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# Antimicrobial Activity of Extracts of the Oyster Culinary Medicinal Mushroom *Pleurotus ostreatus* (Higher Basidiomycetes) and Identification of a New Antimicrobial Compound

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**ABSTRACT:** *Pleurotus ostreatus* is an edible mushroom that also has high medicinal values. In this study, *P. ostreatus* was tested for its ability to inhibit the growth of fungi and bacteria. The freeze-dried fruiting body, broth from submerged culture, and mycelial biomass of *P. ostreatus* were extracted using alcohols and water as solvents. The extracts were then tested for their antimicrobial activity against the growth of fungi and bacteria. It was observed that the water extract from fruiting bodies had the strongest effect in inhibiting the growth of most fungi. The most sensitive test microfungi to the inhibition were *Candida albicans*, *Cryptococcus humicola*, and *Trichosporon cutaneum*, and the most sensitive test bacteria were *Staphylococcus aureus* followed by *Escherichia coli*. Water extracts from culture broth or mycelial biomass were moderately inhibitive to the growth of fungi and bacteria. The alcohol-based solvents from all samples had much less antimicrobial activity against most test microorganisms. An antimicrobial compound was purified from the water extracts of fruiting bodies with Sephadex G l00 column chromatography and characterized by infrared absorption spectrum (IR), nuclear magnetic resonance (NMR), and mass spectroscopic analysis. We have identified this compound to be 3-(2-aminophenylthio)-3-hydroxypropanoic acid. This purified compound had a minimum inhibitory concentration of 30 µg/mL and 20 µg/mL against the growth of fungi and bacteria, respectively.

**KEY WORDS:** medicinal mushrooms, antimicrobial activity, oyster mushroom, *Pleurotus ostreatus*, mushroom extracts, 3-(2-aminophenylthio)-3-hydroxypropanoic acid

**ABBREVIATIONS: IR:** infrared absorption; **MSO:** dimethyl sulfoxide; **ME:** malt extract; **MIC:** minimum inhibitory concentration; **NA:** nutrient agar; **NMR:** nuclear magnetic resonance; **PDA:** potato dextrose agar; **RCMB:** The Regional Center for Mycology and Biotechnology, Cairo, Egypt; **TLC:** thin layer chromatography; **YMB:** yeast-malt broth

## I. INTRODUCTION

Infectious diseases are the second leading cause of death worldwide and remain a major threat to human health.[1] Antimicrobial compounds have played an important role in the treatment of these diseases. Bacteria,[2] fungi,[3] and plants are capable of producing a wide array of antimicrobial compounds and have been the main sources of antimicrobial drugs. Continuing exploration of new compounds from these sources will yield promising results. However, organisms that produce these compounds can themselves be pathogenic or poisonous to humans, requiring careful handling that can complicate the manufacturing processes.[4]

Edible mushrooms are nontoxic and have been used for food consumption since ancient times.[5] Mushrooms are sources of highly valued nutritional ingredients[6] including essential amino acids,[7] glycopeptides,[8] various carbohydrates such as pentoses, methyl-pentoses, hexoses, amino sugars, sugar alcohols, sugar acids, polysaccharides, glycogen, and

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ß-glucan,[9,10] dietary fiber,[11] unsaturated fatty acids,[12] vitamins such as thiamine, riboflavin, niacin, biotin, and ascorbic acid,[13] and various minerals such as potassium, phosphorus, sodium, calcium, magnesium, copper, zinc, and iron.[14] Some mushroom species are medicinally valuable due to their antimicrobial,[15] antiviral,[16] antioxidant,[17,18] antihypertensive,[19] cholesterol-lowering, cardiovascular diseases preventative,[20,21] liver protective,[22] anti-fibrotic,[23] anti-inflammatory,[24] anti-diabetic,[25] and anti-cancer[18] activities.

The antimicrobial activities of many species of mushrooms in various parts of the world have been extensively studied.[15,27,28] Both fruiting body and mycelium of many mushroom species contain antimicrobial compounds.[29] For example, *Lentinus edodes* and *Pleurotus ostreatus* mushrooms have antibacterial and antifungal properties.[30] Some species in the *Pleurotus* genus showed an ability to produce both antifungal and antibacterial agents that can be used to control fungal and bacterial infections.[31] Aqueous and ethanol extracts from fruiting bodies or mycelia of *P. ostreatus* possess different antimicrobial and antineoplasic activities,[32] so suitable solvents must be identified to effectively extract the active ingredients from these mushrooms.[33] In addition, mushrooms at different developmental stages may contain different types and amounts of antimicrobial compounds that require different solvents to maximize the yields of specific antimicrobial compounds.[34]

In this study, we investigated the levels of antimicrobial activity against selected fungal and bacterial species of extracts of *P. ostreatus* from both water and alcoholic solvents. We then isolated, purified, and identified a new antimicrobial compound.

