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Antibacterial and Antifungal Activities of *Stereum ostrea*, an Inedible Wild Mushroom

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Antibacterial and antifungal activities of liquid culture filtrate, water and ethanol extract (solid culture) of *Stereum ostrea* were evaluated against 5 bacteria and 3 plant pathogenic fungi. To determine the minimal inhibitory concentration (MIC), we studied 5~300 mg/*ml* concentrations against bacteria and fungi separately. The MIC was 10 mg/*ml* for *Bacillus subtilis* and 40 mg/*ml* for *Colletotrichum gloeosporioides* and *Colletotrichum miyabeanus*. Liquid culture filtrate was more effective against Gram positive than Gram negative bacteria, and *Staphylococcus aureus* was the most inhibited (20.3 mm) bacterium. Water and ethanol extracts were effective against both Gram positive and Gram negative bacteria, and water extract was better than ethanol extract. In water and ethanol extract, inhibition zones were 23.6 and 21.0 mm (*S. aureus*) and 26.3 and 22.3 mm (*Pseudomonas aeruginosa*), respectively. For plant pathogenic fungi, the highest and lowest percent inhibition of mycelial growth (PIMG) was found 82.8 and 14.4 against *C. miyabeanus* and *Botrytis cinerea* in liquid culture filtrate, respectively. In water extract, the PIMG was found to be the highest 85.2 and lowest 41.7 for *C. miyabeanus* and *C. gloeosporioides*, respectively. The inhibitory effect of ethanol extract was better against *C. miyabeanus* than *C. gloeosporioides* and *B. cinerea*. Among 3 samples, water extract was the best against tested pathogenic fungi. This study offers that the extracts isolated from *S. ostrea* contain potential compounds which inhibit the growth of both bacteria and fungi.

KEYWORDS: Antimicrobial activities, Crude extracts, Inhibition, MIC, PIMG

Stereum ostrea is one of the colorful mushroom belongs to Stereaceae, Basidiomycota. The fungus *S. ostrea* is inedible due to its tough, leathery texture and is often called the 'False turkey tail', since it mimics *Trametes versicolor*. Like the 'True turkey tail', *S. ostrea* has somewhat fuzzy cap that displays zones of brown and reddish brown colors. The *S. ostrea* is distinguished by its relatively large size and it tends to develop individual, sliced-funnel-shaped fruit bodies, rather than laterally fused flat ones. It is saprophytic on the dead hardwoods, growing in dense overlapping clusters and widely distributed in the world (Kuo, 2005). This fungus has long been used for folk remedies even without any knowledge of which compounds are responsible. The ethnobotanical uses of this mushroom to heal both human and plant diseases have been accumulated but scientific evidences are not yet well known. Recently, some new compounds such as a sesquiterpene, three aromatic compounds and a known compound methyl 2,4-dihydroxy-6-methylbenzoate were isolated from a culture broth of the fungus *Stereum* sp. The novel sesquiterpene was determined to be stereumone and the three new aromatic compounds were elucidated together with the known compound. The combination of these compounds showed evident nematocidal activity against nematode *Panagrellus redivivus* (Li *et al*., 2006). In our earlier research, we screened antimicrobial activities of nine Korean wild mushrooms and found good inhibitory effect of liquid culture filtrate of *S. ostrea* (Imtiaj and Lee, 2007).

To find out the effective antimicrobial compounds we studied liquid culture filtrates, water and ethanol extract of solid culture of this mushroom extensively, expecting that the cultures contain the similar compounds as the fruiting body. Here, we reported antibacterial and antifungal activities of *S. ostrea*.

