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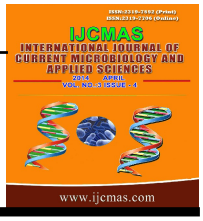

Original Research Article

Mycelial Growth and Bioactive Substance Production of *Pleurotus ostreatus* in Submerged Culture

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A B S T R A C T

The aim of the current work is to study the potential of edible mushrooms as a mycelial biomass source use as food additives and bioactive source (Exopolysaccharide) for antimicrobial and antioxidant activity. Also, it aims to study the effect of various carbon sources such as glucose, maltose, sucrose, starch, lactose and fructose on mycelial biomass and exopolysaccharide production by *Pleurotus ostereatus* at different fermentation periods. The optimal period for mycelial biomass and exopolysacchride production was 15 days. The optimal sugar for mycelial biomass production was starch. The optimal sugar was glucose for high efficiency of exoploysaccharide production; antimicrobial and antioxidant activities. The concentrated *exopolysaccharide* filtrate of *Ploroteus ostreatus* showed wide antibacterial inhibition (63.15%, 59.25%, 55.31%,36.84%,17.24% for *B.subtilis*, *B.cereus*, *E.coli*, *Staph aureus* and *Pseudomonas aeruginosa*, respectivelly) when glucose used as the only carbon source.. *Pleurotus ostreatus* cultivated in a medium containing glucose as the only carbon source showed the highest antioxidant activity (59.90%).The bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harnessed as potent antibacterial and fungi toxicants.

Keywords

Pleurotus, Myelial, Exopolysaccharide, Antimicrobial, Antioxidant.

Introduction

Pleurotus spp. (Oyster mushrooms) comprises the group of edible white-rot fungi with important medicinal properties and biotechnological and environmental applications. It represents a major and untapped source of potent new pharmaceutical products. A wide range of activities including antitumour, cardiovascular and antimicrobial are reported in mushrooms. (Cohen et al., 2002). In developing countries like Egypt mushroom progress is a boon in the field of food, medicine, and in generating employment.

In recent years Basidiomycetes and other higher fungi including some recognized medicinal mushrooms have been recognized. Medicinal mushrooms have been re-investigated as sources of novel

antibiotics mainly as a result of increasing difficulty and the cost of isolating novel bioactive compounds from the Actinomycetes and Streptomycetes. The research possesses an idea about the antibiotic activity of some of the important wild mushrooms (Karwa and Rai, 2009).

The petroleum ether, chloroform, acetone and water extracts of polysaccharides from mushroom *Osmoporus odoratus* has been observed that the antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa*; the water extract alone showed antibacterial activity against the tested organisms and the results were comparable with that of amphicillin rather than chloramphenicol (Sivakumar et al., 2006).

Determination of antimicrobial activity profile of *Lycoperdon perlatum, Cantharellus cibarius, Clavaria vermiculris, Ramaria formosa, Maramius oreades* and *P. pulmunarius* tested against a panel standard pathogenic bacteria and fungi indicated that the concentration of bioactive components directly influence the antimicrobial capability of the isolates (Ramesh and Pattar, 2010).

Quershi et al. (2010) have studied that the antimicrobial activity of various solvent extracts (40μg/ml) of *Ganoderma lucidum* was tested against six pathogenic species of bacteria. Acetone extract exhibited maximum antibacterial activity (31.60±0.10), while the most susceptible bacterium observed was *Klebsiella pneumoniae.*

The antimicrobial effect of ethanol extracts of *Pleurotus sajorcaju, P. florida* and *P. aureovillosus* were tested against four species of Gram-positive bacteria, five species of Gram-negative bacteria and one species of yeast. *Pleurotus* species had a narrow antibacterial spectrum against Gram-negative bacteria and strongly inhibited the growth of the Gram-positive bacteria tested, including *Bacillus subtilis*, and *M. luteus* (Loganathan et al., 2008).