## II. MATERIALS AND METHODS

### A. Mushroom Strain and Culture Conditions

The mycelia of *P. ostreatus* were grown and maintained on potato dextrose agar medium (PDA), which consisted of 4 g/L potato extract (Sigma-Aldrich, Missouri), 20 g/L dextrose (Pharmacia, New York), and 20g/L agar (Sigma-Aldrich). The mycelia were then transferred to a spawn medium, which was a mixture of cereal grains, calcium carbonate (Sigma-Aldrich), and calcium sulphate (Sigma-Aldrich) =100: 2: 1 (wt:wt:wt), and incubated at 22°C for 21 days. The spawns were then inoculated into 1–1.2 Kg of a rice straw medium (a mixture of rice straw, wheat bran, and gypsum powder = 100:5:5; wt:wt:wt) in plastic bags (24 × 60 cm) fitted with a cotton plug. The bags were sterilized with an autoclave for 40 min at 121°C under 1.5 × atmospheric pressure before the spawn inoculation. The inoculated bags were incubated in a dark mushroom house at 18°C with 95% humidity, and exposed to light for 2 h daily. The bags were ventilated with fresh air for 1 h daily until the mycelia fully penetrated into the substrate. After 15 days, the bags were opened to allow the production of fruiting bodies.

For submerged culture, the mycelial biomass of *P. ostreatus* were grown in 250 mL Erlenmeyer flasks containing 100 mL of a yeast-malt broth (YMB) medium, which consisted of 3 g/L yeast extract (Sigma-Aldrich), 3 g/L malt extract (Sigma-Aldrich), 10 g/L glucose (Pharmacia), and 5 g/L peptone (Pharmacia). The flasks were shaken at 120 rpm on a shaker and incubated at 24°C for 15 days. To separate the mycelial biomass from the cultural broth, the submerged cultures were filtered with a 0.2 μm microfilter and the filtrates were concentrated by a Virtis BT4KZL-105 freeze-dryer (SP Industries, Warminster, Pennsylvania) and stored at –20°C until use.

### B. Fungal and Bacterial Strains Used for Testing Antimicrobial Activity of Mushroom Extracts

Twenty test fungal strains, as listed in Table 1, were cultured on a malt extract agar (MEA) medium consisting (g/L) of 20 g malt extract, 20 g glucose, 1 g peptone, and 20 g agar. Ten test bacterial strains, as listed in Table 2, were cultured on a nutrient agar (NA) medium consisting (g/L) of 3 g beef extract (Sigma-Aldrich), 5 g peptone, and

**TABLE 1: Diameters of Inhibitory Zone Against Fungal Growth by Different Solvent Extracts from Fruiting Bodies, Broth, and Mycelia of *Pleurotus ostreatus***