Materials and Methods

Microorganisms and culture conditions. *Stereum ostrea* IUM1139 was obtained from "Culture Collection of Wild Mushroom Species (CCWM)", University of Incheon, Korea and used in this experiment. The mushroom strain was maintained on potato dextrose agar (PDA) medium at 25°C for further study. Three Gram-negative bacteria such as *Escherichia coli* CCARM1258, *Klebsiella pneumoniae* CCARM10161 and *Pseudomonas aeruginosa* CCARM2171 and two Gram-positive bacteria *Bacillus subtilis* IUB3251 and *Staphylococcus aureus* CCARM3230 were used in this study. *B. subtilis* was obtained from Applied Microbiology Laboratory, Department of Biology, University of Incheon and remaining 4 bacteria were obtained from "Culture Collection of Antibiotic Resistant Microbes (CCARM)", Korea. Three plant pathogenic fungi, including *Botrytis cinerea*, *Colletotrichum gloeosporioides* and

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Table 1. List of microorganisms used in this study

Microbes	Strains
Gram-positive bacteria	*Bacillus subtilis* IUB3251 and *Staphylococcus aureus* CCARM3230
Gram-negative bacteria	*Escherichia coli* CCARM1258, *Klebsiella pneumoniae* CCARM10161 and *Pseudomonas aeruginosa* CCARM2171
Plant pathogenic fungi	*Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Colletotrichum miyabeanus*

Colletotrichum miyabeanus, were obtained from "Center for Fungal Genetic Resources (CFGR)", Korea and used for this study (Table 1). The strains of bacteria and plant pathogenic fungi were maintained on nutrient agar (NA) medium at 37°C and PDA at 25°C, respectively.

Collection of crude extract. The fungus *S. ostrea* was cultured both in potato dextrose broth (PDB) and PDA medium separately. PDB culture was incubated at 25°C, on rotary shaker 140~150 rpm and PDA culture was incubated at 25°C for 30 days. After incubation, solid culture was dried in fume hood (HK-FH1800, Korea), powdered and then extracted both in distilled water and 70% ethanol (1 g: 15 *ml*) separately for 72 hours at 25°C. To obtain filtrates, the liquid culture, water extract and ethanol extract were filtered through 2 layers of Whatman No. 1 filter paper. The 3 filtrates were concentrated by a rotary evaporator (Eyela, Tokyo Rikakikai Co. Ltd., Japan) until semi-solid state substances were obtained. The semi-solid state substances were then freezing dried at −80°C (Operon, Korea).

Determination of minimal inhibitory concentration (MIC). The MIC test was aimed to find out the lowest concentration of the sample that inhibits the growth of tested microorganisms. The sample was used against 5 bacteria and 3 phytopathogenic fungi following modified technique described by Hirasawa *et al*., 1999. Different concentrations (5~300 mg/*ml*) of water extract was diluted in sterilized distilled water and tested using filter paper disc method separately. The test was first carried out by using high concentration of the extract and that was further diluted until no inhibitory zone was found. The sterile paper discs (8 mm diameter, Toyo Roshi Kaisha Ltd., Japan) were soaked with 50 *μl* of the aliquot and placed on both bacteria (10^{4-6} CFU/*ml*) and fungi (10^{4-5} spores/*ml*) seeded plate. Both bacterial and fungal cultures were first incubated at 4°C for 12 hours to allow proper diffusion of the extract into the medium and then incubated at 37°C and 25°C, respectively. After incubation of all cultures, inhibitions of bacterial and fungal growth were observed. Each experiment was done in 5 replicates.

Assay for antibacterial activity. For antibacterial activities, the samples were evaluated following a modified filter paper disc method (Norrel and Messely, 1997). The samples were diluted to 50 mg/*ml* with sterilized distilled water. The sterile filter paper discs were soaked with 50 *μl* of the solution and placed on bacteria seeded plate (10^{4-6} CFU/*ml*) of nutrient agar. For positive control, tetracycline 1000 ppm and streptomycin 2000 ppm were used as similar manner. The plates were first incubated at 4°C for 12 hours to allow proper diffusion of the extract into the medium and then re-incubated at 37°C for 24 hours. After incubation period, the inhibition zone was observed and measured (mm). An average inhibition zone was calculated for 5 replicates.