Mushrooms are rich sources of antioxidants (Barros et al.,2007). In the last years several protocols have reported to determine their antioxidant activity based on spectrophotometric techniques progressively, electrochemical techniques have been tested and developed as alternative tools for the evaluation of different food extracts, expressed in terms of "antioxidant power" due to their quickness , simplicity and low cost (Blasco et al 2004). On the other hand, Ascorbic acid and phenols compounds are common antioxidants in mushrooms. Electrochemical measurement at positive potentials will then correspond to the oxidation of "total phenolic" and "ascorbic acid" plus all the compounds with natural antioxidant properties and electrochemical activity which are present in foods. (Blasco et al 2004).

This work aims to discuss: Firstly optimizing the conditions for maximum mycelial and bioactive polysaccharides production from several types of sugars with inoculation by *Pleurotus ostreatus*. Secondly: Studying the antimicrobial and antioxidant properties of *Pleurotus ostreatus* on some gram positive, gram negative bacteria, some fungi and some yeasts.

Materials and Methods

Source of Basidiomycetes

Throughout the current investigation, a fungal strain was tested for their potential to produce bioactive substances. *Pleurotus ostreatus* was obtained from the Culture Collection of the Laboratory of Mushroom, Food Technology Institute, Agriculture Researches Center, Cairo, Egypt.

Microorganisms

Microorganisms used for the antimicrobial test were gram positive bacteria: such as *Bacillus subtilis, Bacillus cereus and Staphylococcus aureus* .*G*ram negative bacteria like: *Salmonella typhi, Escherichia coli and pseudomonas aeruginosa* .Pathogenic fungi like *Candida albicans* and and *Aspergillus niger were used for antimicrobial tests. Microorganisms* were collected from the Culture Collection of the Department of Agriculture Microbiology, Faculty of Agriculture, Cairo University.

Mycelial cultivation through spore germination

Sabouraud dextrose agar slant was heavily inoculated with spores collected from the gilled mushroom, *Pleurotus ostreatus*. Incubation was carried out at ambient temperature for 7 days. Several sub culturing exercises were carried out until a pure culture was obtained. The mycelium culture thus obtained was used as inoculum in subsequent experiments.

Fermentation medium

The polysaccharide production was carried out on submerged fermentation culture. Mushroom complete medium (MCM, Oxoid) was used for the production of the bioactive substance using different carbon sources i.e. glucose, lactose, fructose, maltose, sucrose, starch with concentration 20 g/l . The fermentation medium was inoculated with 5 % (v/v) of the seed culture and then cultivated in a 250 ml flask containing 50 ml of MCM medium. The culture was then incubated at 25°C with shaking at 200 rpm for 10 and 15 days (Iwan, 2009).

Estimation of mycelium dry weight exopolysaccharide

Samples collected from shake flasks were centrifuged at 5000 rpm for 20 min. The dry weight of mycelium was measured after drying at 70°C for overnight to a constant weight (Iwan, 2009). Yield percentage was calculated in relation to 20 g/l initial sugar concentration.

Estimation of extracted exopolysaccharide

Certain amount of supernatant was mixed with three volumes of absolute ethanol and left for 24 hrs at 4 C°. The resulting precipitate was then separated by centrifugation at 5000 rpm for 10 minutes (Bae et al., 2000). The dry weight exopolysaccharide was measured after drying at 70 C° for overnight to a constant weight (Iwan,2009). The rest amount of crude exopolysaccharide supernatant used in antimicrobial assay. Yield percentage was calculated in relation to 20 g/l initial sugar concentration.

