ORIGINAL ARTICLE

Antimicrobial activity of oil from the root of *Cinnamomum porrectum*

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Abstract

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The steam-distilled oil from the root of *Cinnamomum porrectum* was tested for its antimicrobial activity against human pathogens including bacteria, yeasts and dermatophytes. It exhibited strongest activity against *Streptococcus mutans* (MIC 0.01 mg/ml) followed by *Candida albicans* and dermatophytes (0.5-1.0 mg/ml), *Bacillus subtilis* (2 mg/ml), and susceptible strains of *Staphylococcus aureus* (4-16 mg/ml). It showed moderate activity against *Cryptococcus neoformans* (MIC 16-64 mg/ml) but no activity against *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus*. These data indicate that *C. porrectum* oil has antibacterial and antifungal activity.

Key words : *Cinnamomum porrectum*, oil, antibacterial, antifungal

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ฤทธิ์ต้านจุลินทรีย์ของน้ำมันจากรากเทพธาโร (*Cinnamomum porrectum*)

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น้ำมันเทพธาโร (*Cinnamomum porrectum*) ที่ได้จากการกลั่นส่วนราก เมื่อนำมาทดสอบฤทธิ์ต้านจุลินทรีย์ต่อเชื้อก่อโรคในคน ทั้งแบคทีเรีย ยีสต์ และเชื้อราก่อโรคกลาก พบว่ามีฤทธิ์ดีที่สุดในการต้านเชื้อ *Streptococcus mutans* (MIC 0.01 มก./มล.) รองลงมาได้แก่ *Candida albicans* และราก่อโรคกลาก (0.5-1.0 มก./มล.) *Bacillus subtilis* (2 มก./มล.) และ *Staphylococcus aureus* สายพันธุ์ที่ไวต่อยา (4-16 มก./มล.) น้ำมันเทพธาโรมีฤทธิ์ปานกลางในการยับยั้ง *Cryptococcus neoformans* (MIC 16-64 มก./มล.) แต่ไม่ยับยั้ง *Pseudomonas aeruginosa* และ *S. aureus* ที่ดื้อยาเมธิซิลลิน ผลการทดลองแสดงให้เห็นว่าน้ำมันเทพธาโรมีฤทธิ์ต้านแบคทีเรียและต้านรา

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The bark and leaves of *Cinnamomum* spp. are commonly used as spices in the home kitchen and their distilled essential oils or synthetic analogs are used as flavoring agents in the food and beverage industry (Jham *et al*., 2005). *Cinnamomum porrectum* (Roxb.) Kosterm (family Lauraceae) or Theptaro is the symbolic tree of Phang Nga province. It is endemic in the south of Thailand including Krabi, Phang Nga, Pattalung and Trang provinces. Its bark and leaves have been used in Thai traditional medicine as an anti-flatulent (Wuttithammawej, 1997). Recent scientific studies have shown that essential oils of *Cinnamomum* plants; *C. cassia*, *C. camphora*, *C. iners*, *C. osmophloem* and *C. zeylanicum* have antimicrobial activity (Mishra *et al*., 1991; Tiwari and Tiwari, 1997; Samy *et al*., 1998; De *et al*., 1999; Ferhout *et al*., 1999; Mastura *et al*., 1999; Chang *et al*., 2001). We have therefore investigated the antimicrobial activity of oil from the roots of *C. porrectum* against human pathogenic bacteria and fungi.

Materials and methods

Preparation of oil

Dried root of *C. porrectum* (7 kg) was chopped into small pieces and soaked in water (30 l) for 3 days, then steam-distilled for 9 h to obtain the oil (128 ml). The yield of the oil was 1.83% v/w. The major component of the oil (95.5-98.0%) as confirmed by GCMS is safrole (Tunsuwan *et al*., 2006).

Microorganisms and media

The microorganisms used in this study were pathogenic bacteria, (*Staphylococcus aureus* ATCC25923, *S. aureus* ATCC6538, methicillin-resistant *S. aureus* (MRSA DMST20654 and a clinical isolate MRSA SH-05-1), *Bacillus subtilis*, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853, and *Streptococcus mutans* DMST18777); yeasts (*Cryptococcus neoformans* ATCC90012, ATCC90013, and a clinical isolate SH-MU-1, *Candida albicans* ATCC20028, NCPF3153, TISTR5779 and a clinical isolate SH-01); dermatophytes (clinical isolates of *Microsporum gypseum* SH-MU-4, *Trichophyton rubrum* SH-MU-1, and *Trichophyton mentagrophytes* SH-MU-2).

