Antioxidant and Antimicrobial Activities of Fruiting Bodies of *Phellinus gilvus* Collected in Korea

Ki-Nam Yoon, Hyung Seok Jang

Department of Clinical Laboratory Science, Ansan University, Ansan 15328, Korea

국내에서 수집된 마른진흙버섯 자실체의 항산화 및 항균 효과

윤기남, 장형석

안산대학교 임상병리과

This study was initiated to evaluate the antioxidant and antimicrobial activities of methanol extract (ME) and hot water extract (HWE) obtained from the fruiting bodies of medicinal mushroom, *Phellinus gilvus*. The free radical scavenging activity of ME from *P. gilvus* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) were 93.65% at 2 mg/mL, which was comparable with the positive control, butylated hydroxytoluene (BHT, 96.97%) at the same concentration. The ferrous ion-chelating ability of ME and HWE was significantly higher than that of BHT at all concentration levels. The antimicrobial assay of ME was performed against six bacteria and one species of fungus. ME exhibited antibacterial activity against 5 out of 6 bacteria: *Staphylococcus aureus*, *Streptococcus mutans*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*; whereas, ME did not show antimicrobial activity against gram-negative bacterium *Vibrio vulnificus* and fungal species *Candida albicans*. The minimum inhibitory concentration (MIC) of ME against 5 strains of bacteria, such as *S. aureus*, *S. mutans*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, was 100, 100, 50, 100, 200 mg/mL, respectively. The results suggest that good antioxidant and microbial activities of *P. gilvus* fruiting bodies might be used for natural antioxidant and antimicrobial agents.

Key words: Antimicrobial, Antioxidant, Medicinal mushroom, *Phellinus gilvus*

Corresponding author: Hyung Seok Jang
Department of Clinical Laboratory Science,
Ansan University, 155 Ansandaehak-ro,
Sangnok-gu, Ansan 15328, Korea
Tel: 82-31-400-6935
Fax: 82-31-363-7702
E-mail: himylife@ansan.ac.kr

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Received: October 5, 2016
Revised 1st: November 20, 2016
Revised 2nd: November 25, 2016
Revised 3rd: November 29, 2016
Revised 4th: November 29, 2016
Accepted: November 29, 2016

Introduction

Mushrooms have become attractive as a functional food because of high contents of protein, crude fiber, minerals and vitamins [1] and also contain physiologically beneficial bioactive substances that promote good health [2]. The secondary metabolites produced from mushrooms possess antibacterial, antifungal, antioxidant, antiviral, antitumor, anti-allergic, antiatherogenic, hypoglycemic, anti-inflammatory

and hepatoprotective activities [3,4].

Reactive oxygen species (ROS) were produced by a result of normal cellular metabolism in a living organism. At low to moderate concentrations, ROS function in physiological cell processes, but at high concentrations, they provoke adverse effects to cell components, such as lipids, proteins, and DNA and caused degenerative diseases including cancer, cardiovascular disease, neural disorders, and diabetes. Protection against free radicals can be enhanced by intake of dietary

antioxidant. Experimental evidence suggested that foods containing antioxidant nutrients may be of major importance in disease prevention possibly caused by free radicals. The intake of natural antioxidant sources such as vegetables, fruits, and mushrooms may be effective over the long term for health. Therefore, antioxidant from natural products may be of great benefit for improving the quality of life by preventing or postponing the onset of degenerative diseases [5].

Antibiotic resistance has become global problems in recent years. Even though a number of natural and synthetic antimicrobial agents have been isolated and synthesized against pathogenic microorganisms, infectious diseases remain one of the major threats to human health [6]. The increasing antibiotic resistance of pathogenic microorganisms caused by overuse of newly developed antibiotics has led to the screening of novel sources for potential antimicrobial activity [7]. Although mushrooms belong to Basidiomycota have not been fully studied on antimicrobial activity, a variety of antimicrobial compounds produced by the fungi suggested that they may be a new and useful sources to combat against pathogenic microorganisms [8]. In the natural environment, the fungi including mushrooms need defense chemical compounds to fight against bacterial and fungal invasion. Therefore, antimicrobial compounds produced by many mushroom species could be useful to develop new antibiotics. Although many antimicrobial compounds have been isolated and identified from mushrooms, the antimicrobial compounds prescribed in the hospital are still originated from microscopic fungi and actinomycetes [9].