Assay for antifungal activity. For quantitative assay, the percent inhibition of mycelial growth (PIMG) was used to determine the antifungal effect of the 3 crude extracts of *S. ostrea*. The crude extracts of liquid culture filtrate, water and ethanol extract from solid culture were added to PDA at the concentrations of 10, 20 and 40 mg/*ml* separately. After autoclaving at 121°C for 15 minutes, these media were poured in 55 mm sterilized Petri dishes. Agar discs (5 mm) were taken from 10 days old cultures of 3 plant pathogenic fungi and placed in the center of the Petri plates separately. For control, same size agar discs of 3 fungi were placed in a same way on a fresh PDA plate. All pairings of cultures were carried out in 5 replicates and incubated at 25°C for 6 days. Inhibitory activity was assessed by measuring the radial growth of mycelium on the treated media (R_2) and the radial growth on fresh PDA as control (R_1). The two measurements were transformed in to PIMG using the formula of Skidmore and Dickinson (1976), where $PIMG = \{(R_1 - R_2)/R_1\} \times 100$.

Results and Discussion

MIC. The MIC is the lowest concentration of substance at which it plays to inhibit the growth of target microorganisms. We studied 5~300 mg/*ml* concentration against 5 bacteria and 3 phytopathogenic fungi separately (Table 2). The MIC of bacterial inhibition was found 10 mg/*ml* (*B. subtilis*), 30 mg/*ml* (*S. aureus* and *P. aeruginosa*) and 40 mg/*ml* (*E. coli* and *K. pneumoniae*). In case of phytopathogenic fungi, MIC was observed 40 mg/*ml* for *C. gloeosporioides* and *C. miyabeanus*, and 75 mg/*ml* for *B. cinerea*. Jonathan *et al*. (2007) studied the antagonistic effect of Nigerian higher fungi and found that the lowest and highest MIC of *Marasmius jodocodo* and *Ter-*

Table 2. The minimal inhibitory concentration (MIC) of water extract isolated from solid culture

	Microbial strains	MIC (mg/*ml*)									
		5	10	20	30	40	50	75	100	200	300
Bacteria	*B. subtilis*	+	–	–	–	–	–	–	–	–	–
	S. aureus	+	+	+	–	–	–	–	–	–	–
	E. coli	+	+	+	+	–	–	–	–	–	–
	K. pneumoniae	+	+	+	+	–	–	–	–	–	–
	P. aeruginosa	+	+	+	–	–	–	–	–	–	–
Fungi	*B. cinerea*	+	+	+	+	+	+	–	–	–	–
	C. gloeosporioides	+	+	+	+	–	–	–	–	–	–
	C. miyabeanus	+	+	+	+	–	–	–	–	–	–

Each paper disc was soaked with 50 μl of aliquot.

Table 3. Inhibitory effect of liquid culture, water and ethanol extracts of solid culture on 5 pathogenic bacteria

Bacterial strains		Inhibition zone (mm)				
		Liquid culture	Water extract	Ethanol extract	Positive control	
					T	S
Gram-positive	*B. subtilis*	15.6±1.1	20.6±2.0	18.6±0.5	25.3±0.5	26.3±1.1
	S. aureus	20.3±1.5	23.6±1.5	21.0±1.0	27.6±0.5	23.3±0.5
Gram-negative	*E. coli*	11.6±0.5	16.6±1.5	14.6±0.5	–	26.3±0.5
	K. pneumoniae	13.6±2.0	18.3±0.5	15.6±0.5	26.3±1.1	25.3±1.5
	P. aeruginosa	14.3±0.5	26.3±1.5	22.3±2.0	–	28.3±2.0

Extracts were used at 50 mg/*ml* concentration. Each paper disc was soaked with 50 μl of aliquot. T: Tetracycline 1000 ppm, S: Streptomycin 2000 ppm. Inhibition zone was measured (n=5) after 24 hours of incubation at 37°C.

mitomyces robustus was 2.75 and 15.75 mg/*ml* against *E. coli* and *Microsporum boulardii*, respectively. This result implies that the extract of *S. ostrea* contains potential therapeutic compounds against some of the medically important bacteria and fungi but the MIC against fungi was generally higher than that of bacteria.