Mycelial biomass and exopolysaccharide production from molasses

The clarification of molasses was done by adding 3 ml concentrated sulfuric acid to 1 Kg molasses mixed with 1000ml tap water. The mixture was heated in a water bath to boiling for 30 minutes, and then it was stand in refrigerator overnight and

sterilized at 121 °C for 15 minutes. Different concentrations of molasses (2%, 4%, 8%) media were inoculated with 5% (v/v) of seed culture and then cultivated in a 500 ml flask containing 100ml of MCM medium. The culture was then incubated at 25 °C with shaking at 200 rpm for 15 days. Samples collected from shake flasks were centrifuged at 5000 rpm for 20 minutes. The dry weight of mycelium was measured after drying at 70°C overnight to a constant weight.(Iwan, 2009). Certain amounts of supernatant was mixed with three volumes of absolute ethanol and left for 24h at 4°C. The resulting precipitate was then separated by centrifugation at 5000 rpm for 10 minutes (Bae et al., 2000). The dry weight of exopolysaccharide was measured after drying at 70 °C for overnight to a constant weight (Iwan, 2009). The rest amount of the supernatant used in the antimicrobial assay.

Antimicrobial activities

Microbial growth by optical density method was used to evaluate the antibacterial activities of the culture filtrates. Bacteria for testing the antibacterial activities were grown in nutrient agar (NA) (M- Lab). The antibacterial activity of the mushroom culture filtrate (Crude exopolysaccharide) were evaluated by adding 50ml of mushroom filtrate to 50ml of fresh potato dextrose broth (v/v) and then autoclaved at121 °C for 15 min (Imtiaj and Lee, 2007) . Cooled liquid medium containing mushroom filtrate was inoculated with each tested bacterium separately in 250ml conical flasks and incubated at 35 °C for 10 and 15 days incubation periods. The bacterial growth was determined by measuring the optical density (OD) at 600nm (Imtiaj and Lee, 2007) after 10 days and 15 days incubation period. For control experiment, 50ml of fresh PDB (M-lab) was added to 50ml sterile distilled water (without the mushrooms filtrates) and inoculated with each bacterium. The OD was measured with the growth inhibition of the microorganism as expressed in percentage with the equations of:

$$\textbf{Growth inhibitions (\%)} = \frac{OD1 - OD2}{OD1} \times 100$$

OD_1= OD of microorganisms tested in medium without culture broth of *P. ostreatus*.OD_2= OD of microorganisms tested in medium with mushroom filterate (Sterilized crude exopolysaccharide filterate of *P. ostreatus.*

Antioxidant activity

The DPPH[2,2-Diphenyl-Picrylhdrazyl] scavenging activity was measured using spectrophotometry (Lee et al., 2002). DPPH scavenging effect calculated according to the equation. DPPH scavenging effect (%)= A0- AP/A0 X 100.Where A0 is the absorbance of the control and AP is the absorbance of sample. Vitamin C (Ascorbic acid) was used as control.(Adebayo et al., 2012).This experiment was done to evaluate the antioxidant activity of extracted exopolysaccharide (EEPS) produced in media containing glucose or maltose or 2% molasses as only carbon sources. Also, to evaluate the antioxidant activity of crude exopolysaccharide (CEPS) in medium containing glucose as only carbon source.

Statistical analysis

The data in triplicate for the parameters in various experiments were subjected to ANOVA (Analysis of variance) (Silva et al., 2009).

Results and Discussion

Effect of carbon source on mycelia biomass production

*Pleurotus ostreatus grow in Mushrooms complete medium containing starch as carbon source; produce the highest mycelia biomass with high significant after 10 and 15 days of incubation period (6.29 and 6.82 g/l, respectively a*s shown in (Table.1) with the highest percentage of biomass yield (31.45 and 34.10, respectively).

As general results showed decline in pH with incubation time and with all carbon sources applied. It was observed that decreasing in pH does not affect the mycelia biomass production as *Pleurotus ostreatus* produce the highest mycelia biomass production when it cultivated in a medium containing starch as only carbon source(Table.2).

Mycelium production by *P.ostreatus* using molasses as cheap carbon source material.

Different concentrations of molasses as a raw material (2%,4%,and 8%) were tested for mycelium production with low cost. It was observed that molasses give the maximum mycelium production (18.62 g/l) with the highest significant after 15 days incubation period with the highest percentage of biomass yield (93.40%) with reduction in pH till 4.7 (Table.2, Fig.2).