P. gilvus, a white rot fungus, belongs to family Hymenochaetaceae of Basidiomycota and known to as 'Sanghwang' and consumed for medicinal purposes in Korea, China, and Japan for thousand years [10]. The functional and physiological activities of the *P. gilvus* have been reported by several researchers. The hot water extract of fruiting bodies from *P. gilvus* has been reported to contain inhibitory effect on pulmonary inflammation [11]. The polysaccharide extracted from fruiting bodies of *P. gilvus* exhibited inhibitory effect on melanoma of rats [12]. The polysaccharide extracted from *P. gilvus* also showed wound healing effect on diabetic rats [13].

Hence, the aim of this study was to evaluate the antioxidant

and antimicrobial activities of ME and HWE from fruiting bodies of *P. gilvus*. In addition to this, profiles of phenols and flavonoid compounds concentrations of the mushroom were also analyzed.

Materials and Methods

1. Mushroom sample

Fruiting bodies of *P. gilvus* were collected from Bukhan mountain of Seoul city in the summer of 2015 and identified by Dr. Kyung-Rim Lee (mycologist). The voucher specimens were deposited in the "Culture Collection of Mushrooms", Incheon National University. The fruiting bodies were fully air dried at 45°C and ground into a fine powder.

2. Preparation of the extracts

To obtain the ME, 20 g of the mushroom powder was extracted with 200 mL of 80% methanol at orbital shaker (150 rpm) for 24 hr at 25°C. Then, the mixture was filtered through Whatman no. 4 filter paper, and the remaining residue was extracted with 200 mL of methanol twice as described above. To obtain the HWE, 20 g of the mushroom powder was boiled for 3 hr in 200 mL of distilled water, cooled to 25°C and filtered with filter paper. Then, the remaining residue was extracted with 200 mL of 80% methanol or distilled water twice as described above, respectively. The ME and HWE were evaporated to dryness at 50°C under reduced pressure in a rotary evaporator, and remaining solvent was removed with a freeze-drier [14]. Extracts were kept in the dark at -80°C for prior to use.

3. Scavenging effect on DPPH free radicals

The hydrogen atoms or electron donation ability of the ME and HWE was measured by the method bleaching of the purple colored DPPH methanol solution [15]. One mL of 0.1 mM DPPH radical solution in methanol was mixed with 1 mL aliquots of varying mushroom extract concentrations (0.125, 0.25, 0.5, 1.0 and 2.0 mg/mL). The reaction was carried out by shaking vigorously and then allowing it to stand for 30 min in the dark before measuring the absorbance at 517 nm using a spectrophotometer. Inhibition of the DPPH free radical in

percent (I%) was calculated as:

$$I\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100,$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. BHT was used as the positive control.

4. Chelating effects on ferrous ions

The chelating effect of the ME and HWE was determined according to the method of Dinis *et al.* [16]. Briefly, various concentrations (0.063 ~ 1.0 mg/mL, 2 mL) of the extracts in methanol were added to a solution of 2 mM FeCl_2 (0.05 mL). The reaction was initiated by adding 5 mM ferrozine (0.2 mL). Total volume was adjusted to 5 mL with methanol, and the mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of the ferrozine- Fe^{2+} complex formation was calculated using the following formula:

$$\text{Ferrous ion chelating effect (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100,$$

where A_{control} is the absorbance of the control (control contained FeCl_2 and ferrozine; complex formation molecules), and A_{sample} is the absorbance of the test compound. BHT was used as the positive control.

5. Reducing power assay

The reducing power of the ME and HWE was determined according to the method of Gulcin *et al.* [17]. Each extract (1 ~ 8 mg/mL) in methanol (2.5 mL) was mixed with 200 mM sodium phosphate buffer (pH 6.6, 2.5 mL) and 1% potassium ferricyanide (2.5 mL), and the mixture was incubated at 50°C for 20 min. Then, 10% trichloroacetic acid (2.5 mL) was added, and the mixture was centrifuged at 200 × g (6K 15; Sigma, Munich, Germany) for 10 min. The upper layer (2.5 mL) was mixed with deionized water (2.5 mL) and 0.1% ferric chloride (0.5 mL). Finally, the absorbance was measured at

700 nm against a blank. BHT was used as the positive control.