| | RCMB* | Fruiting bodies | | | Broth | Mycelia | Control** | |
|---|---|---|---|---|---|---|---|---|
| Fungal species | Number | ethanol | methanol | water | water | water | ethanol | methanol |
| *Candida albicans* | RCMB (005 003) | 1 ± 0 | 3 ± 0 | 30 ± 2 | 12 ± 1 | 3 ± 0 | 1 ± 0 | 1 ± 0 |
| *Candida dubliniensis* | RCMB (005 004) | 4 ± 0 | 0 | 17 ± 1 | 7 ± 1 | 6 ± 1 | 1 ± 0 | 0 |
| *Cryptococcus humicola* | RCMB (050 001) | 6 ± 0 | 6 ± 1 | 30 ± 3 | 0 | 10 ± 1 | 2 ± 0 | 1 ± 0 |
| *Trichosporon cutaneum* | RCMB (049 001) | 0 | 0 | 30 ± 2 | 0 | 15 ± 2 | 0 | 0 |
| *Geotrichum candidum* | RCMB (034 001) | 6 ± 0 | 6 ± 0 | 25 ± 3 | 0 | 3 ± 0 | 2 ± 0 | 1 ± 0 |
| *Aspergillus flavus* | RCMB (002 002 “5”) | 0 | 0 | 23 ± 2 | 11 ± 2 | 0 | 0 | 0 |
| *Aspergillus fumigatus* | RCMB (002 008 “2”) | 3 ± 0 | 0 | 25 ± 3 | 7 ± 1 | 0 | 0 | 0 |
| *Aspergillus niger* | RCMB (002 007 “2”) | 0 | 0 | 12 ± 1 | 5 ± 0 | 0 | 0 | 0 |
| *Aspergillus terreus* | RCMB (002 006 “1”) | 0 | 0 | 14 ± 1 | 11 ± 2 | 0 | 0 | 0 |
| *Penicillium expansum* | RCMB (001 001 “1”) | 0 | 0 | 8 ± 0 | 0 | 3 ± 0 | 0 | 0 |
| *Penicillium roqueforti* | RCMB (001 009 “1”) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eupenicillium ludwigii* | RCMB (001 020 “1”) | 0 | 7 ± 1 | 15 ± 2 | 0 | 0 | 0 | 2 ± 0 |
| *Trichoderma viride* | RCMB (017 003 “2”) | 3 ± 0 | 3 ± 0 | 12 ± 2 | 12 ± 1 | 0 | 0 | 0 |
| *Drechslera rostrata* | RCMB (022 002) | 0 | 6 ± 1 | 23 ± 2 | 0 | 0 | 0 | 0 |
| *Curvularia clavata* | RCMB (019 001) | 3 ± 0 | 0 | 23 ± 2 | 5 ± 0 | 6 ± 1 | 1 ± 0 | 0 |
| *Alternaria chlamydospora* | RCMB (009 003) | 0 | 3 ± 0 | 19 ± 1 | 0 | 0 | 0 | 0 |
| *Botrytis cinerea* | RCMB (048 002) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Fusarium moniliforme* | RCMB (008 005 “2”) | 0 | 3 ± 0 | 25 ± 3 | 5 ± 0 | 3 ± 0 | 0 | 1 ± 0 |
| *Mucor rouxii* | RCMB (015 002) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Syncephalastrum racemosum* | RCMB (016 001 “2”) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Numbers represent the mean diam ± SD (mm) of inhibition zone with triplicated samples

*RCMB Number: Code numbers for test microorganisms in the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Egypt

**Control: Solvent only

**TABLE 2: Diameters of Inhibitory Zone Against Bacterial Growth by Different Solvent Extracts from Fruiting Bodies, Broth, and Mycelia of *Pleurotus ostreatus***

| | RCMB* | Fruiting bodies | | | Broth | Mycelia | Control | |
|---|---|---|---|---|---|---|---|---|
| **Bacterial species** | **Number** | **ethanol** | **methanol** | **water** | **water** | **water** | **ethanol** | **methanol** |
| *Staphylococcus aureus* | RCMB (B 001 001"3") | 5 ± 0 | 0 | 25 ± 2 | 12 ± 2 | 5 ± 0 | 2 ± 0 | 2 ± 0 |
| *Streptococcus pyogenes* | RCMB (B 002 001 "2") | 0 | 0 | 13 ± 1 | 17 ± 2 | 5 ± 0 | 0 | 0 |
| *Bacillus subtilis* | RCMB(B 003001"2") | 5 ± 0 | 0 | 16 ± 1 | 12 ± 1 | 0 | 1 ± 0 | 2 ± 0 |
| *Bacillus megaterium* | RCMB (B 003 002) | 0 | 0 | 8 ± 1 | 0 | 0 | 2 ± 1 | 0 |
| *Bacillus thuringiensis* | RCMB (B 003 003) | 7 ± 1 | 10 ± 1 | 16 ± 1 | 17 ± 2 | 0 | 1 ± 1 | 2 ± 1 |
| *Escherichia coli* | RCMB (B 004 001 "4") | 0 | 0 | 23 ± 3 | 12 ± 1 | 0 | 2 ± 1 | 2 ± 1 |
| *Klebsiella pneumoniae* | RCMB (B 005 001 "2") | 0 | 0 | 19 ± 2 | 12 ± 1 | 5 ± 0 | 0 | 0 |
| *Pseudomonas aeruginosa* | RCMB (B 008 001 "2") | 0 | 10 ± 1 | 19 ± 2 | 13 ± 1 | 0 | 0 | 1 ± 1 |
| *Shigella dysenteriae* | RCMB (B 006 001) | 0 | 6 ± 0 | 13 ± 1 | 13 ± 1 | 5 ± 0 | 3 ± 1 | 1 ± 0 |
| *Salmonella enterica* | RCMB (B 007 001) | 0 | 6 ± 0 | 8 ± 1 | 13 ± 1 | 0 | 2 ± 1 | 2 ± 0 |

Numbers represent the mean diam ± SD (mm) of inhibition zone with triplicated samples

*RCMB Number: Code numbers for test microorganisms in the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Egypt.