Antibacterial assay. The results acquired from the paper disc method, 3 different extracts were showed less or more inhibitory effect against 5 bacteria (Table 3 and Fig. 1). Liquid culture filtrate was more effective against Gram positive than Gram negative bacteria and *S. aureus* was the most inhibited (20.3 mm) bacterium. Water and ethanol extract were effective against both Gram positive and Gram negative bacteria, and water extract was better than ethanol extract. In water and ethanol extract, inhibition zones were 23.6 and 21.0 mm (*S. aureus*) and 26.3 and 22.3 mm (*P. aeruginosa*), respectively. Inhibition of *B. subtilis* was found to be good both in 3 extracts. As positive control, tetracycline 1000 ppm or streptomycin 2000 ppm was used against the same bacteria. The tested concentration of tetracycline was not effective against *E. coli* and *P. aeruginosa* where extracts from *S. ostrea* showed less or more inhibitory effect on those bacteria. Jonathan and Fasidi (2003) reported that two edible Nige-

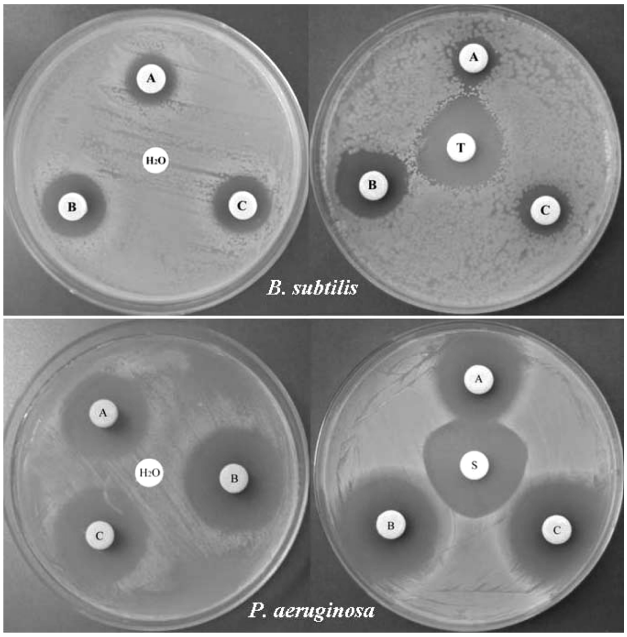

Fig. 1. Inhibitory effect of different crude extracts (50 mg/*ml*) on *B. subtilis* and *P. aeruginosa*. A: Liquid culture filtrate, B: Water extract, C: Ethanol extract, H_2O: Negative control, T: Tetracycline 1000 ppm and S: Streptomycin 2000 ppm (positive control). Each filter paper disc contains 50 μl of aliquot.

rian macro-fungi *Lycoperdon pusilum* and *L. giganteum* were selectively active on a few clinical pathogenic microorganisms. Al-Fatimi *et al*. (2006) used a paper disc method and stated that the fungus *Podaxis pistillaris* was found to exhibit a strong antibacterial activity against several Gram-positive and Gram-negative bacteria such as *S. aureus*, *Micrococcus flavus*, *B. subtilis*, *Proteus mirabilis*, *Serratia marcescens* and *E. coli*.

Antifungal assay. The liquid culture filtrate, water and ethanol extract (solid culture) of *S. ostrea* were used against 3 plant pathogenic fungi. To evaluate PIMG, 3 crude extracts were added to PDA at 10, 20, 40 mg/*ml* concentrations separately. In liquid culture filtrate, the highest and lowest PIMG of *C. miyabeanus* and *B. cinerea* was 82.82 and 14.40 at 40 and 10 mg/*ml* concentrations, respectively. But in water extract, PIMG of *C. miyabeanus* and *C. gloeosporioides* was found to be the highest (85.20) and lowest (41.73), respectively. The inhibitory effect of ethanol extracts was better against *C. miyabeanus* (86.05) than *C. gloeosporioides* and *B. cinerea*. The inhibitory effect of water and ethanol extracts from solid culture were about similar to *C. gloeosporioides* and *B. cinerea*. Among 3 crude samples, inhibitory activity of water extract was found to be the best against used 3 plant pathogenic fungi (Fig. 2 & 3). Chu *et al*. (2005) studied that the antifungal peptide isolated from *Pleurotus ostreatus* and found good inhibitory effect against *Fusaerium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora piricola*. They also added 3 doses (1, 5 and 25 μM) of Pleurostin to PDA (4 *ml*) and examined the growth phenotype of the pathogenic fungi. Jonathan and Fasidi (2003) studied the antifungal effects of two *Lycoperdon* spp. and the best antifungal activity was recorded