6. Determination of bioactive components

Phenolic compound concentration in the ME and HWE of the mushroom was determined by a colorimetric assay, based on procedures described by Singleton and Rossi [18] with minor modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was measured spectrophotometrically at 765 nm. Gallic acid was used to calculate the standard curve. The results were mean value ± standard deviation (SD) and expressed as µg of gallic acid equivalents (GAEs) per mg of extract.

Flavonoid concentration of the ME and HWE was determined by a colorimetric method described by Jia *et al.* [19] with minor modifications. The ME and HWE solution (1 mL) of the mushrooms was diluted with 4.3 mL of 80% aqueous ethanol, and 0.1 mL of 10% aluminum nitrate and 0.1 mL of 1 M aqueous potassium acetate were added. After 40 min at 25°C, the solution was mixed well, and the intensity of the pink color was measured by spectrophotometer at 415 nm. Quercetin was used to calculate the standard curve. The results were mean value ± standard deviation (SD) and expressed as µg of quercetin equivalents (QEs) per mg of extract.

7. Determination of antimicrobial efficacy

1) Microbial test organisms

Three species of gram-positive bacterial strains such as *S. aureus* KCTC 1621, *S. mutans* KCTC 3065, *B. subtilis* KCTC 1021, and 3 species of gram-negative bacterial strains including *E. coli* KCTC 1045, *P. aeruginosa* KCTC 2513, and *V. vulnificus* KCTC 2959, and one fungal species of *C. albicans* KCTC 7965 were obtained from Korean Collection for Type Cultures (KCTC). The characteristics, culture media, and culture conditions of the microorganisms used in this study were listed in the Table 1.

Table 1. List of microorganisms used for antimicrobial activity in this study

Microorganisms		Characters	Culture condition and media	
<i>Staphylococcus aureus</i>	KCTC 1621	Gram+	36°C	Nutrient agar
<i>Streptococcus mutans</i>	KCTC 3065	Gram+	36°C	Brain heart infusion agar
<i>Bacillus subtilis</i>	KCTC 1021	Gram+	36°C	Nutrient agar
<i>Escherichia coli</i>	KCTC 1045	Gram—	36°C	Nutrient agar
<i>Pseudomonas aeruginosa</i>	KCTC 2513	Gram—	36°C	Nutrient agar
<i>Vibrio vulnificus</i>	KCTC 2959	Gram—	36°C	Nutrient agar added 3% NaCl
<i>Candida albicans</i>	KCTC 7965	Yeast	30°C	Sabouraud dextrose agar

2) Antimicrobial assay

Antimicrobial activity of the ME of the mushroom was determined by the agar well diffusion method [20], with slight modification. Briefly, the ME and HWE were dissolved in 3% dimethylsulfoxide (DMSO) to a final concentration of 200 mg/mL, and filter sterilized through 0.45- μ m membrane filter. Small wells (5 mm in diameter) were made in the agar plates by sterilize cork borer. One hundred μ L of the ME and HWE with 200 mg/mL concentration was loaded into the different wells. An overnight culture of each microbial isolate was emulsified with nutrient broth to a turbidity that was equivalent to 0.5 McFarland (10^5 cfu/mL). In order to determine the antimicrobial efficacy of the extracts, aliquot of test culture (100 μ L) was evenly spread over the surface of the solidified agar. Bacteria were cultured on Mueller Hinton agar, and fungus on Sabouraud dextrose agar. Antibiotics such as streptomycin (30 μ g/well), tetracycline (30 μ g/well), and fluconazole (40 μ g/well) were used for positive controls for determining inhibition zone of selected bacteria and fungus, respectively. DMSO was used as negative control for testing selected microorganisms. All the plates previously loaded with respective extracts and test microorganisms were incubated for 24 hr at 37°C for bacteria and 48 hr for fungus at 28°C. After incubation, inhibition zone was measured in millimeters. All the tests were carried out in triplicate and their means and standard deviation (SD) were recorded.

