ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF EXO AND ENDO EXTRACTS OF WHITE OYSTER MUSHROOM (PLEUROTUS FLORIDA)

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Abstract: context: pleurotus species (oyster mushroom) have been used by people worldwide for their medicinal properties, dietary benefit and other purposes. They contain various compounds which biologically active with therapeutic effects. Pleurotus florida which also known as white oyster mushroom is an edible mushroom that is popular due to its low production cost, nutritional contents and easy cultivation method. Aims: this study was designed to evaluate the potential antioxidant and antimicrobial properties of pleurotus florida exopolysaccharide and endopolysaccharide extracts to be an alternative source for existing antioxidant and antimicrobial agent.
Methods: In this study, the antioxidant activities of different concentrations of Pleurotus florida extracts were evaluated using total phenolic content (TPC), DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) assay and the antimicrobial activities were evaluated using agar disk-diffusion method.
Results: Out of the 5 concentrations tested for antioxidant properties for both extracts, concentration of 1mg/ml of the IPS extract showed the highest TPC and FRAP activity, while for the DPPH radical scavenging activity concentration of 0.2mg/ml of IPS extract showed the highest scavenging activity. For the antimicrobial evaluation, all the 3 concentrations that were used showed no antimicrobial activity against E. coli, S.aureus and P. aeruginosa.
Conclusion: In conclusion, white oyster mushroom has shown potent antioxidant effects which makes it a good antioxidant candidate that can be incorporated in medicine and diet, and as the concentration of Pleurotus florida mushroom increases the antioxidant effects increase. However, both exo-and endopolysaccharide extract doesn't exhibit any antimicrobial activity in low concentrations.

Keywords: Antioxidant; Antimicrobial; Endopolysaccharide; Exopolysaccharide; Mushrooms; Pleurotus florida.

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INTRODUCTION

Mushrooms are eukaryotic, non-photosynthetic organic entities that structure trademark fruiting bodies. They have been utilized by people for millennia as food and medication. With their low fat, and, high protein and cholesterol substance, mushrooms are considered by numerous individuals to be an ideal wellspring of nourishing fixings, plentiful in minerals, polysaccharides and polyphenols. Many mushrooms are useful l in treating human diseases because they possess many pharmacological activities like acting as metabolism activators, preventing/controlling microbial/viral infections and intoxication, helping in immunomodulation and immune-balancing and as antioxidants with energy boosting properties and rejuvenating.

Among edible mushrooms, *Pleurotus* (oyster mushroom) species have been utilized by human everywhere on the world for their dietary benefit, medical effects and other beneficial impacts. They inhibit tumor growth, modulate the inflammation and immune system, have antithrombotic actions and hypoglycemic, prevent hypertension and atherosclerosis, lower blood lipid concentrations and other actions. *Pleurotus florida* also known as white oyster mushroom is an comestible mushroom that becoming popular due to its cheap production cost, high nutritional values, and easy to cultivate. Fungal polysaccharides, derived from *Pleurotus* have shown multiple beneficial therapeutic action, including immunomodulation, anticancer, antimicrobial, hypocholesterolemic, and hypoglycemic actions.

Antioxidants are substances that are available at low concentrations and remarkably prevent or delays oxidation of compounds. Antioxidants are effective due to they can donate their own electrons to reactive oxygen species (ROS) and neutralize the adverse effects of a latter. Antioxidants from our

diet are important because its helps in engulfing ROS and neutralize the oxidative stress. The nutrient antioxidant deficiency is one of the causes of many degenerative and chronic diseases. When an antioxidant destroys a free radical, this antioxidant becomes oxidized. Thus, the antioxidant resources must be regularly replaced in the body. Multiple tests were used to evaluate the antioxidant activity of *Pleurotus florida;* the well-known method that works towards determining the total phenolic content (TPC) in plant tissues. Besides, DPPH assay is widely used to evaluate the radical scavenging effects of plant extracts. Lastly, The Ferric reducing antioxidant power (FRAP) test which is a typical method measuring the reduction of ferric ions (Fe3+)-ligand to the intensely blue ferrous complex (Fe2+) by means of antioxidants in acid environments.

In order to fight infectious disease, a chemicals potential to have antimicrobial effects that are potent in preventing, limiting or eliminating the growth of microbial predators has to be developed. The majority of these antimicrobial agents are derived from natural products where they were used by many organisms naturally to defend against microbial attack. Agar disk-diffusion testing is used routinely to measure the antimicrobial susceptibility testing.

