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Screening of Antibacterial and Antifungal Activities from Korean Wild Mushrooms

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Abstract: Antibacterial and antifungal activities of nine Korean wild mushrooms were evaluated. The 20 days old liquid culture filtrates of *Sterium ostrea*, *Pycnoporus cinnabarinus*, *P. coccineus*, *Oudemansiella mucida* and *Cordyceps sobolifera* showed good antibacterial effects. In paper disc method, *S. ostrea* showed the best inhibitory effect against the growth of three bacteria. Inhibitory effect of culture filtrates was better against *Staphylococcus aureus* (Gram-positive bacterium) than the *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria). The culture filtrates of the mushrooms were also used against mycelial growth and mycelial weight of three plant pathogenic fungi *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Colletotrichum miyabeanus* and showed good inhibitory effect. *O. mucida* was the best mushroom showing antifungal activity. The effectiveness of culture filtrates was found to be varied against in different bacterial and fungal microorganisms. This study indicates that culture filtrates of *C. sobolifera*, *O. mucida, S. ostrea*, *P. cinnabarinus* and *P. coccineus* were the most effective against both bacteria and fungi. The culture filtrates showing the best inhibitory effect have been considered for further extensive study.

Key words: Antimicrobial activities · culture filtrate · growth · inhibition · medicinal mushroom

INTRODUCTION

Mushroom is used as food stuffs containing both nutritive and medicinal values [1-3]. It contains all the essential amino acids [4, 5]. Mushrooms are not only sources of nutrients but also preventing diseases such as hypertension, hypercholesterolemia and cancer [6, 7]. Recently researchers isolated and identified some compounds, originating from mushrooms, show other medical properties, such as immuno-modulatory, liver protective, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial activities [8-11]. Antimicrobial compounds isolated from mushroom (*Lentinula edodes*) liquid cultures include lentinamicin, β-ethyl phenyl alcohol [12] and lentin, an antifungal protein [13]. Mycelial-free culture of *L. edodes* [14] exhibited greater antimicrobial activity against gram-positive than gram-negative bacteria. The *Bacillus subtilis* and *Staphylococcus aureus* are the most highly inhibited bacteria among the tested microorganisms. From these reports, it is focused that mushrooms are a vital sources of medicinal compounds that may use to cure different disorders and prevent pathogenic microorganisms.

To find the effective antimicrobial compounds we studied mycelial liquid cultures of mushrooms, expecting that the cultures will produce the similar compounds as the fruiting bodies. Here, we report antibacterial and antifungal activities of culture filtrates of 9 Korean wild mushrooms.

MATERIALS AND METHODS

Used microorganisms: Nine mushrooms such as *Armillaria mellea* IUM1986, *Calvatia craniiformis* IUM469, *Cordyceps sobolifera* IUM1613, *Dictyophora indusiata* IUM68, *Oudemansiella mucida* IUM929, *Pholiota adiposa* IUM132, *Pycnoporus cinnabarinus* IUM253, *Pycnoporus coccineus* IUM1126 and *Stereum ostrea* IUM1139 are obtained from "Culture Collection of Wild Mushroom Species (CCWM)", University of Incheon and used in this experiment (Table 1). The mushroom strains were maintained on Potato Dextrose Agar (PDA) medium at 25°C for further study.

Two Gram-negative bacteria such as *Escherichia coli* CCARM1258, *Pseudomonas aeruginosa* CCARM2171 and one Gram-positive bacterium *Staphylococcus aureus* CCARM3230 were used in this study. These 3 bacteria were obtained from "Culture Collection of Antibiotic Resistant Microbes (CCARM)". Three plant pathogenic fungi include *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Colletotrichum miyabeanus* were obtained from "Center for Fungal Genetic Resources (CFGR)" and used for this study (Table 1). The bacterial strains were maintained on nutrient agar (NA) medium at

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Table 1: List of microorganisms used in this study