Effect of carbon sources on exopolysaccharide production.

The highest polysaccharides production was obtained by the utilization of glucose as carbon source after 15 days of incubation period (1.07g/l) with the highest significant and with the highest percentage of exopolysaccharide yield (5.35%)(Table.3).

Exopolysaccharide production by *P.ostreatus* using Molasses as cheap carbon source material

Different concentrations of molasses as a raw material (2%, 4%, and 8%) were tested for exopolysaccharide production with low cost. It was observed that medium supplemented with 8% molasses give the maximum exopolysaccharide production (14.05%) with the highest significant and with the highest percentage of exopolysaccharide yield (70.25%) after 15 days incubation period (Table.4).

Antimicrobial activity of exopolysaccharide produced by *P.ostreatus*

The antimicrobial activity of exopolysaccharide in the crude exopolysaccharide of *P.ostreatus* is expressed by the growth inhibition of the tested microorganisms as shown in (Table. 5). Growth of *P.ostreatus* in medium containing glucose as carbon source exhibited the highest growth inhibition of the most tested microorganisms using crude and diluted exopolysaccharide. The crude exopolysaccharide extract showed varying degree of inhibition on the tested microorganisms. The results of optical density showed that the concentrated crude exopolysaccharide filtrate of *Pleurotus ostreatus* was highly significantly effective against gram positive bacteria than gram negative bacteria after 15 days incubation time .Also, results of optical density showed that concentrated crude exopolysaccharide culture filtrate was highly significantly effective against *E. coli* and *S. aureus after 15 days incubation time.*

The concentrated culture filtrate of *Pleurotus ostreatus* showed slight inhibitory effect on *Pseudomonas aeruginosa* (Table 5). *Candida albicans* is more susceptible to the concentrated culture filtrate of the mushroom when compared to *A.niger.* Crude exopolysaccharide produced by P.ostreatus in a medium containing maltose shows the highest growth inhibition against Aspergillus niger after 10 days incubation i.e.23.80 %.

The highest percentage inhibitory effect on bacterial growth was recorded in *B.subtilis* (63.15%) while the least was recorded (3.44%) on *Pseudomonas aeruginosa* after 15 days incubation time. Results showed that the highest percentage inhibitory effect on bacterial and fungal growth was achieved when glucose used as the only carbon source with all microorganisms under study (Table.5).

Effect of antioxidant activity of crude exopolysaccharide of *P.ostreatus* cultivated in media containing different carbon sources.

It could be noticed that crude exopolysaccharide produced in a medium containing glucose shows the highest antioxidant activity 59.9% at 150 µl with the highest significant (Table.6). Although, the antioxidant activity of exopolysaccharide of *P.ostreatus* cultivated in medium containing 2% molasses is lower than the antioxidant activity of exopolysaccharide of *P.ostreatus* cultivated in medium containing pure sugars by about 10%. The huge amount of exopolysaccharide produced by *P.ostreatus* cultivated in a medium containing 2% molasses offsets that decrease in antioxidant activity.

Many kinds of mushrooms frequently require starch, glucose, sucrose and etc., for their submerged culture. In order to investigate the effects of different carbon sources on growth and extracellular polysaccharide of *Ploroteus ostreatus*, the fungus was cultivated in the medium containing various carbon sources for 10 and 15 days. Initial pH was adjusted to 5.5 (Table 1 and Table 2) showe the cell concentration (dry weight, w/v) and the extracellular polysaccharides production. The maximum exopolysaccharide production was obtained from medium containing glucose with a final mycelial biomass concentration of 1.07g/l. This result is in agreement with the results reported by other investigators, who demonstrated that glucose is clearly a good carbon source for exopolysaccharide production in submerged cultures of mushrooms (Xu et al. 2003). Meanwhile, this result is in disagreement with (Iwan , 2009) who found that the highest exopolysaccharide production of *Pleurotus ostreatus* is obtained in a medium containing maltose as only carbon source. Meanwhile 16.6% inhibition of spore germination of *Aspergillus niger* was observed from the medium containing glucose as carbon source. The maximum mycelial biomass level was found in medium containing Starch. The fungus grew very well in the medium containing Starch with a total biomass concentration of 6.82 g/l, being significantly higher than the medium containing maltose, lactose and other sugars. Sistorm and Michilis (1955) explained that fructose is next best utilized carbon by many fungi. Often adaptation is required before growth begins, but then growth is often as rapid as with glucose. It was unexpected, therefore, the inhibition of spore germination was low when fructose was used (Table 1). It seems that glucose as a carbon source is necessary for production of such bioactive compounds. This was explained that “a

