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Contributed Paper

# Antibacterial, Antioxidant Properties and Bioactive Compounds of Thai Cultivated Mushroom Extracts against Food-borne Bacterial Strains

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## ABSTRACT

A fine-dried edible mushroom was extracted and investigated for its antibacterial activity against food-borne pathogenic bacteria, i.e. *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Salmonella typhimurium* and *Staphylococcus aureus*. The extracts of *Flammulina velutipes*, *Ganoderma lucidum*, *Pleurotus ostreatus* and *Pleurotus pulmonarius* inhibited both Gram-positive and Gram-negative bacteria. Water extract of *Pleurotus pulmonarius* significantly inhibited the growth of Gram-positive, i.e. *Bacillus cereus* and *Micrococcus luteus* (15 mm), while Gram-negative, i.e. *Escherichia coli* and *Salmonella typhimurium* resisted to most extracts. Only water extract from *Pleurotus pulmonarius* showed antibacterial activity against all tested bacteria. The minimum inhibitory concentrations (MICs) of *Bacillus cereus* had the lowest MIC range (1.25-5.00 mg/ml), whereas those of *Salmonella typhimurium* had the highest MICs (12.5-22.5 mg/ml) in *Flammulina velutipes* in ethylacetate extract, *Ganoderma lucidum* in methanol extract, *Pleurotus ostreatus* in ethylacetate extract and *Pleurotus pulmonarius* in water extract. Mushroom crude extracts were investigated for antioxidant capacity using the ABTS system. Results showed an effective antioxidant activity against ABTS radicals (91-94 %) and vitamin E derivatives (97 %), respectively. The $IC_{50}$ values of *Ganoderma lucidum*, *Pleurotus pulmonarius* and *Flammulina velutipes* were $2.81 \pm 0.02$ mg/ml to $10.57 \pm 0.27$ mg/ml. *Ganoderma lucidum* exhibited maximum antioxidant potential as compared to *Pleurotus pulmonarius* and *Flammulina velutipes*, respectively. Positive correlations were found between total phenolic content in the mushroom extracts and their antioxidant activities. Water-extracted bioactive compounds produced by *Pleurotus pulmonarius* were characterized and identified on the basis of $^1$H-NMR as a polysaccharide. The study reveals that cultivated mushroom extracts have higher free radical scavenging potential with high levels of antioxidant compounds. These compounds showed broad-spectrum activity, were non-toxic, and might be applicable for human use. Results suggest that cultivated mushrooms may have potential as natural antibacterial and antioxidant properties and could be used as potential natural source for the development of nutraceuticals.

**Keywords:** antibacterial activity, antioxidant activity, cultivated mushroom, food-borne pathogenic bacteria, polysaccharide

## 1. INTRODUCTION

Food-borne pathogenic bacteria are the group of bacteria that cause food spoilage and they may produce toxins, off-flavors, lytic enzyme and rotting. The most dangerous bacteria consist of *Bacillus cereus*, *Enterobacter aeroginese*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus* and *Salmonella typhimurium* that cause toxin-contaminated foods which promote diarrhea in human and animal [1]. The lytic enzyme derived from these pathogens includes lipase, protease and carbohydrase. These enzmes can deteriorate food sensorial properties [2]. Moreover, their enterotoxins are significant to human health because toxin-contaminated foods have been associated with liver and kidney tumors [3]. Multidrug resistance in pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs in the treatment of infectious disease. This has led to the urgent need for new antibiotics to treat infections caused by this group of resistant bacteria including the infection by food-borne pathogenic bacteria. Edible mushrooms in the genera of *Lentinula*, *Hericium*, *Grifola*, *Flammulina*, *Pleurotus* and *Tremella* have been reported to possess medicinal properties [4] such as anti-carcinogenic, anti-inflammatory, immune-suppressing and antimicrobial activities [5, 6]. The cell wall glucans are well known for their immunomodulatory properties, and many of the externalized secondary metabolites (extracellular secretion by the mycelium) combat bacteria [7] and viruses [8]. The exudates from mushroom mycelia are active against protozoa such as the parasite that causes malaria, *Plasmodium falciparum* and other microorganisms [9]. Some edible mushrooms are considered to be a good source of proteins and phenolic antioxidants [10]. Secondary metabolites including phenolic compounds, polyketides, terpenes and steroids derived from many polypores and agarics have been responsible for their antimicrobial activity [11]. These compounds could inhibit lipoprotein oxidation [12] and inhibit the occurrences of atherosclerosis and cancer [11]. Glucosylceramide isolated from *Pleurotus citrinopileatus* was found to be active against *Escherichia coli* and *Staphylococcus aureus* with $IC_{50}$ values of 275.1 µM and 323.2 µM, respectively [13]. The importance of the Chinese Shiitake mushroom (*Lentinus edodes*) has been demonstrated to increase the host resistance to bacterial and viral infection [14]. Several compounds extracted from Shiitake mushroom revealed antifungal and antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Thus, chloroform and ethyl acetate extracts of dried mushroom have antibacterial activity against *Streptococcus mutans* and *Prevestella intermedia* [15]. Researchers revealed antimicrobial activity of several mushroom extracts [5] such as chloroform and ethyl acetate extracts of *Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portensosum* which exhibited antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* [15]. *Ganoderma*, *Cantharellus*, *Lentinus*, *Russula*, *Agaricus* and *Pleurotus* extracts have been reported to show antimicrobial potentials which possess the different bioactive compounds [16, 17, 18]. The extracts of *Agaricus bisporus*, *Auricularia auricula*, *Lentinula edodes* and *Pleurotus* were against

*Mycobacterium smegmatis* and *Candida albicans* [19]. A few studies have been reported on the antimicrobial activity of other edible mushrooms and their bioactive compounds. The extractable products from mushrooms were the supplement diet which enhances health and fitness and can be classified as medicinal therapy. Thus, mushrooms could offer a particularly rich source of the new potential medicines.