3) Minimum inhibitory concentration (MIC)

The MIC of the mushroom extract was performed with ME since the HWE did not exhibit inhibition zone at 200 mg/mL concentration in the agar gel diffusion test. Therefore, antibacterial activity of MIC against 5 bacterial strains such as

S. aureus, *S. mutans*, *B. subtilis*, *E. coli*, and *P. aeruginosa* were further studied with ME by applying the broth dilution method [21] with minor modifications. The ME was diluted to give the final concentrations of 25~400 mg/mL. One hundred μ L of 10^5 CFU/mL of the bacterial strains was inoculated in tubes with equal volume of nutrient broth and the ME. The tubes were incubated aerobically at 37°C for 24~48 hr. The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 hr when compared with the negative control tubes was considered as initial MIC. The dilutions that showed no turbidity were incubated further for 24 hr at 37°C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 hr was regarded as final MIC.

8. Statistical analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan's multiple range tests (SPSS 11.5 version). Differences were considered significant at $p < 0.05$.

Results

1. DPPH radical scavenging activities

The scavenging activities of the ME, and HWE from the fruiting bodies of *P. gilvus* on DPPH radicals increased with increasing concentration. At 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL concentration of the extracts, the scavenging abilities of the ME, and HWE on the DPPH radical ranged from 89.86%~93.65%, and 88.18%~91.45%, respectively (Fig. 1). These results indicated that the ME and HWE possessed good scavenging activity on DPPH radicals at the concentrations

Fig. 1. Scavenging activity of methanol and hot water extract from fruiting bodies of *Phellinus gilvus* against 1,1-diphenyl-2-picrylhydrazyl. Values expressed as mean $\pm$ SD (n=3). Fr. MeOH, fractions extracted with 80% methanol; Fr. HW, fractions extracted with hot water; BHT, butylatedhydroxytoluene. *** $p\leq 0.001$; ** $p\leq 0.01$; * $p<0.05$ vs. BHT group.

Fig. 2. Chelating effect of methanol and hot water extract from fruiting bodies of *Phellinus gilvus* at different concentrations. Values are expressed as mean $\pm$ SD (n=3). Fr. MeOH, fractions extracted with 80% methanol; Fr. HW, fractions extracted with hot water; BHT, butylatedhydroxytoluene. *** $p\leq 0.001$; ** $p\leq 0.01$; * $p<0.05$ vs. BHT group.

tested. However, BHT, the positive control showed excellent scavenging abilities of 96.19~96.97% at the concentration range of 0.125~2.0 mg/mL, respectively.

2. Chelating activity of ferrous ion

The chelating activity of the ME and HWE from the fruiting bodies of *P. gilvus* toward ferrous ions was investigated at five different concentrations (0.125, 0.25, 0.50, 1.0, and 2.0 mg/mL). BHT was used as reference standard. As shown in Fig. 2, the chelating capacity of the ME and HWE increased with dose-dependent manner. The chelating effect of ME and HWE were 72.70% and 79.95% at 2.0 mg/mL, respectively, whereas chelating effect of BHT, the positive control was 68.87% at 2.0 mg/mL.

Fig. 3. Reducing power levels of methanol and hot water extract from fruiting bodies of *Phellinus gilvus* at different concentrations. Values are expressed as mean $\pm$ SD (n=3). Fr. MeOH, fractions extracted with 80% methanol; Fr. HW, fractions extracted with hot water; BHT, butylatedhydroxytoluene. *** $p\leq 0.001$; ** $p\leq 0.01$; * $p<0.05$ vs. BHT group.

3. Reducing power assay

The reducing power of ME and HWE at five different concentrations (0.5, 1.0, 2.0, 4.0, and 6.0 mg/mL) ranged from 1.88 to 2.24, and 1.84 to 1.98, respectively, suggesting that reducing power of the ME, and HWE of *P. gilvus* fruiting bodies increased with increasing concentration of the extracts (Fig. 3). However, the reducing power of the reference antioxidants, BHT was much higher than those of the ME and HWE, indicating that reducing power of the ME, and HWE were moderate.

