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Anti-*staphylococcus aureus* Activity of *Phellinus igniarius* Aqueous Extract

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Abstract: In this study, anti-bacterial activity of aqueous extract of *P. igniarius* against selected gram positive and gram negative bacteria using agar diffusion, broth macrodilution and agar dilution methods were investigated. The agar diffusion method revealed that *P. igniarius* aqueous extract showed inhibition zone against only *S. aureus* ATCC 24923. The susceptibility test against MSSA and MRSA revealed that *P. igniarius* aqueous extract showed inhibition zone against all of MSSA strains but cannot inhibit growth of MRSA. The broth macro dilution and agar dilution methods reveal MIC and MBC of *P. igniarius* against *S. aureus* ATCC 25923 was 1.25 and 2.5 g L^{-1}, respectively while MICs and MBCs against MSSA were 1.25-2.5 g L^{-1} and 0.25-0.5 g L^{-1}, respectively. The aqueous extract of *P. igniarius* showed inhibitory effect on growth of MSSA but not MRSA.

Key words: *P. igniarius*, *P. igniarius* aqueous extract, fungus extract, MSSA, MRSA

INTRODUCTION

Phellinus igniarius or *Phellinus igiarius* is the wooden fungus belong to family Hymenochaetaceae. It has been reported as the Eskimos folklore (Blanchette *et al*., 2002). The fungus in Phellinus group has been intense study about it immunological and anticancer activity. *P. igniarius* has been reported antiproliferative, antimetastatic (Tuzz-Ying Lin *et al*., 2008) and anticacinogenic effect (Shon and Kyungo-Soo, 2001).

Furthermore, it also has been reported vasorelaxation (Kang *et al*., 2006), antioxidant and cytotoxic (Wang *et al*., 2005a, b), inhibition of cytochrome P450 isozyme and ornithine decarboxylase activity (Shon and Kyungo-Soo, 2004). However, *P. igniarius* never been reported it antibacterial activity. This is the first reported of *P. igniarius* antibacterial activity.

MATERIALS AND METHODS

Fungi sample and extraction: The *P. igniarius* was identified by Natural medicinal mushroom museum, Mahasarakham University, Thailand. The fungus was collected on July 2008 from forest in Cambodia. The 10 g of fungi were grinded and boiled in 1 L of water for 15 min and filtrated. The filtrates were spray dried. The yield of spray dried extract was 1-2.5% of dried weight of fungi. In this study used one batch of extraction throughout the studies.

Tested microorganism: All microorganisms were obtained from Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. Eight bacteria, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtillis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917 were used as gram positive bacteria *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Klebsiella pneumonia* ATCC 10031 were used as gram negative bacteria.

Twelve clinical isolates of *S. aureus* were included in this study. The tested isolates were divided into 2 groups, 6 of each, according to susceptibility to methicillin where, MRSA referred to methicillin-resistant strains and MSSA referred to methicillin-susceptible strains.

Antimicrobial assay

Agar diffusion susceptibility test: Susceptibility determinations were made as described in the standard guideline technique (Lorian, 1996). All test bacteria were cultured overnight on Tryptic Soy Agar (TSA) slant at 37 °C. Bacteria were washed from surface agar slant with sterile normal saline solution (0.9% NaCl) then adjusted to match turbidity of standard Mcfarland No. 0.5 before used as starter solution. Twenty milliliter of Mueller Hinton Agar (MHA) was putted in cultivation plates and swabbed starter solution on agar surface by using swab cotton.

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Phellinus igniarius extract was dissolved in sterile water and put in sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) were placed on the inoculated agar surface. The various concentrations of *P. igniarius* extract solution were filled in the cylinders (300 μL cylinder^{-1}). After pre-diffusion at room temperature for 1 h, the plates were incubated at 37°C for 19 h. The normal saline solution filled in the cylinder was used as control and 10 mg L^{-1} gentamicin sulphate solution or 1 μg mL^{-1} oxacillin were used as standard in same cultivation plate.

MICs and MBCs determination using agar dilution and broth macro dilution methods: MICs of crude water extract of *P. igniarius* solution were determined by agar dilution method (Merck) (Lorian, 1996) while MBCs were determined by broth macro-dilution method were (Lorian, 1996) and reference antibiotics oxacillin (Sigma Chemical Co., St. Louis, USA). Inoculates were prepared in the same medium at density adjusted to 0.5 McFarland turbidity standard (10^8 colony-forming units (Cfu mL^{-1}) and two fold dilution for the broth macro-dilution procedure. The inoculated tube were incubated at 37°C and the MICs were recorded after 24 h of incubation. The MIC was defined as the lowest concentration of *P. igniarius* solution or oxacillin at which the microorganism tested did not showed visible growth while MBC was defined as the minimum bactericidal concentration with negative subcultures on agar medium. Values were means of triplicate.

RESULTS AND DISCUSSION

Phellinus sp. were reputed as oriental and Russian folklore especially *P. igniarius* has been record to use as remedy in Russian (Blanchette *et al*., 2002). Among of *Phellinus* sp., *Phellinus linteus* is the most studied of it biological activities. *P. linteus* has been investigated it anti-*Staphylococcus aureus* (Hur *et al*., 2004), the aqueous extract of its show anti-methicillin resistant *S. aureus*. However *P. igniarius* has never been investigated it antibacterial activity.