Mushrooms	Strain	Bacteria	Strain	Plant pathogenic fungi
Armillaria mellea	IUM1986	*Escherichia coli*	CCARM 1258	*Botrytis cinerea*
Calvatia craniiformis	IUM0469			
Cordyceps sobolifera	IUM1613			
Dictyophora indusiata	IUM0068	*Pseudomonas aeruginosa*	CCARM 2171	*Colletotrichum gloeosporioides*
Oudemansiella mucida	IUM0929			
Pholiota adiposa	IUM0132			
Pycnoporus cinnabarinus	IUM0253	*Staphylococcus aureus*	CCARM 3230	*Colletotrichum miyabeanus*
Pycnoporus coccineus	IUM1126			
Stereum ostrea	IUM1139			

Table 2: Antibacterial activity of culture filtrates harvested from 9 mushrooms

	Inhibition zone (mm)[a]		
Scientific name	*E. coli*	*P. aeruginosa*	*S. aureus*
A. mellea	1.00±0[b]	1.33±0.58	6.33±0.58
C. craniiformis	-	1.33±0.58	8.00±2.65
C. sobolifera	1.00±0	1.33±0.58	9.00±1.00
D. indusiata	-	1.67±0.58	8.33±0.58
O. mucida	1.67±0.58	2.33±0.58	9.00±2.00
P. adiposa	-	1.00±0	7.33±1.53
P. cinnabarinus	2.00±0	4.33±0.58	10.00±1.00
P. coccineus	1.67±0.58	3.33±0.58	8.33±1.15
S. ostrea	3.67±0.58	6.33±0.58	13.33±1.15

[a]Inhibition zone was measured after 24 h of incubation at 35°C, [b]Inhibition zone was measured by diameter of total inhibition zone minus diameter of paper disc

35°C and plant pathogenic fungi were cultured on PDA at 25°C for further study.

Collection of liquid culture filtrate: The mushrooms were cultured both in potato dextrose broth (PDB) and in 12% citrus extract liquid medium separately and incubated at 25°C, on rotary shaker 140~150 rpm for 20 days. To obtain the culture filtrate from 9 mushrooms, the liquid cultures which contained mycelium were filtered through 2 layers of Whatman No. 1 filter paper. Thus the filtrates were ready for further use.

Antibacterial activity: The antibacterial activity of culture filtrate was evaluated in liquid culture medium measuring Optical Density (OD). Filtered culture filtrate (PDB) through Whatman No. 1 filter paper was incorporated with fresh PDB at 50% concentration (v/v) and autoclaved at 121°C for 15 min. Cooled liquid medium was inoculated with each bacterium separately in 250 ml conical flask and incubated at 35°C. The bacterial growth was determined by measuring OD at 600 nm after every alternative 6 h and it was done until 36 h. For control, fresh PDB was also inoculated by each bacterium and OD was measured with same method.

A modified filter paper disc method [15] was also used to determine the antibacterial activity. The culture filtrate (12% citrus medium) was concentrated by a rotary evaporator until a semi-solid state substance was obtained. The semi-solid state substance was freezing dried at -80°C and diluted to 10% solution (0.1 g ml^{-1}) with sterilized distilled water for experiment. The sterile paper discs (8 mm diameter, Toyo Roshi Kaisha Ltd., Japan) were soaked with 80 μl of the solution and placed on a bacterial seeded plate (10^5 CFU ml^{-1}) of nutrient agar. The plates were incubated at 35°C for 24 h and the inhibition zone was observed and measured. An average inhibition zone was calculated for 4 replicates.

Antifungal activity: Two parameters were observed in this test, the Percent Inhibition of Mycelial Growth (PIMG) and the Percent Inhibition of Mycelial Weight (PIMW). Culture filtrates of mushrooms were diluted with PDB medium separately to make 50% concentrations (v/v) of each filtrate and 2% agar was added to make solid. After autoclaving at 121°C for 15 min, these were poured in sterilized Petri dishes. Agar discs taken from 10 days old cultures of 3 plant pathogenic fungi and were placed in the center of the Petri plates separately. For control, agar discs of 3 fungi were placed in a same way on a fresh PDA plate. All pairings were carried out in 4 replicates and incubated at 25°C. Inhibitory activity was assessed after 10 days of incubation by measuring the radial growth on culture filtrates (R_2) and the radial growth on fresh PDA as control plate (R_1). The two measurements were transformed in to PIMG using the formula PIMG = $\{(R_1-R_2)/R_1\}\times100$ [16].

Determining the percent inhibition of mycelial weight (PIMW) of the 3 plant pathogenic fungi, culture filtrates were diluted similarly without agar and autoclaved.