Although a wide variety of edible mushrooms particularly in the tropical region has been consumed regularly, the information regarding the antimicrobial potentials of mushrooms in the tropical region is limit. In this study, we screened for antibacterial activity from Thai edible mushrooms against food-borne pathogenic bacteria. Elucidation of bioactive compound from mushroom extract was carried out by nuclear magnetic resonance (NMR) analysis. We aim to discover some novel natural compounds with low toxicity that could be used further to treat infections caused by multi-drug resistant strains of food-borne pathogenic bacteria.

## 2. MATERIALS AND METHOD

### 2.1 Preparation of Mushroom Extracts

Edible mushrooms (Table 1) were air-dried in an oven at 40°C before extraction. A fine-dried mushroom powder sample (20 g) was extracted by stirring with 100 ml of a series of organic solvent as hexane, ethyl acetate, 95% ethanol, 95% methanol and sterile distilled water at 30°C, 150 rpm for 24 hr and filtered through Whatman No.4 filter paper. The residue was then extracted with additional 100 ml of same solvent. Each solvent extract was combined and evaporated to dryness. The organic solvent in the extracts was removed by a rotary evaporator. For the entire analysis, compounds of extract were dissolved in 10% dimethylsulfoxide (DMSO), and filter sterilization was done through a 0.22 mM membrane filter. Extracts were kept in the dark at 4°C before use.

**Table 1.** List of Thai edible mushrooms used in this study.

| Mushroom Family | Common name | Strain/Species of Mushrooms |
|---|---|---|
| Family Agaricaceae | Shimeji mushroom | *Hypsizgus marmoreus* |
| Family Amanitaceae | - | *Amanita vaginata* (Fr.) Quel. var. *fulva* |
| Family Auriculariaceae | Jelly ear mushroom | *Auricularia auricular* (Hook.) Underw. |
| Family Ganodermataceae | Lingzhi mushroom | *Ganoderma lucidum* (Fr.) Karst. |
| Family Pleurotaceae | Indian oyster mushroom | *Lentinula edodes* (Berk.) Sing. |
| | | *Lentinus polychrous* Lev. |
| | | *Lentinus squarrosulus* Mont. |
| | Oyster mushroom | *Pleurotus ostreatus* (Fr.) Guel. |
| | | *Pleurotus ostreatus* (Fr.) Kummer. |
| | | *Pleurotus pulmonarius* (Fr.) Quelet . |
| | | *Pleurotus sajarcaju* (Fr.) Sing. |
| Family Russulaceae | Chestnut mushroom | *Agrocybe cytindracea* (Fr.) Gill. |
| Family Tremellaceae | White jelly mushroom | *Tremella fusiformis* Berk. |
| Family Tricholomataceae | Enokitake mushroom | *Flammulina velutipes* (Fr.) Curt. |
| Family Volvariaceae | Straw mushroom | *Volvariella volvacea* (Bull. Ex. Fr.) Sing. |

**2.2 Antimicrobial Activity**

**2.2.1 Microorganisms**

The following strains of tested bacteria were used: *Bacillus cereus* ATCC 11778, *Enterobacter aerogenes*, *Escherichia coli* O157:H7, *Micrococcus luteus* ATCC 9341, *Proteus vulgaris* ATCC13315, *Salmonella typhimurium* ATCC13311, and *Staphylococcus aureus* ATCC 25923. Each bacterial stain was sub-cultured on Nutrient agar (NA) (Merck, Germany) to ensure the purity of the culture. The bacteria were grown in Nutrient broth (NB) overnight at 37°C before use in antimicrobial assay.

**2.2.2 Screening of Antibacterial Activity of Mushroom Extracts**

Antibacterial activity of mushroom crude extracts was determined by paper disc diffusion assay. Each bacterial strain was grown in NB at 37°C for 48-72 hr and the bacterial suspensions were adjusted to the concentration of $10^6$ cfu/ml. A sterile cotton swab was used to apply each bacterial suspension onto the entire surface of the NA plate. Twenty µl of 100 mg/ml of crude extracts in DMSO were applied to the blank discs (1 mg/disc) and placed on a bacteria-seeded plate and incubated at 37°C for 72 hr. Amoxicillin (20 mg/disc) was used as a positive control. Antibacterial activities were determined by measuring the diameter of the inhibition zone, and the mean value was calculated. All experiment was performed in triplicates.