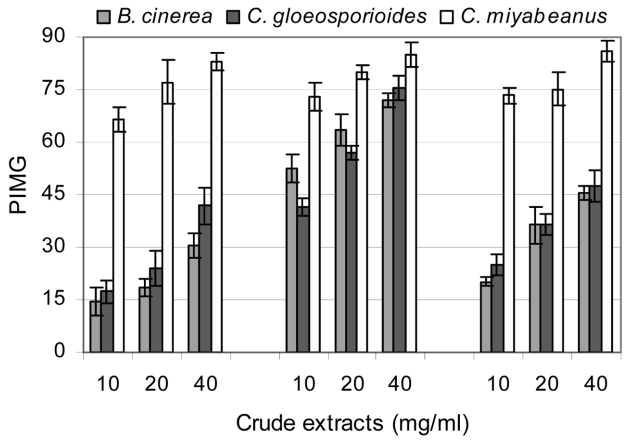

Fig. 2. Inhibitory effect of liquid culture filtrate (left), water (middle) and ethanol extract (right) of solid culture on the mycelial growth of 3 plant pathogenic fungi. Percent inhibition of mycelial growth (PIMG) was measured (n=5) after 6 days of incubation at 25°C.

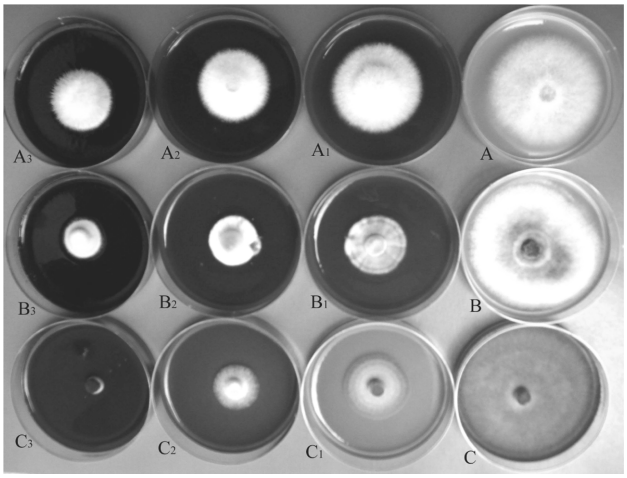

Fig. 3. Inhibitory effects of ethanol extract on the mycelial growth of *B. cinerea* (A), *C. gloeosporioides* (B) and *C. miyabeanus* (C). A, B and C indicate control (fresh PDA) of respective fungi. $A_1/B_1/C_1$, $A_2/B_2/C_2$ and $A_3/B_3/C_3$ contain 10, 20 and 40 mg/*ml* crude extract, respectively. Percent inhibition of mycelial growth (PIMG) was calculated (n=5) after 6 days of incubation at 25°C.

in *Lycoperdon giganteum* ethanol extract against *Microsporum boulardii*. Even though they focused that the higher fungi are promising antifungal agents, the observed values for all other extracts against pathogenic fungi were low. Lam and Ng (2001) designed *Lyophyllum* antifungal protein (LAP) isolated from mushroom (*L. shimeji*) which exerted antifungal activity against *Physalospora piricola* and *Mycosphaerella arachidicola* but not against *Rhizoctonia solani* and *Colletotrichum gossypii*. Tokimoto *et al*. (1987) reported antifungal effect of *L. edodes* and identified the compound as straight-chain alcohol with 8~9 carbons having double and triple bonds is active on filamentous fungi.

In this study, different crude extracts of *S. ostrea* have been used *in vitro* to evaluate the inhibitory effect against disease causing bacteria and fungi. It can therefore be suggested that crude extracts contain potential antimicrobial compounds and the obtained results may also be useful for evaluating substances of interest. Further investigations to isolate and characterize the potential antimicrobial compounds are underway.

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