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# Antibacterial Activity of *Phellinus gilvus* Aqueous Extract

C. Sittiwet and D. Puangpronpitag
Biomedical Research Unit, Department of Chemistry, Faculty of Science,
Mahasarakham University, 44150, Thailand

**Abstract:** Anti-bacterial activity of *P. gilvus* aqueous extract was screened using agar diffusion method. The aqueous extract of *P. gilvus* showed inhibition zone against 3 out of 8 tested bacteria (*L. plantarum* ATCC 14917, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 10031). The MICs against *L. plantarum* ACC 14917, *K. pneumonia* ATCC 10031 and *E. coli* ATCC 25922 were 45, 90 and 360 mg $L^{-1}$ respectively while MBC were 90, 180 and 720 mg $L^{-1}$, respectively. The aqueous extract from *P. gilvus* showed good inhibitory effect against gram negative bacteria with low MIC and MBC.

**Key words:** *P. gilvus*, *P. gilvus* aqueous extract, *in vitro* antibacterial activity

## INTRODUCTION

The *Phellinus* species have been intense investigated about its biological activities. Among of these group *Phellinus linteus* is the most attention and have many reported about it biological activities. Mushroom in this group has been use for immunological remedies in Korea and Russia.

*Phellinus gilvus* is the mushroom belong to family Hymenochaetaceae. It has bracket or kidney shape with reddish brown cap with yellow margin. The extract from *P. gilvus* has been reported inhibitory effect on pulmonary inflammation (Jang *et al*., 2004). The polysaccharide from *P. gilvus* showed inhibitory effect on melanoma in rats (Bae *et al*., 2005a). Interestingly, polysaccharide isolated from *P. gilvus* showed wound healing effect in normal (Bae *et al*., 2005c) and diabetic rats (Bae *et al*., 2005b). Earlier reported the water extract of *Phellinus linteus* showed no inhibitory effect on bacteria growth. However, the antibacterial activity of *P. gilvus* never been investigated. In this study the antibacterial activity of *P. gilvus* against selected bacteria strains was investigated.

## MATERIALS AND METHODS

**Mushroom sample and extraction:** The *P. gilvus* was identified by Natural Medicinal Mushroom Museum, Mahasarakham University, Thailand. The mushroom was collected on September 2008 from forest in Mahasarakham province Thailand. The 10 g of mushroom was grinded and boil in 1 L of water for 15 min and filtrated was spray dried. The yield of spray dried extract was 1-2.5% of dried weight of mushroom. 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* ATCC25922, *Salmonella typhimurium* ATCC 14028 *Klebsiella pneumonia* ATCC 10031 were used as gram negative bacteria.

**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.

*Phellinus gilvus* 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 plant 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 NSS filled in

**Corresponding Author:** Chaiyasit Sittiwet, Biomedical Research Unit, Department of Chemistry, Faculty of Science, Mahasarakham University, 44150, Thailand Tel: +6684-0170171 Fax: +6643-754241

the cylinder was used as control and 10 mg $L^{-1}$ gentamicin sulphate solution was 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 ME were determined by Agar dilution method (Merck) (Lorian, 1996), while MBCs were determined by broth macro-dilution method were (Lorian, 1996) and reference antibiotics gentamicin sulphate (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 ME or gentamicin sulphate 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* spp. were reputed as oriental and Russian folklore. Among of *Phellinus* spp., *Phellinus linteus* is the most studied of it biological activities. *Phellinus linteus* has been investigated it anti-staphylococcus aureus (Hur *et al*., 2004), the aqueous extract of its show anti-methicillin resistant *S. aureus* at high dose. However *P. gilvus* has never been investigated it antibacterial activity.

*Phellinus gilvus* is reputed in Russian folklore to have anti-carcinogenic and immune-modulatory properties (Gilbertson *et al*., 1980). It showed highest activity of immune stimulation (murine spleenocyte cells) compared with *P. linteus* and *P. baumii* (Chang *et al*., 2008). It also showed inhibitory effect on tumor and cancer cell such as human gastric adenocarcinoma melanoma benzo(a)pyrene-induced forestomach carcinogenesis in mice. *Phellinus gilvus* was shown wound healing properties in normal and diabetic rats (Bae *et al*., 2005). Furthermore it also showed anti-inflammatory in pulmonary cells (Jang *et al*., 2004).

