhibited good antibacterial activity against S. aureus (MIC = 1.25, 0.15625 and 0.3125 mg/ml respectively).*
Conclusion*: These results supported the use of this preparation on chronic wound infection of cancer patients and the antimicrobial compounds of the preparation should be further studied to be used in cancer patients.*

Keywords: *Thai medicinal preparation, Khampramong Temple, Antimicrobial*

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Thai medicinal preparation of Khampramong Temple, Sakon Nakhorn Province in Thailand, has been used for cancer treatment more than ten years ago. The efficacy of this preparation in the treatment of cancer patients has been studied. There were 1,153 patients participated in the present study. The results showed significant improvement in both treatment effectiveness and mood state after the cancer patients were involved in all treatments session of this temple[1]. This preparation makes cancer patients get well and prolong their lives. However, there is no research on the biological activities of medicinal plants in this preparation which correlated with cancer and antimicrobial activity. Antimicrobial activity is necessary for infectious treatment in cancer patients. There are many anticancer drugs which exhibited antimicrobial activity such as antitumor antibiotics; the anthracycline group [daunorubicin] and quinone group [mitomycin C][2]. Thus, the present study on antimicrobial activity of this cancer preparation and their individual component will provide strong support for using this preparation in cancer patients.

The cancer preparation of Khampramong temple [KP] is composed of eleven plants including,

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Acanthus ebracteatus Vahl., *Angelica sinensis* (Oliv) Diels., *Artemisia vulgaris* Linn, *Hydnophytum formicarum* Jack., *Orthosiphon grandiflorus* Bold., *Polyalthia cerasoides* (Roxb) Benth ex Bedd ST, *Pygmaeopremna herbacea* (Roxb) Mold, *Rhinacanthus nasutus* (L) Kurz, *Salacia chinensis* L, *Smilax glabra* Roxb, *Tectona grandis* L.f. The antimicrobial activities of some plants such as *Hydnophytum formicarum, Rhinacanthus nasutus*, *Tectona grandis* and *Polyalthia cerasoides* have been reported[3-6]. However, there is no report on antimicrobial activity of this cancer preparation. In the present study, the authors aimed to determine the antibacterial activity of cancer preparation and its plant component extracts.

Material and Method

Plant materials

Thai medicinal preparation and its plant components used for cancer treatment in Khampramong Temple of Thailand were collected. The place of collection, plant parts, voucher specimen and traditional use are shown in Table 1. The voucher specimens were deposited at the Herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkla Province, Thailand.

Preparation of crude extract

Plant materials were collected from all regions of Thailand and Laos. They were washed, cut into small pieces and dried by oven 50°C. They were ground into powder and macerated in 95% ethanol for 3 days, three times, then filtered and dried using a rotary evaporator. The percentage yield for each crude extract was determined. All dried extracts were stored at -20°C until use.

Determination of antibacterial activity

Microorganism test

Bacterial strains used for testing include two Gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633), one Gram negative bacterium *Escherichia coli* (ATCC 25922) and one fungus *Candida albicans* (ATCC 90028). All bacteria were cultured in nutrient agar (NA) at 37°C for 24 hours, while fungus was cultured in Sabouraud dextrose agar (SDA) at 37°C for 48 hours.

Preparation of inocula

Isolated colonies of three bacteria and one fungus were cultured in Mueller-Hinton broth (MHB) at 37°C for 2-6 hours. Then suspension was adjusted to 0.5 McFarland standard turbidity.

Preparation of test disc

All ethanolic extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration 500 mg/ml. Then 10 µl of the prepared ethanolic extracts were applied to 6 mm sterile paper discs (5 mg/disc).

Disc diffusion method

The discs containing extracts were impregnated onto the seeded Mueller-Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for fungus. Plates were incubated at 37°C for 18 to 24 hours (bacteria) or 37°C for 48 hours (fungus), then activity was determined by measuring the inhibition zone (clear zone) around the disc. Gentamicin (1 µg) and amphotericin B (1 µg) were used as positive controls for bacteria and fungus, respectively[7].