METHODOLOGY

Source of Endopolysaccharide and Exopolysaccharide *Pleurotus florida* Mushroom Extracts

Pleurotus florida Endopolysaccharides (EPS) and Exopolysaccharides (IPS) extracts were obtained from Malaysian Nuclear Agency, Malaysia.

Chemicals

Folin-ciocalteu reagent, sodium carbonate, 2,4,6-Tri-(2-Pyridyl)-1,3,5-Triazine, Gallic acid, L-Ascorbic acid, methanol, Ferric chloride, Ferrous sulphate, Dimethyl sulfoxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol 95%, Mueller-hinton agar powder, 40mM Hcl, sodium acetate buffer buffer, normal saline, distilled water and Gentamicin antibiotic discs (10 µg) were used in this study.

Preparation of different concentrations of IPS and EPS extracts of *Pleurotus florida*

Both IPS and EPS extracts were prepared on various concentrations of 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml. The extract was diluted using distilled water.

Total Phenolic Content Assay

0.5 mL of both IPS and EPS extracts from each concentration was added to 4.5 mL of distilled water. Later, the solution was mixed with Folin–Ciocalteu phenol reagent, Na2CO3, and lastly distilled water was added to the solution. After that, the mixtures were incubated for 60 min in the dark room and the absorbances were measured at 725 nm. Ethanol was used as a blank. Similar concentrations of gallic acid were prepared and the same procedure was done to be used as a standard.

DPPH Radical Scavenging activity

Each mushroom extract with different concentrations were mixed with 1 mL of DPPH (0.1 mM) dissolved in methanol. Then, the solution was incubated in the dark for 15 minutes at room temperature. After incubation, the solution was read at 517nm absorbance. L-ascorbic acid was used as positive control and methanol was used as blank. A negative control that contains all reagents except the tested samples was prepared and the absorbance was taken to be used in the equation below: The capability to scavenge the DPPH radical was calculated using Equation (1):

DPPH scavenging effect (%) = (Ablank-Asample)/Ablank*100	[1]

Ferric Reducing Antioxidant Power Assay

FRAP reagent was added into test tube that contains IPS and EPS extracts and the solutions were incubated at room temperature in the dark for 30 minutes. After incubation, the solution was read at 593 nm with dimethyl sulfoxide used as blank.

Agar disk diffusion method

Antimicrobial properties were tested against *E. coli, S.aureus* and *P.aeruginosa*. Mueller Hinton agar was used as the media. The plates were seeded with microbes' suspension using sterile swab. Sterile discs were soaked in the various concentrations along with the negative control (distilled water) and gentamicin 10µg/disc (as positive) was placed on the petri plates. The plates were incubated at 37 C for 24 h and the diameter of the zone formed around the paper disc was measured after incubation period.

RESULTS and discussion

Evaluation of Antioxidant properties of *Pleurotus florida*

Total Phenolic Content

The standard curve for Gallic acid was plotted and the standard curve equation is as follow:

$y = 4.6655x - 1.0383$ ($R^2 = 0.9066$)

The TPC results is as shown in **Table 1**. The values are presented as Mean ± Standard deviation (SD) of triplicate samples and expressed in mg gallic acid equivalent (GAE)/ g of dry weight sample (DW). From the results, it is shown that as the concentration of IPS and EPS extracts increases, the TPC value increases. Meaning the highest concentration has the highest total phenolic content.

Table 1. Mean Total Phenolic concentrations of different concentrations of IPS and EPS Pleurotus florida extracts (n=3)

Concentration of extract (mg/ml)	n	Total Phenolic Content (Mean ± SD)	p-value
0.2 IPS	3	$0.2346 \pm 0.00^{b,c,d,e}$	(P<0.05)
0.4 IPS	3	$0.2410 \pm 0.00^{a,c,d,e}$	
0.6 IPS	3	$0.2544 \pm 0.00^{a,b,d,e}$	
0.8 IPS	3	$0.2648 \pm 0.00^{a,b,c,e}$	
1 IPS	3	$0.2704 \pm 0.00^{a,b,c,d}$	
0.2 EPS	3	$0.2296 \pm 0.00^{b,c,d,e}$	
0.4 EPS	3	$0.2306 \pm 0.00^{a,c,d,e}$	
0.6 EPS	3	$0.2314 \pm 0.00^{a,b,d,e}$	
0.8 EPS	3	$0.2328 \pm 0.00^{a,b,c,e}$	

1 EPS	3	$0.2340 \pm 0.00^{a,b,c,d}$	

In this study, the results showed an increase in total phenolic content as the concentration increases for both IPS and EPS extract, with the lowest TPC value of 0.2346 ± 0.00 mg GAE/g and the highest TPC value of 0.2704 ± 0.00 mg GAE/g for IPS and the lowest TPC value of 0.2296 ± 0.00 mg GAE/g and the highest TPC value of 0.2340 ± 0.00 mg GAE/g for EPS respectively.