**2.3 Determination of Minimum Inhibitory Concentration (MIC)**

MIC tests were used to determine the lowest concentration of each mushroom extract that could inhibit the growth of test bacteria by the modified technique described by Hirasawa et al., (1999) [15]. Bacterial suspensions were prepared to contain approximately $10^6$ cfu/ml. They were inoculated on 96-well microlitre plates containing mushroom extracts (10-100 mg/ml) on NB and incubated at 37°C for 48 hr. Bacterial growth was monitored by microlitre plate reader at 600 nm and culture broth (0.1 ml) were spread on NA to confirm the absence of bacterial growth. MIC was defined as the concentration of mushroom extracts that inhibited the visible growth of tested strain.

**2.4 Antioxidant Activity**

The ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic) radical cation scavenging activity was performed with slight modifications described by Re et al. (1999) [20]. The ABTS radicals were produced by the reaction between 7 mM ABTS in water and 140 mM potassium persulfate, stored in the dark at room temperature for 12 hr. Prior to use, the solution was diluted with ethanol to get an absorbance at 734 nm. Free radical scavenging activity was assessed by mixing 200 µl of test sample with 1.8 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measures exactly after 6 min. The percentage inhibition was calculated according to the formula: $[(Ao-A1)/Ao] \times 100$, where, Ao was the absorbance of the control, and A1 was the absorbance of the sample.

Trolox, a derivative of vitamin E, was used as a standard. Antioxidant activity of each sample was expressed as Trolox equivalent antioxidant capacity (TEAC) which represented the concentration (µM) of Trolox, having the same activity as 1 mg of sample. All determinations were carried out in triplicates. The $IC_{50}$ was calculated from dose-response curve.

### 2.5 Bioassay-guided Separation

Mushroom extract which showed broad spectrum antibacterial activity was fractionated by preparative Thin layer chromatography (TLC), using Whatman 20×20 $cm^2$ silica gel plates with fluorescent indicator and eluted with benzene-acetone-acetic acid (7:3:0.5 v/v/v). Following development of the TLC plates, the active fraction with activity against tested bacteria was visualized under UV light (254 nm). Structural analyses were performed using proton NMR spectroscopy (300 MHz).

### 2.6 $^1$H-NMR Spectroscopy of Mushroom Extracts

$^1$H-NMR spectra were obtained using a Bruker DRX 400 spectrometer, with a 5-mm inverse probe. The samples being dissolved in DMSO-$d_6$ followed by a drop of $D_2O$, for OH group exchange. Chemical shifts (d) are expressed relative to the resonance of $Me_4Si$ (d = 0) obtained in a separate experiment. Coupling constants and chemical shifts were obtained from a first-order analysis of the spectra.

### 2.7 Statistical Analysis

All assays were carried out in triplicates and results are expressed as mean ± standard deviation (SD). The data were analyzed using SPSS software. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used to analyze the difference among scavenging activity and $IC_{50}$ of various extracts for antioxidant assays with least significance difference (LSD) at $P \leq 0.01$ as a level of significance. Experimental results were further analyzed for Pearson correlation coefficient of total phenolic with antioxidant assays.

## 3. RESULTS

### 3.1 Total Yields of Thai Edible Mushroom Extracts using Different Solvents

Fifteen edible mushroom species (Table 1) were extracted and screened for their antibacterial activity. Total yields of the mushroom extracts were varied depending on mushroom strains and solvents used (Table 2). The yields from mushroom extractions were in the range of 0.3-27.8 %, and always higher in water extracts, followed by ethanol, methanol, ethyl acetate, and hexane extracts (Table 2), respectively. Straw mushroom (*Volvariella volvacea*) in water extract showed significantly highest yield of crude extract (55.6 mg) followed by oyster mushroom (*Pleurotus ostreatus* (Fr.) Kummer (44.5 mg) and *Pleurotus sajarcaju* (Fr.) Sing. (40.3)) in water extract, while shiitake mushroom (*Lentinula edodes* and *Lentinus polychrous*) in hexane extract gave least yield of crude extract (0.6 mg).

### 3.2 Antibacterial Activities of Thai Edible Mushroom Extracts

In an attempt to find new drugs to treat infections, 75 Thai edible mushroom extracts were tested for antibacterial activity by agar disc diffusion assay against food-borne pathogenic bacteria. Forty-seven extracts showed inhibitory effects against tested bacteria, and 23 extracts showed broad-spectrum activity against both Gram-positive and Gram-negative bacteria. However, only 4 extracts, i.e. Flammulina velutipes in ethyl acetate extract, *Ganoderma lucidum* in methanol extract, *Pleurotus pulmonarius* in water extract and *Pleurotus ostreatus* in ethyl acetate extract showed inhibition zones against test bacteria greater than 10 mm

(Table 3). Negative control (DMSO) displayed no inhibition zone, whereas amoxicillin (20 mg/disc) (a positive control) displayed a large inhibition zone (20-25 mm) against all tested bacteria. Ethyl acetate extracts showed broad-spectrum activity against all tested bacteria in *Flamulina velutipes*, *Ganoderma lucidum*, *Hypsizygus marmoreus*, *Pleurotus ostratus* (Fr.) Kummer, *Pleurotus pulmonarius* and V*olvariella volvacea*, while hexane extracts in *Auricularia auricular*, *Flammulina velutipes*, *Ganoderma lucidum*, *Lentinus polychrous*, *Pleurotus ostreatus* (Fr.) Guel and *Tremella fusiformis* showed weak activity against only *Bacillus cereus*. *Ganoderma lucidum* in methanol extract showed significantly highest activity against *Bacillus cereus* and *Micrococcus luteus* (20 mm), while, aqueous extract of *Pleurotus pulmonarius* showed significantly highest activity against all bacteria tested (Table 3). The cultivated mushroom strains, i.e. *Flammulina velutipes*, *Ganoderma lucidum*, *Pleurotus pulmonarius* and *Pleurotus ostreatus* (Fr.) Guel. showed significant broad-spectrum activity against all tested bacteria (Table 3 and Figure 1). They were tested for the MIC values of extracts.