Minimal inhibitory concentrations (MICs)

Minimal inhibitory concentrations (MICs) were determined using broth microdilution method from previous report with some modifications[8]. The inoculum was adjusted to 0.5 McFarland standards and diluted with sterile MHB at 1:200 to give a final concentration of 5×10^5 CFU/ml. Serial two-fold dilutions of each crude extract were prepared. The 50 µl of each concentration of crude extract solution and 50 µl of the inocula were added into 96-well microplates. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a plate shaker and incubated at 35-37°C for 16-18 hours.

MICs of the tested samples were detected after adding 10 µl of resazurin[9,10] (blue compound, 7-hydroxy-3H-phenoxazin-3-one 10-oxide) and incubated at 37°C for 2 h. The result was interpreted by the change of color of resazurin. MIC value is the lowest dilution of crude extract solution that can inhibit microorganism by generating blue color of resazurin. The assay was performed in triplicate. Positive control, negative control and viable control of microorganism were included.

Results and Discussion

Khampramong preparation [KP] and eleven plant extracts with percentage of yield and plant parts used in their studies were shown in Table 1. They were tested for their antimicrobial activity by disc diffusion test against three strains of bacteria (*S. aureus*, *B. subtilis* and *E. coli*), one strain of fungus (*C. albicans*)

Table 1. Botanical data and biological activity of plants

Scientific name : Family	Thai name	Place for specimen collection	Voucher specimen number	Part used	Biological activity	Thai Traditional use[11]
Acanthus ebracteatus Vahl: Acanthaceae	Ngueak-Pla-Moh	Bangkok	SKP 001 010501	all part	Anti-inflammatory[12]	Chronic ulcer healing
Angelica sinensis (Oliv) Diels: Umbelliferae	Goad-Cheang	India	SKP 199 011901	root	Antioxidant [13], Antiproliferative effect[14]	Antipyretic, Relief cough
Artemisia Vulgaris Linn: Compositae	Goad-Chu La Lum Pa	Bangkok	SKP 051 012201	all part	Antioxidant[15]	Antipyretic with rash, Relief cough
Hydnophytum formicarum Jack: Rubiaceae	Hua-Roi-Roo	Laos	SKP 165 080601	rhizome	Antimicrobial activity, Antioxidant[3]	Anticancer and nourish the heart
Orthosiphon grandiflorus Bold: Laminaceae	Yha Nuad Meaw	Bangkok	SKP 095 150701	all part	Antioxidant[16]	Diuretic, Kidney dysfunction
Polyalthia cerasoides (Roxb) Benth ex Bedd ST: Annonaceae	Phe Moab	Chonburi	SKP 011 160301	bark	Antimicrobial activity, Antioxidant[6]	Pain relief and to cure *tuberculosis*
Pygmaeopremna herbacea (Roxb) Mold: Verbenaceae	Hua Khao Yen Neua	Laos	SKP 202 160801	rhizome	Anti-inflammatory[17]	Healing lymphatic system
Rhinacanthus nasutus (L) Kurz: Acanthaceae	Thong Pan Chang	Bangkok	SKP 001 181401	all part	Antimicrobial activity[4], Cytotoxic activity[18]	To cure tinea versicolor and dermatophytosis
Salacia chinensis L: Celastraceae	Kampangchedchun	Songkla	SKP 044 190301	bark	Cytotoxic activity[19]	Pain relief and Antipyretic
Smilax glabra Roxb: Smilacaceae	Hua Khao Yen Tai	Laos	SKP 179 190701	rhizome	Cytotoxic activity[19,20], Anti-HIV-1 protease and HIV-1 Integrase[21] Immunomodulatory activity[22]	Healing lymphatic system
Tectona grandis L.f: Labiatae	Sak-Hin	Chiang-mai	SKP 095 200701	stem	Antimicrobial activity[5]	Nourish blood and elements of body, Antipyretic

Table 2. % yield and antimicrobial activity of ethanolic crude extracts expressed as diameter of inhibition zones (mm.) by disc diffusion method and minimal inhibitory concentration (MIC, mg/ml)