4. Bioactive compounds

Total phenolics and flavonoid contents of the ME and HWE from the fruiting bodies of *P. gilvus* were investigated. The contents of phenolic compound of the ME and HWE from fruiting bodies of the mushroom ranged from 31.17 and 12.83 μ g gallic acid equivalent (GAE)/mg, respectively. The flavonoid content of the ME and HWE ranged from 15.29 and 27.46 μ g quercetin equivalent (QE)/mg, respectively (Table 2).

5. Antimicrobial assay

1) Agar well diffusion method

Antimicrobial activity of ME and HWE of fruiting bodies of *P. gilvus* against 6 bacteria such as *S. aureus*, *S. mutans*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, *V. vulnificus* and 1 species of fungus, *C. albicans* was conducted using agar well diffusion method. The ME of the fruiting bodies of *P. gilvus* exhibited the various degrees of antimicrobial effects against 5 out of 7

test microorganisms, whereas gram-negative bacterium *V. vulnificus* KCTC 2959 and fungal strain *C. albicans* KCTC 7965 did not show inhibition zone, indicating that the antimicrobial activity of the ME was not effective to all bacteria and the fungus tested. However, the HWE of *P. gilvus* did not exhibit inhibitory activity against 7 microbial strains such as six bacteria and one fungus. As summarized in Table 3, three gram-positive bacteria such as *S. aureus* KCTC 1621, *S. mutans* KCTC 3065, and *B. subtilis* KCTC 1021 have shown susceptibility against the ME at 200 mg/mL with inhibition zone diameter of 13.4, 14.5, and 17.7 mm, respectively, whereas 2 out of 3 gram-negative bacteria such as *E. coli* KCTC 1045, and *P. aeruginosa* KCTC 2513 showed susceptible reaction against the ME with the inhibition zone diameter of 9.3, and 7.5 mm at the same concentration tested. These results suggest that the highest inhibitory activity was determined against *B. subtilis* KCTC 1021, and the weakest inhibitory activity was determined against *P. aeruginosa* KCTC 2513. However, the inhibition zone diameter of

streptomycin and tetracycline, the positive controls ranged from 16.9~21.4 mm, and 11.8~23.8 mm, respectively.

2) Minimum inhibitory concentration

The MIC of the ME of *P. gilvus* was tested using broth dilution method. Since the HWE of the fruiting bodies of *P. gilvus* did not show inhibition zone against all microbial strains tested in the agar well diffusion method, HWE was excluded for the MIC test. The ME did not show inhibition zone against a bacterial strain *V. vulnificus*, and a fungal strain *C. albicans* at 200 mg/mL, these two microbial strains were excluded in the MIC test. The 5 bacterial strains, which showed susceptible reaction to the ME were used for the MIC test. In the MIC test, *S. aureus* KCTC 1621 strain showed no visible growth at 100 mg/mL of ME, whereas visible growth was found at 25 and 50 mg/mL concentration. The *S. mutans* KCTC 3065 strain showed visible growth at 50 mg/mL of the ME, and no visible growth was found at 100 mg/mL. The *B. subtilis* KCTC 1021 strain exhibited no visible growth at 50 mg/mL of ME and showed visible growth at 25 mg/mL. The *E. coli* KCTC 1045 strain showed no visible growth at 100 mg/mL, whereas visible growth was observed at 50 mg/mL of the ME. The *P. aeruginosa* KCTC 2513 strain exhibited visible growth at 100 mg/mL of the ME, whereas no visible growth was observed at 200 mg/mL (Table 4). Therefore, it is concluded that the MIC values of the ME against 5 bacterial strains such as *S. aureus* KCTC 1621, *S. mutans* KCTC 3065, *B. subtilis* KCTC 1021, *E. coli* KCTC 1045, and *P. aeruginosa* KCTC 2513 were 100, 100, 50, 100, 200 mg/mL, respectively.