**Table 2.** Total yield of crude extract obtained from mushroom species using different extraction solvents.

| Strain/Species of Mushroom | Yield (mg) of crude extract / Solvent | | | | |
|---|---|---|---|---|---|
| | Hexane | Ethyl acetate | Methanol | Ethanol | Water |
| *Agrocybe cytindracea* (Pers.) Fayod. | 0.7±0.1 k | 2.1±0.7 j | 19.3±1.1 e | 8.0±0.7 g | 16.5±1.1 f |
| *Amanita vaginata* (Bull. Ex. Fr.) Vitt. | 9.6±0.7 g | 12.6±1.1 f | 28.8±1.5 cd | 21.8±1.3 d | 29.2±1.5 cd |
| *Auricularia auricular* (Hook.) Undrew. | 0.8±0.1 k | 1.4±0.7 j | 2.7±0.7 i | 1.6±0.1 j | 6.0±0.1 h |
| *Flammulina velutipes* (Curtis) Sing. | 0.8±0.3 k | 1.9±0.7 j | 23.8±1.3 d | 14.6±0.9 f | 25.0±1.3 d |
| *Ganoderma lucidum* (Fr.) Karst. | 0.7±0.1 k | 5.4±1.1 h | 7.0±0.7 h | 5.1±0.1 h | 13.5±1.1 f |
| *Hypsizygus marmoreus* | 0.8±0.1 k | 1.5±0.5 j | 3.1±0.1 i | 1.9±0.1 j | 5.9±0.3 h |
| *Lentinula edodes* (Berk.) Sing. | 0.6±0.1 k | 1.6±0.3 j | 2.9±0.1 i | 1.7±0.1 i | 6.8±0.3 h |
| *Lentinus polychrous* Lev. | 0.6±0.1 k | 1.4±0.1 j | 3.5±0.1 i | 2.1±0.1 i | 14.0±0.7 f |
| *Lentinus squarrosulus* Mont. | 0.7±0.1 k | 1.3±0.1 j | 2.5±0.1 i | 1.5±0.1 j | 7.5±0.1 g |
| *Pleurotus ostreatus* (Fr.) Quel. | 1.3±0.3 j | 1.5±0.1 j | 12.0±0.1 f | 7.3±0.3 h | 32.0±1.7 c |
| *Pleurotus ostreatus* (Fr.) Kummer | 1.0±0.1 k | 2.9±1.1 i | 20.1±1.3 e | 9.9±0.5 g | 44.5±1.5 b |
| *Pleurotus pulmonarius* (Fr.) Quel. | 0.8±0.3 k | 1.8±0.7 j | 15.6±1.3 f | 5.7±0.3 h | 24.5±0.7 d |
| *Pleurotus sajarcaju* (Fr.) Sing. | 0.9±0.3 k | 2.5±0.7 i | 19.0±1.1 e | 8.8±0.5 g | 40.3±1.3 b |
| *Tremella fusiformis* Berk. | 0.7±0.1 k | 5.7±1.1 h | 4.0±0.3 h | 1.7±0.1 j | 15.0±0.3 f |
| *Volvariella volvacea*(Bull. Ex. Fr.) Sing. | 0.9±0.3 k | 1.5±0.7 j | 30±1.7 c | 12±1.0 f | 55.6±1.1 a |

* The results are means ± SD. Means with different letter are significantly different from each other. The same letters of homogeneity groups denote non-significant difference of ANOVA test ($P \leq 0.01$) among average of yield of crude extract.