**Control: Solvent only

20 g agar. All microorganisms were obtained from the culture collection of (RCMB) Al-Azhar University, Cairo, Egypt.

### C. Extraction of Antimicrobial Agents

Fresh fruiting bodies were washed with distilled water, cut into small pieces, and lyophilized in the Virtis freeze-dryer. Ten g of the lyophilized fruiting bodies were added into 10 mL of methanol, 70% (v/v) ethanol, or distilled water, and then soaked overnight before they were ground by mortar and pestle. The mixtures were then centrifuged at 10,000 × g for 20 min to remove debris, and the supernatants were concentrated by lyophilization before they were stored at –20°C.

Lyophilized mycelial biomass pellets from submerged culture were suspended at 1 gm/mL in distilled water overnight at 24°C. They were then ground by mortar and pestle, pulverized in an ultra-sonicator (Branson 2510, at RCMB, Cairo) for 30 min at 25 KHZ, and subjected to centrifugation at 10,000 × g for 20 min to pellet the debris. The supernatants were concentrated by lyophilization followed by storing at –20°C until use.

### D. Assay of Antimicrobial Activity

Antimicrobial assays were carried out using an agar diffusion method[35] by spreading the suspension cultures of test fungi and bacteria (pregrown to log-phase) into petri dishes (150 × 20 mm) containing 40 mL of solidified ME and NA media, respectively. The plates were then punched with a 1 cm diameter cork borer to create wells. The lyophilized powders of extracts were suspended in their original extraction solvents at 100 mg/mL, and 100 µL of each suspended extract was added into each well. The control wells were filled only with the same solvents that were used for the extraction. The plates were first incubated for 2 h at 4 ± 2°C followed by 48 h at 28 ± 2°C and 24 h at 37 ± 2°C for fungi and bacteria/yeast, respectively. All experiments were carried out in triplicate. Antimicrobial activities were determined by measuring the diameters in millimeter of inhibition zone. After subtracting the diameters of the inhibition zone from the controls, the standard deviation (SD) of the mean was calculated from the triplicated samples for each test.

### E. Purification of the Active Antimicrobial Compound

Column chromatography was carried out by using a Pharmacia column (40 × 2 cm) packed with Sephadex G l00 (Pharmacia) in a Gilson FC 203B LPLC system (Gilson, Inc., Middletown, Wisconsin) with UV/VIS-151 detector and a pump. The water extracts of the fruiting body were loaded into the column and 42 fractions were collected at 3 mL per tube. All fractions were tested for antimicrobial activity by the agar diffusion method described above.

Fractions having high antimicrobial activity were subjected to thin layer chromatography (TLC) using F254 17 cm silica gel plates (Merk Inc., Kansas), with a solvent mixture consisting of butanol, acetic acid, and water = 4: 1: 1 (v/v/v). The plates were analyzed by a CAMAG TLC Scanner 3 system (CAMAG, Wilmington, Delaware).

### F. Minimum Inhibitory Concentration (MIC) Assay

Fractions with high antimicrobial activity were further investigated to determine their MIC against two bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Aspergillus fumigatus*). Concentrations between 10 and 100 µg/mL of each fraction were tested by the agar diffusion method as described above.

### G. Identification of the Most Active Antimicrobial Compound

The infrared (IR) absorption spectrum of the purified fraction was analyzed using a BRUKER IR Victor 22 Spectra system (Bruker Corp., Billerica, Manning Park , Massachusetts). Nuclear magnetic resonance (NMR) spectra analysis of the purified

fraction was performed in deuterated dimethyl sulfoxide (DMSO-d6) on a Varian Mercury-VX-300 NMR spectrometer (Bruker crop). Mass spectroscopy of the active fraction was performed in a Shimadzu QP-5050 GC-MS (Shimadzu Corp. Kyoto, Japan) using the direct inlet unit to determine the purity of the active compounds.