Table 2. Total Phenolic and flavonoids content in methanol and hot water extracts from fruiting bodies of *Phellinus gilvus*

Samples	Extraction yield % (w/w)	Phenolic content (µg GAEs/mg extract)	Flavonoids content (µg QEs/mg extract)
Methanol extract	10.37	31.17±1.04	15.29±0.56
Hot water extract	7.62	12.83±0.51	27.46±1.87

The values are expressed as means±SD of three parallel measurements.

Abbreviation: GAEs, gallic acid equivalents; QEs, quercetin equivalents.

Table 3. Diameter of inhibition zone of methanol (ME) extract from *Phellinus gilvus* fruiting bodies against selected microorganisms

Microorganism	ME (200 mg/mL)	Streptomycin (30 µg/mL)	Tetracycline (30 µg/mL)	Fluconazole (40 µg/mL)
<i>Staphylococcus aureus</i>	13.4±1.07	19.7±1.12	20.6±0.32	NT
<i>Streptococcus mutans</i>	14.5±0.23	18.1±0.23	19.3±1.03	NT
<i>Bacillus subtilis</i>	17.7±1.32	21.4±1.45	23.8±1.27	NT
<i>Escherichia coli</i>	9.3±0.67	16.9±1.16	17.7±0.32	NT
<i>Pseudomonas aeruginosa</i>	7.5±0.25	–	11.8±1.05	NT
<i>Vibrio vulnificus</i>	–	19.3±0.71	18.5±0.61	NT
<i>Candida albicans</i>	–	–	–	13.6±0.64

Antimicrobial activity was determined by diameter (mm) of the inhibition zone surrounding well.

The data are expressed as means±standard deviation (SD) of triplicate determinations.

Values with different superscripts within same row are significantly different at $p<0.05$.

Abbreviation: –, Inhibition zone was not detected; NT, Not tested.

Table 4. Minimum inhibitory concentration (MIC) of methanol extract of *Phellinus gilvus* fruiting bodies

Extract (mg/mL)	<i>S. aureus</i>	<i>S. mutans</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
25	+	+	+	+	+
50	+	+	-	+	+
100	-	-	-	-	+
200	-	-	-	-	-
400	-	-	-	-	-

Abbreviation: -, Growth of microorganisms was not detected; +, Growth of microorganisms was detected.

Discussion

The antioxidant is capable of reducing the stable DPPH radical (purple) to the non-radical form DPPH-H (yellow). The DPPH scavenging activities of antioxidant are known to as attributed to their hydrogen donating abilities to ROS. In the DPPH radical scavenging assay, the ME and HWE of the mushroom showed the good antioxidant activity, however the BHT, positive control showed excellent DPPH scavenging effect compared with the ME and HWE at the all concentration tested. The methanol extract of *P. florida* cultivated in India showed 38% DPPH scavenging activity at 1.0 mg/mL [22], whereas the DPPH of the ME of *P. gilvus* was 92.76% at 1.0 mg/mL. Mau et al. [23] found that the radical scavenging abilities of methanol extracts from *Grifola frondosa*, *Hericium erinaceus*, *Tricholoma giganteum*, and *Dictyophora indusiata* ranged from 63.3%~92.1% at a concentration of 6.4 mg/mL. It seemed that the scavenging activity of ME and HWE of *P. gilvus* fruiting bodies was more effective than those mentioned mushrooms above.

Ferrous ions are regarded as the strongest pro-oxidant among various metal ions. Chelating activity of ferrous ions can prohibit free radical generation and protect cells from oxidative damage by removing iron, which may be involved in free radical generation [24]. Sarikurku et al. [25] documented that chelating effect on ferrous ion by the methanol extract of *Amanita caesarea*, and *Clitocybe geotropa* were 74.1%, and 37.2% at 2.0 mg/mL, respectively. The chelating effects of methanol extracts from *Auricularia fuscosuccinea*, *Auricularia polytricha*, and *Tremella fusiformis* at 2.0 mg/mL were reported to be 91.28%, 88.04%, and 86.92%, respectively [26]. The ME and HWE determined herein exhibited significantly higher ferrous ion chelating

activity than that of the standard reference, BHT, at the 0.125–2.0 mg/mL. However, the ME and HWE of *P. gilvus* demonstrated strong chelating abilities than those of all mentioned mushrooms above except *C. geotropa*. As ferrous ions are a potent pro-oxidant, the strong chelating effect found in *P. gilvus* fruiting bodies might be used as a natural antioxidant agent.