**Table 3.** Inhibitory effect of some crude extracts derived from mushroom on some food-borne pathogenic bacteria.

| Mushroom | Solvent | Inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | | | Gram-negative bacteria | | |
| | | BC[1] | ML[1] | SA[1] | EA[2] | EC[2] | PV[2] | ST[2] |
| *Agrocybe cytindracea* | Ethyl acetate | 7.2±0.25 d | - | 6.1±0.10 d | 6.2±0.15 d | - | - | - |
| | $H_2O$ | 6.5±0.25 d | 6.6±0.20 d | 6.2±0.25 d | 6.2±0.15 d | - | 6.6±0.15 d | 6.2±0.25 d |
| *Amanita vaginata* | Ethyl acetate | 6.2±0.15 d | - | - | 6.1±0.10 d | - | 6.2±0.25 d | 6.4±0.35 d |
| | Ethanol | 7.0±0.10 d | 6.2±0.15 d | - | - | - | - | 6.5±0.25 d |
| *Auricularia auricula* | Ethyl acetate | 7.0±0.35 d | - | - | 6.2±0.25 d | - | 6.3±0.15 d | 6.1±0.20 d |
| *Flammulina velutipes* | Ethyl acetate | 7.0±0.35 d | 9.0±0.35 c | - | 10.0±0.50 b | 9.0±0.35 c | 8.0±0.15 cd | 7.0±0.10 d |
| *Ganoderma lucidum* | Ethyl acetate | 8.0±0.35 c | 8.5±0.35 c | 8.5±0.25 c | 6.2±0.25 d | 8.5±0.35 c | 7.0±0.20 d | 8.0±0.15 c |
| | Methanol | 20.0±0.50 a | 11.0±0.35 b | 14.0±0.25 b | - | 11.0±0.35 b | - | 13.0±0.25 b |
| | Ethanol | 8.0±0.20 c | 8.5±0.20 cd | 8.5±0.35 cd | 6.2±0.25 d | 8.5±0.20 cd | 7.0±0.15 d | 8.0±0.35 c |
| | $H_2O$ | 8.5±0.25 c | 6.2±0.25 d | - | - | 6.2±0.25 d | - | - |
| *Hypsizygus marmoreus* | Ethyl acetate | 7.2±0.15 d | 6.9±0.35 d | 6.1±0.15 d | 6.5±0.15 d | 6.9±0.35 d | 8.0±0.35 cd | - |
| *Lentinula edodes* | Ethyl acetate | 6.5±0.15 d | 6.3±0.15 d | 6.2±0.35 d | 6.8±0.10 d | 6.3±0.15 d | 6.9±0.25 d | 6.8±0.15 d |
| | $H_2O$ | 6.1±0.10 d | - | 6.1±0.25d | - | - | 6.2±0.15 d | 6.3±0.35 d |
| *Lentinus polychrous* | Ethyl acetate | - | 6.1±0.10 d | - | 6.1±0.35 d | 6.1±0.10 d | 6.2±0.15 d | - |

**Table 3.** Continued.

| Mushroom | Solvent | Inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | | | Gram-negative bacteria | | |
| | | BC[1] | ML[1] | SA[1] | EA[2] | EC[2] | PV[2] | ST[2] |
| *Lentinus polychrous* | $H_2O$ | - | - | 6.1±0.15 d | 6.2±0.25 d | - | 6.4±0.10 d | 6.2±0.25 d |
| *Lentinus squarrosulus* | Ethyl acetate | 7.2±0.15 d | 6.2±0.25 d | - | 6.5±0.10 d | 6.2±0.25 d | - | 6.1±0.10 d |
| *Pleurotus ostreatus (Fr.) Guel.* | Ethyl acetate | 9.0±0.45 c | 9.0±0.75 c | - | 7.0±0.25 d | 9.0±0.75 c | 8.5±0.25 c | 7.0±0.25 d |
| | Methanol | 6.5±0.10 d | 6.3±0.20 d | - | - | 6.3±0.20 d | - | 6.5±0.25 d |
| | Ethanol | 12.0±0.45 b | - | - | 8.0±0.50 cd | - | 6.1±0.25 d | 7.1±0.25 d |
| *Pleurotus ostreatus (Fr.) Kummer* | Ethyl acetate | 7.1±0.25 d | 6.4±0.15 d | 6.1±0.25 d | 6.5±0.15 d | 6.4±0.15 d | 7.0±0.45 d | 6.5±0.15 d |
| *Pleurotus pulmonarius* | Ethyl acetate | 7.1±0.25 d | 6.3±0.20 d | 6.1±0.20 d | 6.2±0.15 d | 6.3±0.20 d | 6.3±0.15 d | 6.3±0.25 d |
| | $H_2O$ | 15.0±0.50 b | 8.0±0.35 cd | 7.5±0.25 d | 8.5±0.35 c | 8.0±0.35 cd | 8.0±0.50 cd | 9.0±0.15 c |
| *Pleurotus sajarcaju* | Ethyl acetate | - | - | - | 6.1±0.15 d | - | - | - |
| *Tremella fusiformis* | Hexane | 9.0±0.35 c | - | - | - | - | - | - |
| *Volvariella volvacea* | Ethyl acetate | 7.0±0.25 d | 6.1±0.15 d | 6.1±0.15 d | 6.2±0.10 d | 6.1±0.15 d | 6.1±0.15 d | 6.1±0.20 d |
| *Amoxicillin* | $H_2O$ | 20±0.50 a | - | - | 20±0.25 a | 25±0.25 a | 20±0.35 a | 20±0.25 a |
| *DMSO* | $H_2O$ | - | - | - | - | - | - | - |

Extracts were used at the final concentration of 20 µl /ml. Each paper disc was soaked with 20 µl of the aliquot. -; Not detect, *Bacillus cereus* (BC), *Enterobacter aerogenes* (EA), *Escherichia coli* (EC), *Micrococus luteus* (ML), *Proteus vulgaris* (PV), *Salmonella typhimurium* (ST), *Staphylococcus aureus* (SA). Inhibition zone was measured (n=3) after 24 hr at inhibition at 37°C. Mean with the same letter is not significantly different by ANOVA test ($P \leq 0.01$) among average of inhibitory effect of crude extracts.