In this study, anti-bacterial activity of aqueous extract of *P. gilvus* against selected gram positive and gram negative bacteria using agar diffusion, agar dilution and broth macro dilution methods had investigated. First the screening of it anti-bacterial was screened using agar diffusion method. The aqueous extract of *P. gilvus* showed inhibition zone against 3 out of 8 tested bacteria (*L. plantarum* ATCC 14917, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 10031) (Table 1). Among 3 of g negative bacterial strains *E. coli* ATCC 25922 and *K. pneumonia* ATCC 10031 are pathogenic bacteria. *K. pneumoniae* can cause bacterial pneumonia; hospital acquired urinary tract infection and wound infection, particularly in immunocompromised individuals while most of *E. coli* strains are harmless because it also be a normal flora of the gut in human. However, virulent strain of *E. coli* causes gastroenteritis, urinary tract infections and neonatal meningitis.

The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of *P. gilvus* aqueous extract was tested using agar dilution and broth macro dilution tests. The MIC against *L. plantarum* ACC 14917, *K. pneumonia* ATCC 10031 and *E. coli* ATCC 25922 were 45, 90, 360 mg $L^{-1}$, respectively while MBC were 90, 180, 720 mg $L^{-1}$, respectively (Table 2).

As mention earlier *P. gilvus* showed wound healing activity in normal and diabetic rats (Bae *et al*., 2005). This study is the first report about it anti-bacterial activity. It may conclude that *P. gilvus* extract can use as wound healing even infectious wound. It may also can used as the remedy of urinary tract infection and other infection causing by *K. pneumonia* and *E. coli*. The studies evidence from *P. linteus* showed very good

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

| | | Inhibition zone diameter (mm) | | | |
|---|---|---|---|---|---|
| Bacteria | Gram | *P. gilvus* (100 g $L^{-1}$) | *P. gilvus* (50 g $L^{-1}$) | *P. gilvus* (25 g $L^{-1}$) | Gentamicin sulphate (10 mg $L^{-1}$) |
| *S. aureus* ATCC 25923 | + | nz | nz | nz | 21.0±2.65 |
| *S. epidermidis* ATCC 12228 | + | nz | nz | nz | 21.7±1.52 |
| *M. luteus* ATCC 9341 | + | nz | nz | nz | 20.3±2.51 |
| *B. subtilis* ATCC 6633 | + | nz | nz | nz | 18.3±0.57 |
| *L. plantarum* ATCC 14917 | + | 12.3±1.53 | nz | nz | 23.3±0.57 |
| *E. coli* ATCC 25922 | - | 16.6±1.53 | nz | nz | 21.6±0.57 |
| *S. typhimurium* ATCC 14028 | - | nz | nz | nz | 18.0±1.00 |
| *K. pneumoniae* ATCC 10031 | - | 14.7±1.12 | 11.7±0.6 | nz | 17.6±1.53 |

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

Table 2: The MICs and MBCs of *P. gilvus* aqueous extract against various bacteria

| | *P. gilvus* | | Gentamicin sulphate | |
|---|---|---|---|---|
| Bacteria | MIC (mg $L^{-1}$) | MBC (mg $L^{-1}$) | MIC (mg $L^{-1}$) | MBC (mg $L^{-1}$) |
| *L. plantarum* ATCC 14917 | 45 | 90 | <0.5 | nd |
| *E. coli* ATCC 25922 | 360 | 720 | <0.5 | nd |
| *K. pneumoniae* ATCC 10031 | 90 | 180 | <0.5 | nd |

nd = Not Determine

anti-bacterial activity from n-butanol extract (Hur *et al*., 2004), which may guide the further study to investigated in lower polar of extract solvent may give higher activity.

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