The reducing power assay was performed on ME and HWE of fruiting bodies from *P. gilvus*. The reducing power of the ME and HWE of the *P. gilvus* increased with increasing concentration of the extracts. The strongest reducing power (2.24) was observed at 6 mg/mL of ME, and the lowest reducing power inhibition (1.98) was exhibited by the same concentration of the HWE. The synthetic antioxidant BHT showed higher reducing power values of 2.92 at 6.0 mg/mL (Table 2). Menaga et al. [27] reported that methanol extract of *P. florida* fruiting bodies showed reducing power of 0.911 at 0.5 mg/mL, which was significantly lower than the reducing power of our methanol extract of 1.88 at 0.5 mg/mL. The reducing power of a hot water extracts of *Hypsizygus marmoreus* was 0.99 at 5.0 mg/mL [28], whereas those of *Agaricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus* showed reducing powers of 0.76, 0.75, 0.70, and 0.61 at 2.0 mg/mL, respectively [29]. Yoon et al. [30] demonstrated that reducing power of methanol extract of *Agaricus brasiliensis* fruiting bodies was 1.54 at 6.0 mg/mL, which was lower than that of methanol extract from *P. gilvus* fruiting bodies at the same concentration tested.

Our results showed that the reducing power of the both extracts from *P. gilvus* increased steadily as the extract concentrations increased, whereas the reducing power of BHT increased slowly as the concentration increased. Reducing power components are generally associated with

the presence of reductones, which exert their antioxidant action by breaking the free radical chain and donating a hydrogen atom [30]. Therefore, it might be concluded that the good DPPH scavenging, ferrous ion chelating, and reducing power activities of the ME and HWE of *P. gilvus* fruiting body might be used for natural antioxidant sources.

All phenols and flavonoids are considered as compounds possessing the highest antioxidant activity because of their chemical structures. It is suggested that phenolic compounds known to have inhibitory effects on free radicals, and reduce the risk of heart disease and age related disorders by slowing the progression of atherosclerosis [31,32]. The total phenol content extracted with methanol (31.17 µg GAE/mg) from fruiting bodies was higher than that of hot water extract (15.29 µg GAE/mg), indicating that methanol is more efficient solvent extracting phenolics from fruiting bodies of *P. gilvus*. Mujic et al. [33] reported that the phenolic compounds extracted with 50% ethanol from *Lentinula edodes*, *Hericium erinaceus*, and *Agrocybe aegerita* were 11.70, 7.89, 23.07 µg GAE/mg, respectively, whereas phenolics extracted by methanol from fruiting bodies of *Ganoderma lucidum*, and *Morchella esculenta* were 26.4, and 25.01 µg GAE/mg, respectively [34]. The total phenolic compounds extracted with methanol from *P. gilvus* fruiting bodies was higher than those of mushrooms mentioned above, however phenolic compound contents extracted with hot water was lower than those of *A. aegerita*, *G. lucidum*, and *M. esculenta* mushrooms. Therefore, the high antioxidant activity exhibited in the ME and HWE was likely due to relatively higher phenolic and flavonoid contents present in the *P. gilvus* fruiting bodies.

The results from this study showed that the HWE did not exhibit the antimicrobial activity against all seven selected microorganisms and the ME showed antimicrobial activity against 5 out of 7 microorganisms tested. However one gram-negative bacterium *P. vulnificus*, and a fungus *C. albicans* were resistant to the ME by exhibiting no inhibition zone in the agar gel diffusion assay. Therefore, it was considered that the ME of fruiting body from *P. gilvus* has a relatively broad-spectrum antibacterial activity except fungus. Rahman et al. [35] documented antimicrobial study of ethanol extract of *Pleurotus florida* against 6 human