Bacteria were grown on Mueller Hinton agar, (MHA, Difco, USA) at 35°C except for *S. mutans* that was incubated in a candle jar at 35°C. Yeasts and dermatophytes were cultured and maintained on Sabouraud's dextrose agar (SDA, Difco, USA) at 25 and 35°C.

Antimicrobial screening

The disk diffusion method (Lorian, 1996) was used to screen the antimicrobial activity of *C. porreetum* oil. Sterile 6-mm diameter paper disks (Schleicher and Schuell, Germany) were impregnated with 2.5, 5 and 10 µl of concentrated oil and 10 µl of oil was diluted in DMSO (100 mg/ml) and placed on the inoculated MHA and SDA agars. Commercially available antibiotic disks of vancomycin (30 µg) and tetracycline (30 µg) were used as standard antibacterials and amphotericin B (20 µg) and ketoconazole (10 µg) as antifungal agents. Disks impregnated with 10 µl of DMSO were used as negative controls. Plates were incubated at 35°C for 18 h for bacteria and 48 h for yeasts. Plates with dermatophytes were incubated at 30°C for 4 days and the inhibition zone diameter was measured. The tests were performed in duplicate and the results were averaged.

Determination of minimum inhibitory concentrations (MICs)

MICs were performed by the macrodilution and microdilution methods according to NCCLS (2004) against bacteria and yeasts, and a modification of NCCLS 38-A (NCCLS, 2002) against dermatophytes. Serial 2-fold dilutions of the oil were mixed with MHB or SDB in the ratio of 1:100 to give the final concentrations of 0.25-128 mg/ml. An equal volume of inoculum suspension ($1x10^6$ cfu/ml) was then added to each tube or well. Tubes and microplates were incubated at 35°C for 18 h for bacteria and 48 h for yeasts. Dermatophytes were incubated at 30°C for 7-15 days. MICs were recorded by reading the lowest concentration that inhibited visible growth.

Results

The antimicrobial activities of the oil from *C. porrectum* and control drugs are shown in Table 1. Diluted and undiluted oil solutions showed inhibitory activity against all microorganisms tested except *P. aeruginosa*. Their inhibition zones ranged from 7.2 -90 mm (Figure 1) and MIC values 0.01->128 mg/ml. *C. neoformans* and the dermatophytes were the most susceptible strains to the oil when tested by the disk diffusion method. Ten

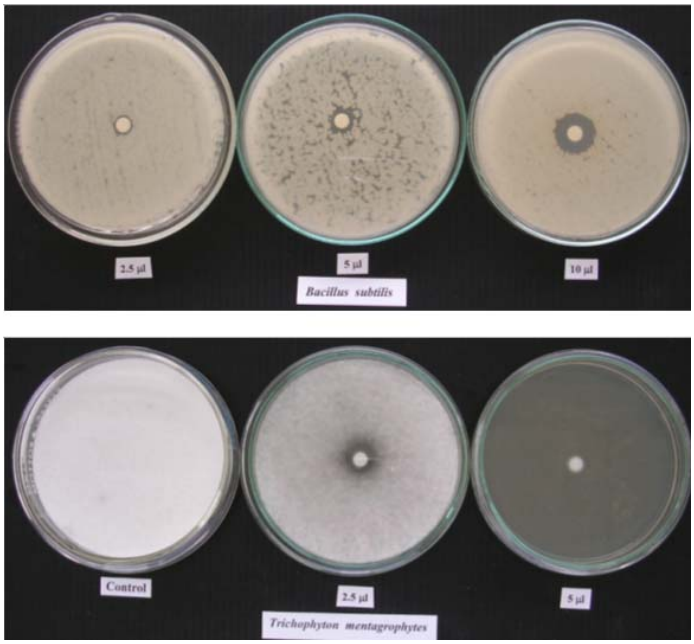

Figure 1. Disk diffusion assays of the *C. porrectum oil* against *Bacillus subtilis* and *Trichophyton mentagrophytes*

Table 1. Antimicrobial activities of *C. porrectum* oil.