Inoculation of fungi was made separately with 100 ml of liquid media in 250 ml of conical flask at 25°C for 10 days. After 10 days of incubation, the entire fungal mycelium was harvested by filtering through previously dried and weighted Whatman filter paper No 1. It was then dried to constant weight at 65°C. Before weighting, the filter paper were allowed to cool and subsequently weighted in a balance. The weight of the mycelium was calculated by deducting the weight of filter paper from the final weight. As control, fresh PDB was inoculated with the test fungi separately. All pairings were carried out in 4 replicates and incubated at 25°C. The inhibition was assessed after 10 days of incubation by measuring the mycelial weight grown in culture filtrates (R_2) and the mycelial weight grown in fresh PDB as control (R_1). The two readings were transformed in to PIMW using the same formula [16].

RESULTS AND DISCUSSION

The outcomes of optical density indicate that the filtrates of *P. cinnabarinus, P. coccineus* and *O. mucida* were highly effective against *E. coli* (Fig. 1). Culture filtrate of *P. cinnabarinus, P. coccineus* and *S. ostrea* was also found to inhibit the growth of *P. aeruginosa* (Fig. 2). *S. aureus* was found to be inhibited by several filtrates of mushrooms including *C. sobolifera, O. mucida, P. cinnabarinus, P. coccineus* and *S. ostrea* (Fig. 3). The mycelial culture filtrate of *Lentinula edodes* was used against the growth of *B. subtilis* and got potential inhibitory effect [14]. Inhibitory effect of *L. edodes* upon Gram-positive and Gram-negative bacteria was observed and the result of inhibition was good for Gram-positive bacteria which are quite similar to our findings [17].

The results obtained from the paper disc method, these three bacteria were less or more inhibited by the culture filtrates (Table 2 and Fig. 4). Culture filtrates of *S. ostrea, P. cinnabarinus, P. coccineus* and *O. mucida* were demonstrated the width inhibition zones of *E. coli*. Inhibition zones were counted 6.33, 4.33, 3.33 and 2.33 mm in *S. ostrea, P. cinnabarinus, P. coccineus* and *O. mucida* for *P. aeruginosa* respectively. In every case, Gram-positive bacterium *S. aureus* was more inhibited than the Gram-negative and the widest inhibition zones (13.33 mm) were found in *S. ostrea*. Culture filtrates of C. *sobolifera* and *O. mucida* were also good (9 mm) against *S. aureus*. Two edible Nigerian macro-fungi *Lycoperdon pusilum* and *L. giganteum* were selectively active on few clinical

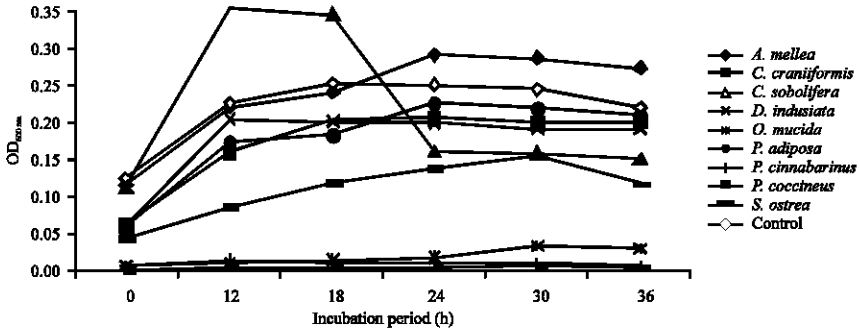

Fig. 1: Growth of *E. coli* measured in 50% (v/v) liquid culture filtrate of mushroom and PDB. The OD value in liquid culture filtrate higher than the OD value of control indicates no inhibition