## III. RESULTS

### A. Antifungal Activity of Different Extracts from *Pleurotus ostreatus*

When the antifungal growth activities in extracts of ethanol, methanol, and water from fruiting bodies were compared, the water extract showed the highest inhibitory activity against most of the fungi tested, producing the largest inhibition zone of 30 mm against both *Candida albicans*, *Cryptococcus humicola* and *Trichosporon cutaneum*, followed by 23–25 mm against *Geotrichum candidum*, *Aspergillus flavus*, *A. fumigatus*, *Drechslera rostrata*, *Curvularia clavata*, and *Fusarium moniliform* (Table 1). The smallest inhibition zones of 8 mm *Penicillium expansum* and the growth of *P. roqueforti*, *Botrytis cinerea*, *Mucor rouxii*, and *Syncephalastrum racemosum* were not affected. In comparison, the ethanol extract from fruiting bodies produced a 6 mm mean diameter of inhibition zone against *Cryptococcus humicola* and *Geotrichum candidum*, and 4, 3, 3, 3, and 1 mm against *Candida dubliniensis*, *Aspergillus fumigatus*, *Trichoderma viride*, *Curvularia clavata*, and *Candida albicans*, respectively (Table 1). The ethanol extract did not have any inhibitory effect on the growth of any other fungi tested. The methanol extract from fruiting bodies showed the largest inhibition zone of 7 mm against *Eupenicillium ludwigii* followed by 6 mm against *Cryptococcus humicola*, *Geotrichum candidum*, and *Drechslera rostrata* (Table 1). The fungal growths of *Candida albicans*, *Trichoderma viride*, *Alternaria chlamydospora*, and *Fusarium moniliforme* were slightly affected, having only a 3 mm inhibition zone.

For the cultural broth and mycelial biomass, only water extracts were used in the antimicrobial assays. The largest inhibitory effect on fungal growth by cultural broth produced an inhibition zone of 12 mm against both *Candida albicans* and *Ttichoderma viride* and 11 mm against both *Aspergillus flavus* and *A. terreus.* The smallest zone sizes were between 5 and 7 mm against *Candida dubliniensis*, *Aspergillus fumigatus*, *A. niger*, *Curvularia clavata*, and *Fusarium moniliforme*. No inhibitory effect was observed on other test fungi.

The water extract of mycelia grown on YMB inhibited the growth of *Trichosporon cutaneum* with 15 mm of inhibition zone, followed by *Cryptococcus humicola* with 10 mm. The growth of *Candida dubliniensis*, *C. albicans*, *Curvularia clavata*, *Geotrichum candidum*, *Penicillium expansum*, and *Fusarium moniliforme* were also slightly inhibited by the water extract from mycelia with inhibition zones ranging from 3 to 6 mm (Table 1). No inhibitory effect was observed on other test fungi.

### B. Antibacterial Activity of Different Extracts of *Pleurotus ostreatus*

The ethanol extract of fruiting bodies from *P. ostreatus* inhibited the growth of *Bacillus thuringiensis* with 7 mm diameter of inhibition zone, followed by both *Staphylococcus aureus* and *Bacillus subtilis* with 5 mm zone. No other bacteria were inhibited (Table 2). The methanol extract inhibited only the growth of *B. thuringinsis* and *Pseudomonas aeruginosa* with 10 mm of inhibition zone, and *Shigella dysenteriae* and *Sh. enterica* with 6 mm. No other bacterial strains were affected. The water extract had a growth inhibitory activity against all bacteria tested with highest inhibition zone of 25 mm on *Staphylococcus aureus*, followed by 23 mm on *E. coli*. The inhibition zones were 19 mm on both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, 16 mm on both *Bacillus subtilis* and *B. thuringinsis*, 13 mm on both *Streptococcus pyogenes* and *S. dysenteriae*, and 8 mm on both *Bacillus megaterium* and *Shigella enterica*.

The water extract of cultural broth produced 17 mm zone against *Sh. enterica* and *Bacillus thuringiensis*, followed by 13 mm on *Pseudomo-*

*nas aeruginosa*, *Streptococcus dysenteriae*, and *S. pyogenes.* For *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, it showed 12 mm of inhibition zone. No inhibition was observed with *Bacillus megaterium* (Table 2).

The water extract of mycelial biomass showed a low inhibitory effect on the growth of bacteria. Only the growths of *Streptococcus pyogenes*, *Klebsiella pneumonia*, and *Streptococcus dysenteriae* were slightly inhibited, having an inhibition zone of 5 mm (Table 2). No inhibition was detected on other bacteria.

### C. Purification of the Antimicrobial Compound by Column Chromatography and TLC

The water extract of the fruiting bodies was subjected to column chromatography with Sephadex G l00. Among the 42 fractions collected, the fraction number 12 had the highest overall antimicrobial activity with inhibition zone of 30 mm against *Candida albicans*, 25 mm against *Aspergillus fumigatus*, 25 mm against *Staphylococcus aureus*, and 23 mm against *Escherichia coli*. Fraction number 12 yielded only a single band on the TLC plate with $R_f$ = 0.63, indicating that this fraction contained only a single compound detectable by the TLC.