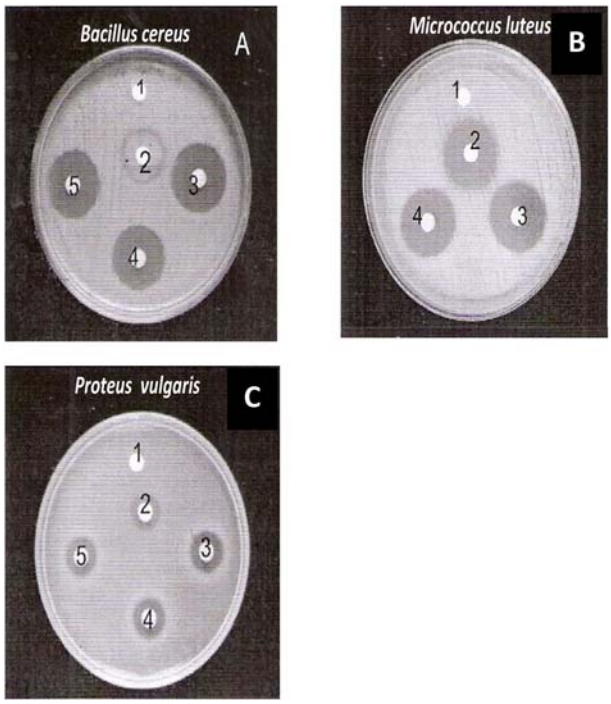


**Figure 1.** Inhibitory effects of *Pleurotus pulmanarius* in water extract on the bacterial growth, *Bacillus cereus* (A), *Micrococus luteus* (B), *Proteus vulgaris* (C). 1 = DMSO 20 µl, 2 = amoxicillin (20 mg/ml), 20 µl and 3, 4, 5 = crude extract 20 µl.

### 3.3 MICs of Most Distinctive Thai Cultivated Mushroom Extracts

The MIC values of selected cultivated mushroom strains, i.e. *Flammulina velutipes*, *Ganoderma lucidum*, *Pleurotus pulmonarius* and *Pleurotus ostreatus* (Fr.) Guel. showed significant broad-spectrum activity against all tested bacteria (Table 4). *Bacillus cereus* was most sensitive as it had lowest MICs range (1.25-5.0 mg/ml), while *Salmonella typhimurium* had highest MICs (12.5-22.5 mg/ml). The lowest MIC value of the extracts was 1.25 mg/ml, while amoxicillin had significantly higher inhibitory effect against most bacteria tested (1.25-2.75 mg/ml). *In vitro* activity of some mushroom extracts suggested that the compound has weak activity against *Salmonella typhimurium*. *Pleurotus pulmonarius* extract had significantly highest activity against all tested bacteria (1.25-15.5 mg/ml), followed by *Pleurotus ostreatus* (Fr.) Guel. extract (1.50-17.5 mg/ml), *Ganoderma lucidum* extract (1.50-37.5 mg/ml) and *Flammulina velutipes* extract (2.5-22.5 mg/ml) (Table 4).

**Table 4.** The minimal inhibitory concentration (MIC) of crude extracts from cultivated mushroom.

| Mushroom | Solvent | MIC (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Gram-positive bacteria | | | | Gram-negative bacteria | | |
| | | BC | ML | SA | EA | EC | PV | ST |
| *Flammulina velutipes* Karst. | Ethyl acetate | 5.00±1.1d | 6.25±1.3e | 6.25±1.1e | 6.25±1.0e | 5.50±0.3d | 2.50±0.5b | 22.5±1.7g |
| *Ganoderma lucidum* (Fr.) Karst. | Methanol | 1.50±0.1ab | 2.75±0.9b | 3.00±0.1c | 25.0±1.7g | 5.25±0.1d | 3.75±0.7c | 12.5±1.0f |
| *Pleurotus pulmonarius* (Fr.) Quelet | $H_2O$ | 1.25±0.1a | 1.75±0.7ab | 2.50±0.5b | 10.0±0.3f | 5.25±0.1d | 5.50±0.9d | 15.5±1.3f |
| *Pleurotus ostreatus* (Fr.) Guel. | Ethyl acetate | 1.50±0.3ab | 3.00±0.5c | 3.25±0.5c | 12.5±0.3f | 5.50±0.3d | 5.25±0.7d | 17.5±1.3fg |
| Amoxicillin (positive control) | $H_2O$ | 1.25±0.1a | 1.25±0.1a | 1.75±0.3ab | 2.25±0.1b | 2.75±0.1b | 2.25±0.3b | 2.25±0.1b |

Each paper disc was soaked with 20 µl of the aliquot with the varied final concentration (1.25-50 mg/ml), amoxicillin was used as a positive control. *Bacillus cereus* (BC), *Micrococus luteus* (ML),*Staphylococcus aureus* (SA), *Enterobacter aerogenes* (EA), *Escherichia coli* (EC),*Proteus vulgaris* (PV), *Salmonella typhimurium* (ST).