Ethanolic extracts	% yield	*Staphylococus aureus* (ATCC 25923)		*Bacillus subtilis* (ATCC 6633)		*Escherichia coli* (ATCC 25922)		*Candida albicans* (ATCC 90028)	
		Disc (mm)	MIC (mg/ml)	Disc (mm)	MIC (mg/ml)	Disc (mm)	MIC (mg/ml)	Disc (mm)	MIC (mg/ml)
Preparation of Khampramong	6.88	11.50 ± 0.5	1.25	12.33 ± 1.26	0.625	10.17 ± 1.26	>5	NI	NI
Acanthus ebracteatus Vahl.	5.60	NI	NI	10.33 ± 0.58	>5	9.67 ± 0.29	>5	NI	NI
Angelica sinensis (Oliv) Diels	7.24	NI	NI	10.67 ± 0.58	2.5	NI	NI	NI	NI
Artemisia vulgaris Linn.	3.64	NI	NI	11.67 ± 0.58	1.25	9.83 ± 0.76	>5	NI	NI
Hydnophytum formicarum Jack	8.32	14.17 ± 0.29	0.15625	12.5 ± 0.5	0.625	10.33 ± 0.57	2.5	NI	NI
Orthosiphon grandiflorus Bold	4.47	NI	NI	9.67 ± 0.289	>5	9.67 ± 0.577	>5	NI	NI
Polyalthia cerasoides (Roxb) Benth ex Bedd.ST	5.14	9.33 ± 0.58	>5	10 ± 1.00	1.25	9.33 ± 1.53	>5	NI	NI
Pygmaeopremna herbacea (Roxb) Mold	4.26	9.33 ± 0.29	>5	9.83 ± 0.76	>5	NI	NI	NI	NI
Rhinacanthus nasutus (L) Kurz	1.28	7 ± 0	2.5	13.33 ± 1.26	0.156	11 ± 2.64	>5	NI	NI
Salacia chinensis L.	4.57	9.17 ± 0.28	0.3125	NI	NI	NI	NI	NI	NI
Smilax glabra Roxb	8.86	8.17 ± 0.29	>5	9.17 ± 0.29	>5	NI	NI	NI	NI
Tectona grandis Lf	2.84	14.17 ± 0.76	0.3125	13 ± 0	0.625	NI	NI	NI	NI
Gentamycin (positive control)	-	25	0.5 mcg/ml	33	0.125 mcg/ml	23	0.5 mcg/ml	ND	ND
Amphotericin B (positive control)	-	ND	ND	ND	ND	ND	ND	21	1 mcg/ml

n = 3, NI = no inhibition, ND = not done

(Table 2). KP showed antibacterial activity against two Gram positive bacteria (MIC = 0.625-1.25 mg/ml), but less active against Gram negative bacteria (MIC > 5 mg/ml) and no antifungal activity against *C. albicans*. However, there is no plant extract which are components of KP showed activity against *C. albicans*. KP inhibited *B. subtilis* better than *S. aureus* and *E. coli* [MIC = 0.625, 1.25 and > 5 mg/ml, respectively]. Among antimicrobial activity of plant ingredients of KP, ten out of eleven plant ingredients were active against *B. subtilis* especially *Rhinacanthus nasutus*, *Tectona grandis* and *Hydnophytum formicarum* (MIC = 0.156, 0.625 and 0.625 mg/ml, respectively). Moreover, *Hydnophytum formicarum* and *Tectona grandis* also showed the highest antibacterial activity against *S. aureus* (MIC = 0.156 and 0.313 mg/ml, respectively). These results support the previous report which found that *Tectona grandis* bark showed antibacterial against methicillin resistant *Staphylococcus aureus* (MRSA)[5]. However, there is no report of antimicrobial activity from stem of this plant. There is only one report of *Hydnophytum formicarum* which hexane and ethyl acetate extracts showed antimicrobial against Gram positive and Gram negative bacteria with MIC value of 0.256 mg/ml[3]. The present study found that the ethanolic extract of *Hydnophytum formicarum* showed higher antibacterial against *S. aureus* than the previous report (MIC = 0.156 and 0.256 mg/ml, respectively). For *Rhinacanthus nausthus*, it has been reported that Rhinacanthin-rich Rhinacanthus extract showed antimicrobial against *S. aureus* but less active against *Candida albicans*[4]. These results can support the use of this preparation as antimicrobial drug in cancer patients