### D. Characterizations of the Purified Compound by IR, NMR, and Mass Spectroscopies

Infrared (IR) spectroscopy of fraction number 12 indicated the presence of a hydroxyl group (OH) by a band at 3417.6 $cm^{-1}$ and the presence of a carbonyl group (C-O) by bands at 1118.6 $cm^{-1}$ and 1245.9 $cm^{-1}$ [Fig. 1(a)]. The existence of an amino group ($NH_2$) was indicated by the presence of band at 3799.5 $cm^{-1}$ and the presence of the aromatic ring can be detected by the presence of a band at 1643.2 $cm^{-1}$. A sulfur molecule was indicated by the presence of a band at 2021.3 $cm^{-1}$ whereas the existence of an alkane (–CH) or ($CH_2$) group can be indicated by the presence of a band at 2923.9 $cm^{-1}$.

Nuclear magnetic resonance (NMR) spectroscopy in $CDCl_3$ [Fig. 1(b)] produced signals at 10.613 ppm that represented a naphthalene with a carboxyl group (–COOH). In addition, an aromatic ring was indicated by a signal at 7.547 ppm; a hydroxyl group (–OH) by a signal at 3.233 ppm; an amino group ($NH_2$) by a signal at 2.509 ppm; a sulfur by 2.562 ppm; and an alkane group by 6.947 ppm. The mass spectrum of mass spectroscopy [Fig. 1(c)] showed many fragmental peaks from the molecular ionization of a pure compound that has a molecular weight of 215 as signaled at M/Z.

On the basis of the characterizations by IR, NMR, and mass spectroscopies as described above, we suggest this compound to be 3-(2-aminophenylthio)-3-hydroxypropanoic acid with a molecular formula of $C_9H_{10}NO_3S$ and the structure formula shown in Fig. 1(d).

### E. Minimum Inhibitory Concentration (MIC) of the Active Antimicrobial Compound

The MIC of the fraction number 12 was tested against two bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Aspergillus fumigatus*). The MIC were 20 µg/mL and 30 µg/mL against the two bacteria and two fungal strains, respectively.

## IV. DISCUSSION

In this investigation, the freeze-dried fruiting body of *P. ostreatus* was extracted by alcohols and water. The cultural broth and mycelia from suspension culture were also freeze-dried, but were extracted only by water due to our preliminary results that indicated a low activity of the alcohol extracts. Our results of these extracts for their antimicrobial activities against 20 fungal and 10 bacterial species showed high degrees of variation between the extraction solvents used and the species of test microorganisms. The extracts from cultural broth had the lowest activity, indicating the possibility