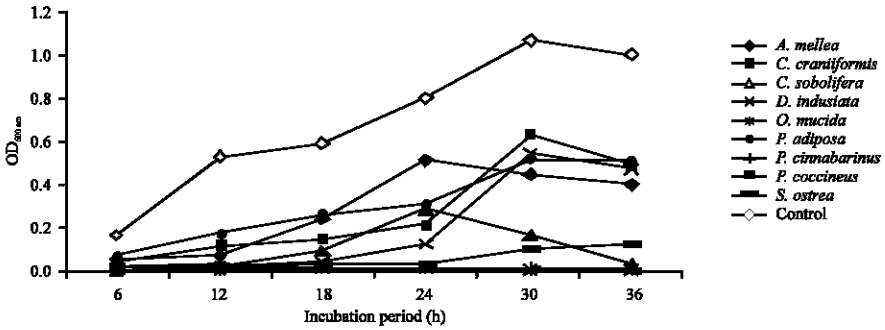

Fig. 2: Growth of *P. aeruginosa* measured in 50% (v/v) liquid culture filtrate of mushroom and PDB. The OD value in liquid culture filtrate higher than the OD value of control indicates no inhibition

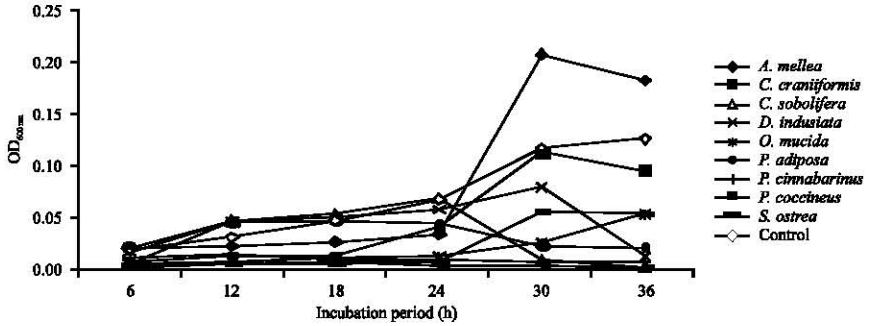

Fig. 3: Growth of *S. aureus* measured in 50% (v/v) liquid culture filtrate of mushroom and PDB. The OD value in liquid culture filtrate higher than the OD value of control indicates no inhibition

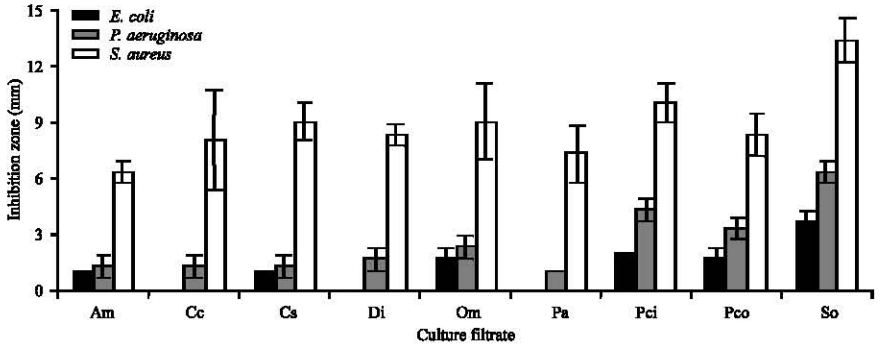

Fig. 4: Inhibition of culture filtrate against 3 bacterial growth measured on nutrient agar. The culture filtrate was obtained from 20 days old culture of 9 mushrooms in 12% citrus medium. (Am: *A. mellea*, Cc: *C. craniiformis*, Cs: *C. sobolifera*, Di: *D. indusiata*, Om: *O. mucida*, Pa: *P. adiposa*, Pci: *P. cinnabarinus*, Pco: *P. coccineus* and So: *S. ostrea*)