pathogenic bacteria. Among them, the highest antibacterial activity was recorded in both gram-positive bacteria *S. marcescens* and *S. aureus*, while the lowest antibacterial activity was observed in gram-negative bacteria *P. aeruginosa*, indicating that gram-negative bacterium was more resistant than gram-positive bacteria to antibacterial agents. Similar antibacterial potentials have been observed in the aqueous extract of *Phellinus ignarius* [36] and methanol extract fruiting bodies of *Flammulina velutipes* [37]. However, the mycelial culture of *Lentinus edodes* inhibited growth of both gram-positive and gram-negative bacteria [38]. Hong and Lee [39] demonstrated that ethanol extract of *Houttuynia cordata* exhibits antibacterial activity against 10 methicillin-resistant *Staphylococcus aureus* (MRSA) by exhibiting their IC₅₀ values ranging from 2.05~4.10 mg/mL. The IC₅₀ values of *H. cordata* extract on MRSA were lower than those of *P. gilvus* methanol extract indicating that ethanol extract of *H. cordata* possessed strong antibacterial activity compared with *P. gilvus* methanol extract.

It is also evident from our results that the gram-positive bacteria such as *S. aureus*, *S. mutans*, and *B. subtilis* were more sensitive to the ME of the *P. gilvus*, whereas gram-negative bacteria such as *E. coli*, *P. aeruginosa*, and *V. vulnificus* were more resistant to the ME. The results from our study are in agreement with literature which showed that the gram-positive bacteria were more sensitive to the antimicrobial agents compared to gram-negative bacteria [40]. It is well known that gram-negative bacteria possess additional outer membrane and a periplasmic space, both of which are absent from gram-positive bacteria. Our results of antibacterial activity on ME may be explained by the differences in the cell wall structure that can produce differences in antibiotic resistant of the gram-positive and gram-negative bacteria [40]. This study has shown that the antimicrobial activity of the *P. gilvus* fruiting bodies are promising natural antimicrobial agents that can be used for potential antibacterial agent. Since many phenolics and flavonoids have been found to be responsible for antioxidant and antimicrobial activities, it was expected that antimicrobial activity of the ME against 5 bacterial species might be due to in part by the phenols and flavonoid compounds present in

the fruiting bodies of *P. gilvus*. However, further extensive studies are recommended for identifying the bioactive components responsible for their antimicrobial activities from this mushroom.

요 약

본 연구에서는 우리나라에 자생하는 약용 버섯인 마른진흙버섯의 자실체를 80%의 메탄올과 열수를 이용해 추출한 물질의 항산화와 항균 효과에 대한 실험을 수행하였다. 마른진흙버섯 자실체의 메탄올 추출물은 2.0 mg/mL 농도에서 DPPH 라디칼 소거능(93.65%)이 양성대조군인 BHT(96.97%)에 비해 낮았으나 상대적 인 효과는 높았다. 철이온 소거능 실험에서 추출물의 모든 농도(0.125~2.0 mg/mL) 범위에서 철이온 소거능 항산화 효과가 BHT에 비해 월등하게 높은 것으로 나타났다. 또한 6종의 세균 *S. aureus*, *S. mutans*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *V. vulnificus*와 1종의 곰팡이 *C. albicans*를 대상으로 200 mg/mL의 농도에서 메탄올 추출물을 이용해 agar well diffusion법으로 항균 효과를 측정한 결과, *V. vulnificus*와 *C. albicans*를 제외한 5종의 세균에서 inhibition zone을 보여 메탄올 추출물은 여러 종류의 그람 양성세균과 그람 음성세균에 항균효과를 나타내는 것으로 나타났다. 또한 메탄올 추출물에 inhibition zone을 나타낸 *S. aureus*, *S. mutans*, *B. subts*, *E. coli*, *P. aeruginosa* 등 5종의 세균을 대상으로 최소저해농도(MIC)를 측정한 결과 각각 100, 100, 50, 100, 200 mg/mL의 MIC를 나타내었다. 따라서 마른진흙버섯 자실체의 메탄올 추출물은 항산화 및 항균 효과가 높아서 이들 추출물은 천연 항산화제와 항균제로 이용이 가능할 것으로 사료된다.

Acknowledgements: None

Funding: None

Conflict of interest: None

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