Conclusion

The Khampramomg preparation which was used for cancer treatment exhibited antimicrobial activity. The plant ingredient which showed the highest antimicrobial activity is *Hydnophytum formicarum* Jack. Thus, these results should support the use of this anticancer preparation of Khampramong temple to treat chronic wound infection of cancer patients. There should be further studied on the antimicrobial compounds of this preparation and its plant ingredients.

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Potential conflicts of interest

None.

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การศึกษาฤทธิ์ต้านจุลินทรีย์ของตำรับยาสมุนไพรวัดคำประมงที่ใช้รักษามะเร็งและสารสกัดสมุนไพรในตำรับ

อรมณี ประจวบจินดา, สุมาลี ปานทอง, อรุณพร อิฐรัตน์

ภูมิหลัง: *ตำรับยาสมุนไพรไทยของวัดคำประมง เป็นตำรับยาที่ใช้ในการรักษาโรคมะเร็งนานมากกว่า 10 ปี ตำรับยาประกอบด้วยสมุนไพร 11 ชนิด ซึ่งยามะเร็งส่วนมากสามารถยับยั้งเชื้อแบคทีเรียได้ ตัวอย่างเช่น ยากลุ่ม anthracycline [daunorubicin] และ ยากลุ่ม quinone [mitomycin C]*

วัตถุประสงค์: *เพื่อศึกษาฤทธิ์ในการต้านจุลินทรีย์ของตำรับยาวัดคำประมงโดยวิธี disc diffusion และ agar dilution*

วัสดุและวิธีการ: *สมุนไพรตำรับ และสมุนไพรเดี่ยวสกัดด้วย 95% ethanol และทำให้แห้งด้วย evaporator ในการศึกษาขั้นต้นทดสอบฤทธิ์การต้านแบคทีเรียด้วยวิธี disc diffusion ทดสอบกับเชื้อแบคทีเรียแกรมบวก 2 ชนิด (Staphylococus aureus และ Bacillus subtilis) เชื้อแบคทีเรียแกรมลบ 1 ชนิด (Escherichia coli) และเชื้อรา 1 ชนิด (Candida albicans) จากนั้นนำสารสกัดสมุนไพรที่มีฤทธิ์ต้านจุลินทรีย์มาทดสอบหาค่า minimum inhibitory concentration (MIC) ด้วยวิธี Agar Dilution*

ผลการศึกษา: *ตำรับยามีฤทธิ์ในการต้านเชื้อ S. aureus, B. subtilis และ E. coli (MIC เท่ากับ 1.25, 0.625 และ 5 mg/ml ตามลำดับ) แต่ไม่มีฤทธิ์ต้านเชื้อ C. albicans สารสกัดพืชสมุนไพรเกือบทุกตัวมีฤทธิ์ต้านเชื้อ B. subtilis ซึ่งสารสกัดทองพันชั่งมีฤทธิ์มากที่สุด (MIC เท่ากับ 0.156 mg/ml) สารสกัดจากหัวร้อยรู สัก กำแพงเจ็ดชั้นมีฤทธิ์ดีในการต้านเชื้อ S. aureus ซึ่งมีค่า MIC เท่ากับ 1.25, 0.15625 และ 0.3125 mg/ml ตามลำดับ*

สรุป: *ผลงานวิจัยสามารถนำตำรับยาสมุนไพรรักษามะเร็งมาใช้ในการรักษาแผลติดเชื้อของผู้ป่วยโรคมะเร็ง และตำรับนี้ควรนำไปศึกษาสารสำคัญที่มีต้านเชื้อแบคทีเรียเพื่อใช้สำหรับการรักษาอาการติดเชื้อในผู้ป่วยมะเร็งต่อไป*