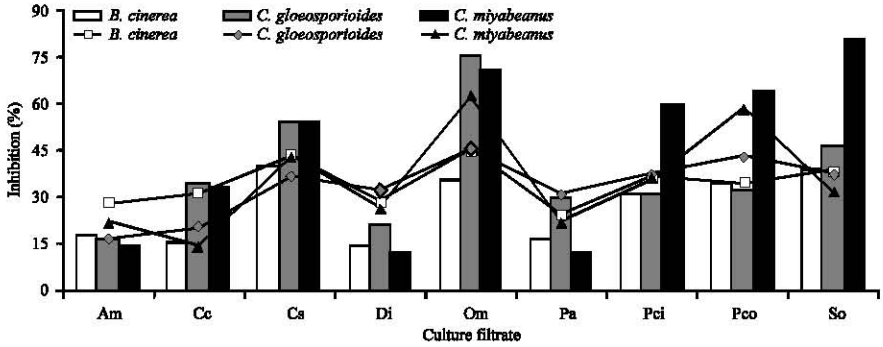

Fig. 5: Inhibition measured in 50% (v/v) liquid culture filtrate of mushroom and PDB against mycelial growth and mycelial weight of 3 plant pathogenic fungi. Bar columns indicate mycelial growth and lines indicate mycelial weight. (Am: *A. mellea*, Cc: *C. craniiformis*, Cs: *C. sobolifera*, Di: *D. indusiata*, Om: *O. mucida*, Pa: *P. adiposa*, Pci: *P. cinnabarinus*, Pco: *P. coccineus* and So: *S. ostrea*)

pathogenic microorganisms [18]. A paper disc method is used 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* [19].

The filtrates harvested from mushroom were used against mycelial growth of *B. cinerea, C. gloeosporioides* and *C. miyabeanus* pathogenic fungi (Table 3 and Fig. 5). Culture filtrate of *P. coccineus*, *P. cinnabarinus* and *C. sobolifera* were obviously inhibited the mycelial growth of *B. cinerea*. The filtrates of *C. sobolifera*,

Table 3: Inhibitory effect of culture filtrates on the mycelial growth and dry weight of 3 plant pathogenic fungi

	Inhibition (%)					
	B. cinerea		*C. gloeosporioides*		*C. miyabeanus*	
Scientific name	PIMG[a]	PIMW[b]	PIMG	PIMW	PIMG	PIMW
A. mellea	17.07	27.54	16.27	16.90	13.60	21.92
C. craniiformis	15.93	31.88	34.83	19.72	33.37	14.11
C. sobolifera	39.87	43.48	54.93	36.62	54.80	42.47
D. indusiata	13.90	28.99	21.13	32.39	12.07	26.03
O. mucida	36.17	45.59	75.67	45.33	70.77	63.01
P. adiposa	16.77	24.64	29.60	30.99	12.13	21.92
P. cinnabarinus	30.43	36.76	31.73	37.33	60.07	35.62
P. coccineus	34.47	35.29	32.17	42.67	64.63	58.90
S. ostrea	39.70	38.24	46.70	37.33	81.33	31.51

[a]Percent inhibition of mycelial growth was measured after 10 days of incubation at 25°C, [b]Percent inhibition of mycelial weight was measured after 10 days of incubation at 25°C

O. mucida were also effective against mycelial growth of *C. gloeosporioides*. On the other hand, filtrates of *S. ostrea*, *O. mucida*, *P. cinnabarinus* and *P. coccineus* were found to be effective against mycelial growth of *C. miyabeanus*. Rests of culture filtrates were more or less effective against used pathogenic fungi.

Mycelium weight of *C. gloeosporioides* and *C. miyabeanus* was mostly inhibited by the filtrates of *O. mucida* (45.33, 63.01%) and *P. coccineus* (42.67, 58.90%) respectively. In case of *B. cinerea*, the highest inhibition was found 43.48 and 45.59% in the filtrates of *C. sobolifera* and *O. mucida* respectively. Among the studied mushrooms *C. sobolifera*, *O. mucida, P. cinnabarinus, P. coccineus* and *S. ostrea* were found to be the most effective against used bacteria and fungi which have already been selected for further extensive study. Mentionable that in this study, a paper disc method was also observed against pathogenic fungi but the result was not focusable. The antifungal effect of two *Lycoperdon* spp. was studied and the best antifungal activity was recorded in *Lycoperdon giganteum* ethanolic extract against *Microsporum boulardil* [18]. They focused that the higher fungus is a promising antifungal agent but the observed values for all other extracts against pathogenic fungi were low. The antifungal effect of *L. edodes* was observed and identified the compound as straight-chain alcohol with 8-9 carbons having double and triple bonds is active on filamentous fungi [20].

In termination, this study has exposed that the different culture filtrates have been used *in vitro* to observe the inhibitory effect against disease causing bacteria and fungi. It can therefore be suggested that, they are promising antimicrobial compounds and this work is already in steps forward in identifying bioactive compound. The obtained results may also be useful for evaluating substances of interest produced by these fungi, such as antimicrobial compounds, enzymes and immunotherapeutic agents.

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