The results are similar with the findings of the study conducted by John et al. (2014). This study found that there is a linear correlation between phenolic content and antioxidant activity. The higher the phenolic content, the higher the antioxidant activity.

DPPH Radical Scavenging activity

IC50 values were determined to calculate the concentration that effective of respective samples needed to scavenge DPPH radical by 50%. From the linear equations of the graphs of ascorbic acid, IPS and EPS *Pleurotus florida* extract, the IC50 is 0.43, 0.85 and 0.97 respectively. The lowest IC50 indicates the highest antioxidant activity.

The reading of free radical scavenging activity is expressed in percentage as shown in **Table 2**. The values are presented as Mean ± Standard deviation (SD) of triplicate samples. The results show that as the concentration of extract increases, the percentage of scavenging activity value decreases.

Table 2. Mean Percentage of Scavenging activity of different concentrations of IPS and EPS extracts of Pleurotus florida (n=3)

Concentration of extract (mg/ml)	n	Percentage of scavenging activity (Mean ± SD)	P value
0.2 IPS	3	$68.875 \pm 2.28^{b,c,d,e,f,g,h,i,j}$	(P<0.05)
0.4 IPS	3	$53.313 \pm 2.61^{a,c,d,e,f,g,h,i,j}$	
0.6 IPS	3	$52.008 \pm 0.97^{a,b,d,e,f,g,h,i,j}$	
0.8 IPS	3	$48.293 \pm 4.95^{a,b,c,e,f,g,h,i,j}$	
1 IPS	3	$23.393 \pm 0.46^{a,b,c,d,f,g,h,i,j}$	
0.2 EPS	3	$40.763 \pm 2.18^{a,b,c,d,e,g,h,i,j}$	
0.4 EPS	3	$40.763 \pm 1.25^{a,b,c,d,e,f,h,i,j}$	
0.6 EPS	3	$44.779 \pm 1.39^{a,b,c,d,e,f,g,i,j}$	
0.8 EPS	3	$33.734 \pm 1.83^{a,b,c,d,e,f,g,h,j}$	
1 EPS	3	$35.542 \pm 1.81^{a,b,c,d,e,f,g,h,i}$	

In this study, the results of IPS extract showed a decrease in antioxidant activity with increasing the concentration, while EPS extract showed mixed results. The highest percentage scavenging is 68.88% belonging to 0.2mg/ml and the lowest percentage scavenging is 23.39% belonging to 1mg/ml for IPS extract. While for EPS extract, the highest percentage scavenging is 44.78% belonging to 0.6mg/ml and the lowest percentage scavenging is 33.73% belonging to 0.8mg/ml.

The percentage scavenging activity was also calculated by the IC50, and it showed values of 0.43, 0.85 and 0.97 for ascorbic acid, IPS and EPS extracts respectively. So, the lowest the IC50 value, the highest the antioxidant activity; meaning, ascorbic acid has the highest antioxidant activity, next is the IPS extract and lastly is the EPS extract. The results were different from the data found. Based on the data found, all the plant extracts showed increased in radical scavenging capacity in concentration-dependent manner.

Ferric Reducing Antioxidant Power test

The standard curve for ferrous sulphate was plotted and the standard curve equation is as follow:

$y = 5.31x + 0.4466$ ($R^2 = 0.98550$)

The antioxidant capacity is as shown in **Table 3**. The values are presented as mean ± standard deviation (SD) of triplicate samples. From the results, it is shown that as the concentration of IPS and EPS extracts increases, the FRAP value increases. It shows that the highest concentration has the highest ability to reduce ferric ions.