### 3.4 ABTS Radical Scavenging Activity

The ABTS radical scavenging test is used to determine the antioxidant activity of hydrophilic and lipophilic compounds. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and chain-breaking antioxidants [21]. The scavenging ability of mushroom extracts and Trolox (vitamin E derivative) on ABTS radicals were 91-94 % and 97 %, respectively. The scavenging activity was better in *Gannoderma lucidum*, followed by *Pleurotus pulmonarius* and *Flammulina velutipes*, respectively. The ABTS radical scavenging activity of various extracts indicates their ability to scavenge free radicals by preventing lipid oxidation via a chain-breaking reaction. The $IC_{50}$ values varied from 2.81 mg/ml to 10.57 mg/ml for all samples tested (Table 5). The $IC_{50}$ values for mushroom extracts were simplified as *Ganoderma lucidum* (2.81 mg/ml), followed by *Pleurotus pulmonarius* (3.89 mg/ml) and *Flammulina velutipes* (10.57 mg/ml), respectively. Methanolic extracts of *Ganoderma lucidum* was assumed to be the strongest inhibitor which showed 94% inhibition of ABTS free radicals at the lowest concentration (2.81 mg/ml) among all tested extracts.

**Table 5.** Comparison of antioxidant activity and $IC_{50}$ value of the aqueous extract of mushroom.

| Crude extracts of mushroom | Antioxidant activity (TEAC) | $IC_{50}$ (mg/ml) |
|---|---|---|
| Flammulina velutipes | 3.38±0.02 c | 10.57±0.27 c |
| Ganoderma lucidum | 12.69±0.41 a | 2.81±0.02 a |
| *Pleurotus pulmonarius* | 9.19±0.50 b | 3.89±0.13 b |
| Standard vitamin E | ND | 0.01±0.0002 d |

TEAC expressed as mM Trolox (Vitamin E derivative) per gram extract, $IC_{50}$ = inhibitory concentration.
The values of $IC_{50}$ are expressed as mean ± SD (n=3). ND = Not detectable.

### 3.5 NMR Structure Elucidation

Water extract of Indian oyster mushroom (Pleurotus pulmonarius) was a light yellow color and the major compound was separated using preparative TLC with the $R_f$ value of 0.48. Thus, the separated compounds were analyzed using $^1$H-NMR, focusing on the interpretation of mainly functional groups. In the $^1$H-NMR spectrum of the main compound, which indicated the presence of polysaccharides, there were signals of different intensities as well, including the anomeric proton signals for four rhamnose residues at d 4.98, 5.04 and 5.06 (2H) (broadened singlet) and one Fuc3HAc residue at d 5.11 (doublet). Only two anomeric proton signals of the minor series were clearly observed, at d 4.94 (broadened singlet, Rha H1) and 5.13 (doublet, Fuc3HAc H1).

## 4. DISCUSSION

The crude extracts of edible mushroom were tested against food-borne pathogenic bacteria. In this study, the mushroom crude extracts were low in yields due to the gel formation leading to the difficulty in filtration [5]. Water extraction resulted in significantly highest yields, while, hexane extraction gave the lowest yield (Table 2). Due to the application of mushroom extracts in traditional medical treatments, the water extraction can be an option of advantageous and safety process. Antibacterial activities from mushroom extracts were detected at varied levels (Table 2). This could be due to the difference in solubility of organic solvents and water of the mushroom constituents in each species. On the other hand, these test strains may have different level of intrinsic tolerance to mushroom crude extracts and the inhibition effect differs from strain to strain. In this study, the water extract of *Volvariella volvacea* gave significantly higher yield than other solvent extracts (Table 2), however, the extract had less antibacterial activities (Table 3). Although, the water extracts of *Flammulina velutipes*, *Ganoderma lucidum*, *Pleurotus pulmonarius* and *Pleurotus ostreatus* (Fr.) Guel significantly inhibited the growth of all tested bacteria, only aqueous extracts from *Pleurotus pulmonarius* showed significant antibacterial activity against all tested bacteria, and also with greater inhibitory activity compared to ethyl acetate extracts. Our results showed higher antibacterial activity of Indian oyster mushroom than those activities found in previous reports [22, 23]. This study indicated that there are differences in antibacterial effects of mushroom strains (Table 3), due to phytochemical differences among species and the sensitivity of microorganisms to the chemotherapeutic compounds which can change even against different strains [23]. Thus, the aqueous

extract of *Pleurotus pulmonarius* may contain antibacterial compounds that can dissolve in water. The difference in the inhibitory effect of water extract may be attributed to the production of secondary metabolites from the shikimic acid and cinnamic acid pathways during lignocellulosic degradation by *Pleurotus* sp., which may have antibacterial activity [24].

Antibacterial assay of hexane extracts from *Auricularia auricular*, *Flammulina velutipes*, *Ganoderma lucidum*, *Pleurotus ostreatus* and *Tremella fuciformis* showed weak activity only against *Bacillus cereus* (6.1-9.0 mm) (Table 3). Ethyl acetate extracts of *Flammulina velutipes*, *Ganoderma lucidum*, *Hypsizygus marmoreus*, *Lentinula edodes* and *Pleurotus ostreatus* significantly inhibited both Gram-positive and Gram-negative bacteria and extracts of *Flammulina velutipes*, *Ganoderma lucidum* and *Pleurotus ostreatus* significantly inhibited *Escherichia coli.* Methanol extract of *Ganoderma lucidum* showed significantly strong activity against all tested Gram-positive bacteria, and also moderate activity against some Gram-negative bacteria (Table 3). *Salmonella typhimurium* was resistant to all mushrooms extracts (12.5-22.5 mg/ml). The standard amoxicillin presented lower MICs (2.25 mg/ml) than the mushroom extracts (Table 4) because antibiotics and pure active compounds revealed more activity than crude extracts. Cell membrane of Gram-negative bacteria contains outer membrane, which is formed by lipoproteins, lipopolysaccharides and phospholipid that show lipophilicity. However, the major compound derived from mushroom extracts showed water-solubility, which makes the compound difficult to transport through outer membrane of the Gram-negative cell [25]. Our results agree with methanol extract of *Ganoderma lucidum* from India, which demonstrated efficient antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella* sp. [23] Broad-spectrum inhibition of the mushroom extracts against different groups of bacteria might concern the physicochemical properties of the bioactive compounds in the solvents used [26]. However, the variation in these antibacterial activities might be due to the differences in their bioactive compositions or concentrations, methods of extraction, and the susceptibility of the different bacterial strains tested [27].