Test microorganisms	*C. porrectum* oil					Control drug		
	Inhibition zone (mm)				MIC	Drug	IZ	MIC
	Dil. oil*	----- Undiluted oil -----			(mg/ml)		(mm)	(µg/ml)
	10.0 µl	2.5 µl	5.0 µl	10.0 µl				
Bacteria								
S. aureus ATCC25923	7.2	7.2	10.8	13.6	4.0	Vancomycin	19.5	0.5
S. aureus ATCC6538	NZ	8.7	13.0	13.2	16.0	Vancomycin	19.5	0.5
MRSA DMST20654	8.0	8.0	10.2	13.5	>128	Vancomycin	19.5	1
MRSA SH-05-1	NZ	NZ	NZ	9.0	>128	Vancomycin	19.5	1
B. subtilis	NZ	8.0	10.2	13.5	4.0	Vancomycin	ND	0.5
S. mutans DMST18777	8.2	ND	ND	34.5	0.01	Vancomycin	39.5	0.5
E. coli ATCC25922	NZ	7.5	9.6	10.5	2.0	Tetracycline	20.0	2.0
P. aeruginosa ATCC27853	NZ	NZ	NZ	NZ	ND	Tetracycline	19.5	4.0
Yeasts								
C. albicans ATCC20028	7.6	ND	8.9	70.9	1.0	Amphotericin B	15.0	1.0
C. albicans NCPF3153	NZ	ND	7.8	18.3	0.5	Amphotericin B	15.0	1.0
C. albicans TISTR5779	NZ	ND	8.0	52.5	1.0	Amphotericin B	15.0	0.5
C. albicans SH-01	NZ	ND	8.1	34.4	1.0	Amphotericin B	14.6	0.5
C. neoformans ATCC90012	10.2	ND	ND	90.0	64.0	Amphotericin B	15.0	4.0
C. neoformans ATCC90013	10.5	ND	ND	90.0	16.0	Amphotericin B	15.0	4.0
C. neoformans SH-MU-1	10.1	ND	ND	90.0	32.0	Amphotericin B	15.5	4.0
Dermatophytes								
T. rubrum SH-MU-2	NZ	11.2	90.0	90.0	0.5	Ketoconazole	ND	0.5
T. mentagrophytes SH-MU-3	NZ	11.5	90.0	90.0	1.0	Ketoconazole	ND	8.0
M. gypseum SH-MU-4	NZ	10.5	90.0	90.0	1.0	Ketoconazole	ND	8.0

***100 mg/ml; Diameter of Petri dish, 90.0 mm**
Standard disk content: vancomycin (30 µg), tetracycline (30 µg), amphotericin B (20 µg)
NZ, no zone; ND, not done

microliters of undiluted oil completely inhibited their growth (90 mm zone). Significant antifungal effects, expressed as MICs of the oil against the three genera of dermatophytes, were seen at the low concentration of 0.5-1.0 mg/ml, while the MICs against *C. neoformans* strains were in the range of 16-64 mg/ml. Although the inhibition zones against *C. albicans* (18.3-70.9 mm) were smaller than those observed with *C. neoformans* when tested with ten µl of undiluted oil, its MICs were much lower (0.5-1.0 mg/ml compared with > 16 mg/ml). Among the bacteria tested, *S. mutans* was the most susceptible strain with the lowest MIC of 0.01 mg/ml, followed by *E. coli* (MIC 2.0 mg/ml), *S. aureus* ATCC25923 and *B. subtilis* (MIC 4.0 mg/ml), and *S. aureus* ATCC6538 (MIC 16 mg/ml). The oil exhibited the lowest inhibitory activity against MRSA.

Discussion

The results obtained from this study showed that oil of *C. porrectum* has strong antibacterial and antifungal activities. The major component (85.5-97%) of *C. porrectum* root distilled oil is safrole and this is also the main component in sassafras root bark. This is used as a popular ingredient in soaps, perfumes, foods and drinks. It was also once used as an ingredient for "root beer" in the United States. It has been used as a topical antiseptic and pediculicide, but it may also be carcinogenic (Rocha and Ming, 1999). Inform-

ation obtained through interviewing traditional healers revealed that *C. porrectum* oil was used by local people to relieve muscle ache and toothache. We found that *C. porrectum* oil had strong antibacterial activity against *S. mutans*. *S. mutans* is considered to be one of the most important cariogenic species of human microbial flora (Loesch, 1986). The suppression of *S. mutans* by *C. porrectum* oil, is of clinical importance. Moreover, *C. porrectum* oil has antifungal activity against dermatophytes, *C. albicans*, and *C. neoformans*. Dermatophytes generally cause infections of the skin, scalp and nail. *C. albicans* and *C. neoformans* are causative agents of candidiasis and cryptococcosis, the top five opportunistic infections in AIDS patients in Thailand (MOPH, 2005). *C. porrectum* oil is a strong candidate for treatment of such fungal infections. However, Chen *et al*. (1999) reported that safrole from betel quid forms stable DNA-adducts in human oral tissue following betel quid chewing, which may contribute to oral carcinogenesis. Therefore, applying oil containing safrole to human tissues should be avoided.

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