Table 3. Mean Ferric Reducing Antioxidant Power of different concentrations of IPS and EPS Pleurotus florida extracts (n=3)

Concentration of extract (mg/ml)	**n**	**Ferric Reducing Antioxidant Power (Mean ± SD)**	**p-value**
0.2 IPS	3	$0.0195 \pm 0.00^{b,c,d,e}$	(P<0.05)
0.4 IPS	3	$0.0270 \pm 0.00^{a,c,d,e}$	
0.6 IPS	3	$0.0421 \pm 0.00^{a,b,d,e}$	
0.8 IPS	3	$0.0524 \pm 0.00^{a,b,c,e}$	
1 IPS	3	$0.0625 \pm 0.00^{a,b,c,d}$	
0.2 EPS	3	$0.0073 \pm 0.00^{b,c,d,e}$	
0.4 EPS	3	$0.0152 \pm 0.00^{a,c,d,e}$	
0.6 EPS	3	$0.0272 \pm 0.00^{a,b,d,e}$	
0.8 EPS	3	$0.0425 \pm 0.00^{a,b,c,e}$	
1 EPS	3	$0.0468 \pm 0.00^{a,b,c,d}$	

In this study, the results showed an increase in ferric reducing antioxidant power as the concentration increases for both IPS and EPS extract, with the lowest FRAP value of 0.0195 ± 0.00 uM FESO4/g and the highest FRAP value of 0.0625 ± 0.00 uM

FESO4/g for IPS and the lowest FRAP value of 0.0073 ± 0.00 uM FESO4/g and the highest FRAP value of 0.0468 ± 0.00 uM FESO4/g for EPS respectively.

The results are similar with the findings of the study conducted by Aryal et al. (2019) where it stated that FRAP assay of antioxidants is reproducible, convenient and concentration dependent. All the extracts from the selected plants showed concentration-dependent reducing power, like the radical scavenging activity.

Evaluation of Antimicrobial properties of *Pleurotus florida*

Table 4 shows antimicrobial activity of three different concentrations (0.2, 0.6, 1mg/ml) of both IPS and EPS extracts of *Pleurotus florida* against *E. coli*, *S.aureus* and *P.aeruginosa*. Gentamicin (10µg/disc) was used as a positive control and distilled water was used as a negative control. The results show that the three different concentrations have zero inhibition zone and thus, zero antimicrobial activity.

Table 4. Mean ± SEM, antimicrobial activity of three different concentrations of IPS and EPS P.florida extract against selected microorganisms (n=3)

Microorganism	Concentration (mg/ml)	n	Diameter of zone of inhibition (mm) Mean ± SEM	P<0.05
E. coli	0.2	3	0.00 ± 0.00^{d}	
	0.6		0.00 ± 0.00^{d}	
	1		0.00 ± 0.00^{d}	
	+ve control		$37.00 \pm 0.00^{a,b,c,e}$	
	-ve		0.00 ± 0.00^{d}	
S.aureus	0.2	3	0.00 ± 0.00^{d}	
	0.6		0.00 ± 0.00^{d}	
	1		0.00 ± 0.00^{d}	
	+ve control		$36.00 \pm 0.00^{a,b,c,e,}$	
	-ve		$0.00 \pm 0.00^{d,}$	
P.aeruginosa	0.2	3	0.00 ± 0.00^{d}	
	0.6		0.00 ± 0.00^{d}	
	1		0.00 ± 0.00^{d}	
	+ve control		$33.00 \pm 0.00^{a,b,c,e,}$	
	-ve		0.00 ± 0.00^{d}	

In this study, three concentrations of both the IPS and EPS extracts were evaluated using this test. The concentrations used were 0.2, 0.6 and 1mg/ml. The results showed zero zones of inhibition against the three different bacterias for both IPS and EPS extracts in comparison with positive control that showed zones of inhibition of 36, 33, and 37 for *S.aureus, P.aeruginosa and E.coli* accordingly.

No articles were found that studies the antimicrobial activities of these concentrations. All the articles found showed studies on higher concentrations; the present study was conducted to investigate the antimicrobial and antioxidant potential of polysaccharides extracted from mushroom species *Pleurotus florida* at low concentration. However, it did not exhibit any antimicrobial effects against all microbes tested.

CONCLUSION

Pleurotus florida, which is also known as white oyster mushroom, has shown a lot of antioxidant potential that can make it an eligible candidate for dietary antioxidants and also, it can be incorporated in pharmaceuticals and supplements as antioxidant products. On the other hand, low concentrations of *Pleurotus florida* didn't show any antimicrobial activity which means that *Pleurotus florida* can be a good antimicrobial agent only in high concentrations.

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