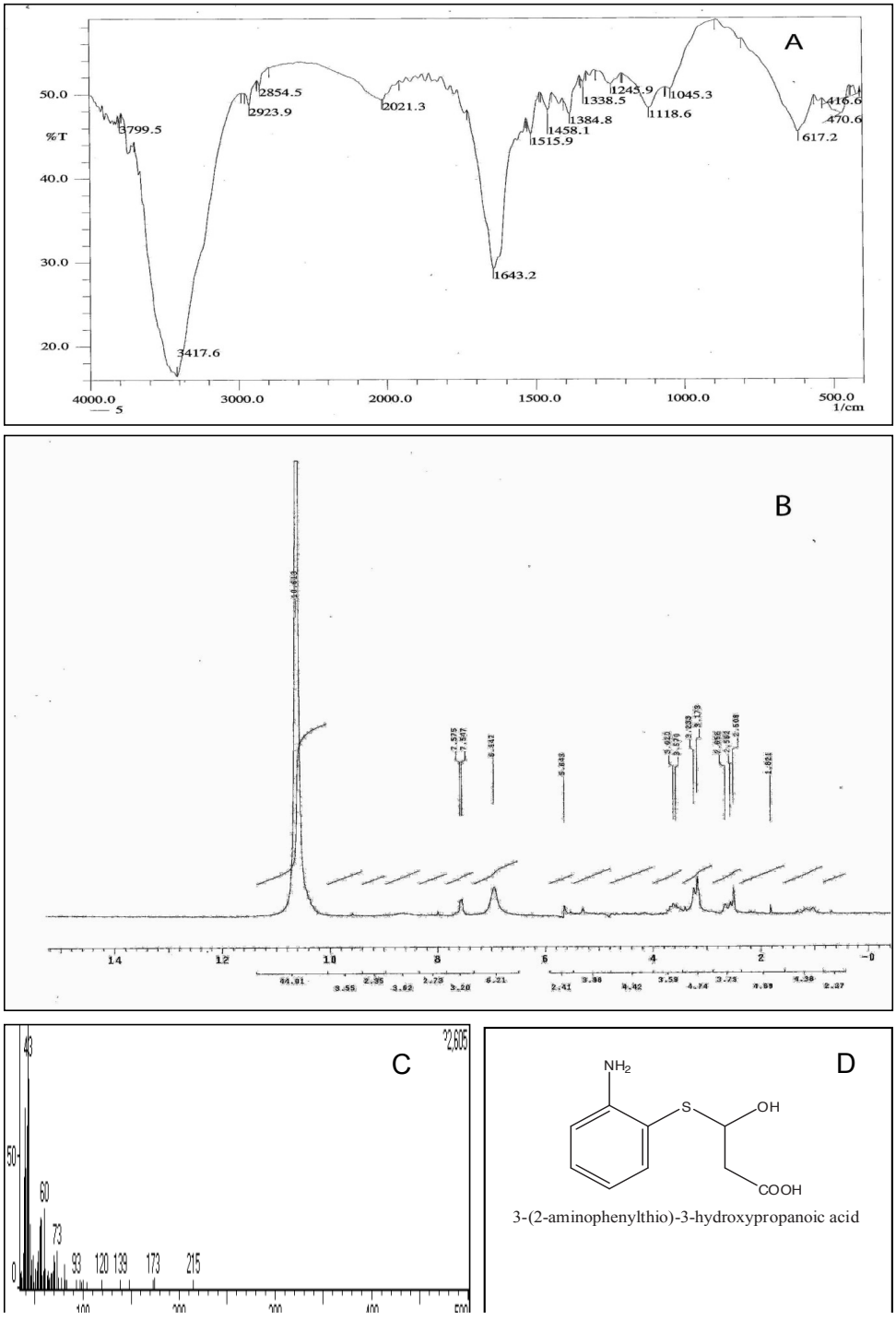


**FIG. 1:** Spectroscopic analyses of the active antimicrobial compound 3-(2-aminophenylthio)-3-hydroxypropanoic, isolated from the water extract of fruiting bodies from *Pleurotus ostreatus.* (**A**) IR spectrum; (**B**) NMR spectrum; (**C**) mass spectroscopic mass spectrum; (**D**) the suggested structural formula of the active compound.

that the active antimicrobial components may not be of secretive nature. Our results clearly indicated that the water extracts from fruiting bodies had the widest spectrum and highest growth inhibitory effect against fungi. The most sensitive fungal species to this water extract were *Candida albicans*, *C. dubliniensis*, *Cryptococcus humicola*, and *Trichosporon cutaneum*, followed by *Geotrichum candidum*, *Aspergillus fumigatus*, *A. flavus*, *A. terrus*, *A. niger*, *Fusarium moniliforme*, *Drechslera rostrata*, *Curvularia clavata*, *Alternaria chlamydospora*, *Eupenicillium ludwigii*, *Trichoderma viride*, and *Penicillium expansum.* This result is in accordance with that the water extracts of fruiting bodies from both *Lentinus edodes* and *Pleurotus ostreatus* contained antimicrobial compounds against certain fungal pathogens.[30] Also, the water extract of fresh fruiting bodies from *P. ostreatus* had an inhibitory effect on the mycelial growth of *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Physalospora piricola*, *Cryptococcus humicola*, *Mycogone* spp., *Aspergillus giganteus*, and *A. niger.*[36]

Water extract from cultural broth and mycelial biomass also exhibited moderate antimicrobial activities against fungal species of *Candida albicans*, *C. dubliniensis*, *Curvularia clavata*, and *Fusarium moniliforme*, but the degrees of inhibition varied. Cultural broth and mycelial biomass extracts often had sharp contracting effects in their antimicrobial activities against certain fungal species. For example, while the broth extract inhibited *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, and *T. viride*, the mycelia extract had no effect at all against these species. On the other hand, the mycelia extract inhibited *Cryptococcus humicola*, *Trichosporon cutaneum*, *Geotrichum candidum*, and *Penicillium expansum*, whereas the broth extract was without an effect on them. Four fungal species including *Penicillium roqueforti*, *Botrytis cinerea*, *Mucor rouxii*, and *Syncephalastrum racemosum* were observed to be recalcitrant to the inhibition by any extracts from *P. ostreatus*.