The used of *P. igniarius* has been recorded in Russian and Alaskans or Eskimos (Branchette *et al*., 2002). Until nowadays Alaskans still used *P. igniarius* as the remedy in elder to 5 years old person even in pregnancy. In oriental, *P. igniarius* has been interesting for treatment as immuno modulator or inhibition of cancer cells. It has been reported antiproliferative and antimetastatic effects of human hepatocacinoma (Tuzz-Ying Lin *et al*., 2008) and antimutagenicity in bacterial model (*Salmonella typhimurium*) (Shon and Kyung-Soo, 2001). It is also showed vasorelaxation effect (Kang *et al*., 2006) and inhibitory effect on cytochrome P450 isozymes and ornithine decarboxylase activities (Shon and Kyung-Soo, 2004).

In this study antibacterial activity of aqueous extract of *P. igniarius* against selected gram positive and gram negative bacteria using agar diffusion, broth macrodilution and agar dilution methods were investigated. The agar diffusion method revealed that *P. igniarius* aqueous extract showed inhibition zone against only *S. aureus* ATCC 24923 (Table 1). Thus it was test inhibitory effect against 12 clinical isolates *S. aureus*. The 1 μg oxicillin disc was tested against clinical isolates of *S. aureus*. The *S. aureus* which showed no inhibition zone with 1 μg oxacillin were grouped as MRSA (methicillin resistant *S. aureus*) while *S. aureus* which give inhibition zone diameter more than 9 mm, were grouped as MSSA (methicillin sensitive *S. aureus*). The susceptibility test against MSSA and MRSA revealed that *P. igniarius* aqueous extract showed inhibition zone against all of MSSA strains but cannot inhibit growth of MRSA (Table 2). The broth macro dilution and agar dilution methods reveal MIC and MBC of *P. igniarius* against *S. aureus* ATCC 25923 was 1.25 and 2.5 g L^{-1}, respectively while MIC and MBC against MSSA were 1.25-2.5 g L^{-1} and 0.25-0.5 g L^{-1}, respectively (Table 3). This is the first reported antimicrobial activity of *P. igniarius*.

Table 1: Inhibition zone diameters of *P. igniarius* aqueous extract solution against various bacteria

		Inhibition zone diameter (mm)			
Bacteria	Gram	*P. igniarius* (100 mg mL^{-1})	*P. igniarius* (50 mg mL^{-1})	*P. igniarius* (25 mg mL^{-1})	Gentamicin sulphate (10 μg mL^{-1})
S. aureus ATCC 25923	+	9.0±0.70	8.0±0.49	nz	21.0±2.65
S. epidermidis ATCC 12228	+	nz	nz	nz	20.7±1.52
M. luteus ATCC 9341	+	nz	nz	nz	21.2±2.51
B. subtillis ATCC 6633	+	nz	nz	nz	19.3±0.57
L. plantarum ATCC 14917	+	nz	nz	nz	23.3±1.0
E. coli ATCC 25922	-	nz	nz	nz	21.6±0.57
S. typhimurium ATCC 14028	-	nz	nz	nz	18.0±1.0
K. pneumoniae ATCC 10031	-	nz	nz	nz	17.6±1.52

Data are Mean±SD (n = 3); nz = No inhibition zone

Table 2: Inhibition zone diameters of *P. igniarius* aqueous extract solution against MSSA and MRSA

	Clear zone diameter (mm)		
Bacteria	*P. igniarius* (100 mg mL^{-1})	*P. igniarius* (50 mg mL^{-1})	Oxacillin (1 μg)
S. aureus ATCC 25923	9.0±0.36	8.2±0.36	22.1±1.27
MSSA1	14.5±0.34	10.2±0.12	20.2±0.71
MSSA2	11.0±1.40	nz	19.3±2.14
MSSA3	16.5±3.12	11.5±2.13	22.31±1.45
MSSA4	13.5±0.74	nz	18.3±0.71
MSSA5	17.5±0.74	13.5±1.42	21.5±2.12
MSSA6	17.0±0.13	15.0±0.73	20.2±2.14
MRSA1	nz	nz	nz
MRSA2	nz	nz	nz
MRSA3	nz	nz	nz
MRSA4	nz	nz	nz
MRSA5	nz	nz	nz
MRSA6	nz	nz	nz

Data are Mean±SD (n = 3); nz = No inhibition zone

Table 3: The MICs and MBCs of *P. igniarius* aqueous extract against MSSA and MRSA

	P. igniarius		Oxacillin	
Bacteria	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (mg mL^{-1})	MBC (mg mL^{-1})
S. aureus ATCC 25923	1.25	0.25	0.25	0.5
MSSA1	2.50	0.50	0.25	0.5
MSSA2	1.25	0.25	0.25	2.0
MSSA3	1.25	0.25	0.50	1.0
MSSA4	2.50	0.50	0.25	0.5
MSSA5	1.25	0.25	0.25	0.5
MSSA6	1.25	0.25	0.25	0.5

In conclusion the *P. igniarius* aqueous extract may consist of antimicrobial active compound that can inhibit growth of *S. aureus* which is involving skin infection. However, *P. igniarius* cannot inhibit growth of MRSA which causing severe infection diseases. This study provides additional information the use of *P. igniarius* as remedy.

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