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain-breaking antioxidant [28]. This method is often used in evaluating total antioxidant power of single compounds and complex mixtures of various mushrooms [29]. In recent years, multiple drug resistance for human pathogenic microorganisms has been developed due to indiscriminate use of commercial antibacterial drugs commonly used in the treatment of infectious diseases. The increasing demand for natural bioactive compounds in the pharmaceutical and food industries have risen in recent years. Since the mushroom contains a significant amount of vitamins, fibers, phenolic compounds and carotenoids, research interests have focused on the determination of antioxidant capacity. Antioxidant capacity of mushroom extracts is determined by the amount of phenolic compounds and their quantity influences the inhibition capacity of the free radicals. In the case of *Ganoderma lucidum*, *Pleurotus pulmonarius* and *Flammulina velutipes* extracts, they had good values of the content of total phenolics with antioxidant properties. Antioxidant activity of the cultivated mushrooms has significant importance because this activity greatly contributes to their nutraceutical properties which enhance nutritional value or functional food. Phenolic compounds such as phenolic acids and tannins are known as major components of

antioxidant in mushrooms. *Gannoderma* sp. and *Pleurotus* sp. contain several types of phenolic compounds such as vanillic acid [30], myricetin, naringin, homogentistic acid, 5-o-caffeoylquinic acid, chrysin, rutin, gentistic acid, gallic acid, protocatechuic acid, caffeid acid, tannic acid, syringic acid and p-coumaric acid [31]. The most antioxidant properties of mushrooms are in the form of phenolic acids and flavonoids, followed by tocopherols, ascorbic acid and carotenoids [32]. The $IC_{50}$ value, defined as the concentration of antioxidant required for 50% scavenging of ABTS radicals is a parameter used to measure antioxidant activity; a smaller $IC_{50}$ value corresponds to a higher antioxidant activity of the mushroom extract. $IC_{50}$ value of the mushroom crude extract was 2.81 mg/ml to 10.57 mg/ml (Table 5). ABTS radical scavenging activity of aqueous extract of mycelia indicates its ability to scavenge free radicals by preventing lipid oxidation via a chain-breaking reaction. These values showed that cultivated mushroom has higher antioxidant activity compared to vitamin E standard (Table 5).

Our research indicated that *Ganoderma lucidum*, *Pleurotus pulmonarius* and *Flammulina velutipes* had antioxidant and antibacterial properties (Table 3, Table 4 and Table 5). Our results agreed with Mondal *et al.* (2013) [33] who reported that Reishi and certain mushrooms had antioxidant, antibacterial and antifungal properties which were much effective against *Staphylococcus aureus* and *Escherichia coli* through agar-well diffusion method. Radical scavenging and antioxidant activities of *Ganoderma lucidum* extracts were higher than those of *Pleurotus pulmonarius* and *Flammulina velutipes*. All mushrooms used in this study were found to have various degrees of antibacterial effects against tested bacteria. The major constituents of the aqueous extract from *Pleurotus pulmonarius* appeared to be polysaccharides, which supported the presumption that antibacterial activity could be due to the presence of polysaccharides [25]. Use of synthetic bacteriocides to control food spoilage bacteria has been discouraged due to their effects on food, acute residual toxicity, long-term degradation and other side-effect in human [23]. The major problems related to the use of chemicals were the resistant of pathogenic bacteria. Use of higher concentration of chemical causes microbial resistance and enhances high level of toxic residues in products. Further, bioactive compounds are biodegradable and are nearly non-toxic residues in nature and safety to develop for commercial purposes with lower cost.

In conclusion, the mushroom mycelium contains many different bioactive compounds with diverse biological activities. *Pleurotus pulmonarus*, *Ganoderma lucidum* and *Flammulina velutipes* mycelium extracts have a strong inhibiting effect which linked with phenolic compounds, and other beneficial or therapeutic health effects in addition to the prevention of some food-borne disease. Results showed that the mushroom mycelium extracts could be used as a rich source of antibacterial and antioxidant in pharmaceutical-type products. It could be suggested that the aqueous extract of potential cultivation mushroom contains potential antibacterial compounds, antioxidant activity and may be useful for evaluating substances of interest. Further research could be structural elucidation of the bioactivecompounds of the polysaccharide substance derived from aqueous extracts of *Ganoderma lucidum*, *Pleurotus pulmonarius* and *Flammulina velutipes*.

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