Alcohol-based solvents including methanol and 70% alcohol were not effective in extracting antimicrobial compounds from the fruiting bodies of *P. ostreatus.* This result is in contrast to that ethanol extract from fruiting bodies of *P. ostreatus* have antimicrobial activities against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis.*[37] It was also reported that the ethanol extracts from the fresh fruiting bodies contained more antimicrobial metabolites than those from dried ones.[38] Also, ethanol extract of lyophilized mycelium of *P. ostreatus* had a strong antibacterial activity against some bacterial strains.[31] However, our results indicated that water extracts from the fruiting bodies and broth from *P. ostreatus* had much higher antibacterial activities against gram-negative and positive bacteria in comparison to the ethanol and methanol extracts. The inhibition of bacterial growth was most effective against *Staphylococcus aureus* and *Escherichia coli* followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *B. megaterium*, *B. thuringiensis*, *Streptococcus pyogenes*, *Shigella dysenteriae*, and *Salmonella enterica*. This result is similar to what was observed that *P. ostreatus* possesses a wide spectrum of antibacterial compounds that can inhibit the growth *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Salmonella typhi.*[23] We observed that the mycelia extract had little or no inhibitory effect on the growth of bacteria, and the broth extract exhibited a high antimicrobial activity against a wide spectrum of bacteria strains suggesting a secretary nature of their antimicrobial compounds that were produced by the mycelia.

Many different antimicrobial compounds have been isolated from mushrooms. Production of cellulase complex enzymes from water extract of *P. ostreatus* showed strong inhibition activity against *Mycobacterium aurum*, *Staphylococcus aureus*, *Streptococcus* sp., *Acinetobacter calcoaceticus*, and *Klebsiella* sp.[39] An antifungal peptide with a molecular mass of 10 kDa isolated from fruiting bodies of the mushroom *Pleurotus eryngii* inhibited mycelial growth in *Fusarium oxysporum* and *Mycosphaerella arachidicola*.[40] Another antifungal peptide agent with inhibitory activity on mycelial growth of *F. oxysporum*, *Mycosphaerella arachidicola*, and *Physalospora piricola* was isolated

from fresh fruiting bodies of *P. ostreatus*.[36] Fatty acids isolated from fruiting bodies of *Gomphus floccosus* possessed antifungal activities against plant pathogens of *Colletotrichum fragariae*, *C. gloeosporioides*, *C. acutatum*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phomopsis obscurans*, and *Phomopsis viticola*.[41]

In the present study, we used IR, NMR, and mass spectrometric analysis that characterized a purified antimicrobial compound from water extract of *P. ostreatus* to be 3-(2-aminophenylthio)-3-hydroxypropanoic acid with MIC of 20 µg/mL against two bacterial strains and 30 µg/mL against two fungal strains. Twenty synthesized phenylenedithiourea derivatives contained both an aromatic amine ring and a carboxyl group, and they all showed high antimicrobial activities.[42] Also, the inhibitory activity of a new antifungal compound, aminophenyl thiozole, suggested to be due to the actions of its carboxyl group and its thiozole group.[43] We suggest that the presence of the amino phenylthio group in our newly identified compound may have played an important role in the antimicrobial activity observed in this report.

Antibiotic resistance of pathogenic microbes is a growing epidemic problem worldwide, and new antimicrobial agents that are available on the market are very limited. The discovery of new antimicrobial compounds is therefore becoming ever important. For example, the European Commission and the European Federation of Pharmaceutical Industries and Associations, together, has launched New Drugs for Bad Bugs programs to explore novel antibiotics. Despite the fact that edible mushrooms are nontoxic and contain various compounds beneficial to human health, they have not been fully exploited as sources of antimicrobial drugs.

## V. CONCLUSIONS

The water extracts from *P. ostreatus* contain antimicrobial compounds that are effective against a wide spectrum of bacteria and fungi, including those that are pathogenic to humans (e.g., *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. pyogenes*, *S. dysenteriae*, *S. enterica*, *C. albicans*, *C. humicola*, *T. cutaneum*, *A. fumigatus*, *A. flavus*, *A. terreus*, *D. rostrata*, and *C. clavata*) (Tables 1 and 2) and offer us the opportunity to explore the possibility of applying the extracts topically or orally in fighting the pathogenic microorganisms. The high nutrition of edible mushroom *P. ostreatus* may also be potentially an added benefit to patients. Finding a novel class of antibiotics that are active against gram-negative bacteria such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. dysenteriae*, and *S. enterica* has become increasingly important and we demonstrated here that our extract is highly effective in inhibiting these bacteria species. More studies will be needed to determine the mode of action of this new compound; we believe that it is worthwhile to exploit the potential of this new compound in treating the infectious bacteria and fungi diseases.

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