Table.1 Myelium production by *P ostreatus* using different sugar types

Type of Sugar	Mycelium production (g/l) at different incubation period			Biomass Yield %		
	0 days	10 days	15 days	0 days	10 days	15 days
Glucose	0.70	2.68	3.15	3.5	13.4	15.75
Maltose	0.70	5.46	6.04	3.5	27.3	30.2
Lactose	0.70	3.08	3.02	3.5	15.4	15.10
Sucrose	0.70	0.98	1.07	3.5	4.9	5.35
Starch	0.70	6.29	6.82	3.5	31.45	34.10
Fructose	0.70	1.47	1.28	3.5	7.35	6.40
LSD(0.01)	0.2805					

LSD carbon sources x incubation time _ (0.01)

Figure.1 Changes in pH during mycelium production using different types of sugars. pH at zero time was 5.5 in all treatments

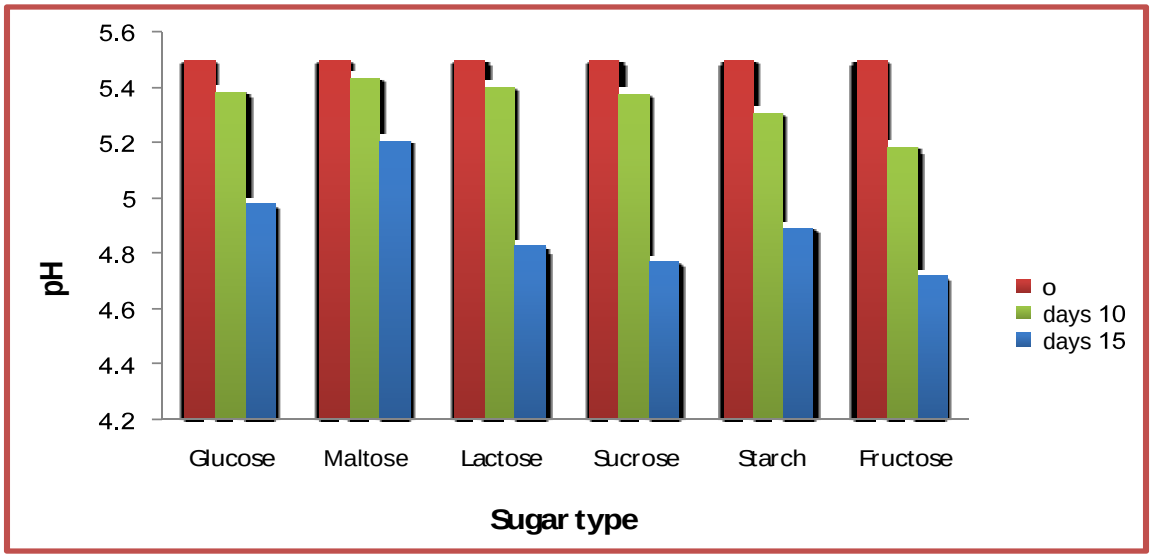

Table.2 Mycelium production by *P.ostreatus* using Molasses as carbon source.

Molasses %	Mycelium production (g/l) at different incubation period.		Yield %	
	0 days	15 days	0 days	15 days
2%	0.90	7.37	4.50	36.85
4%	0.90	13.00	4.50	65.00
6%	0.90	18.62	4.50	93.40
LSD(0.01)	0.4233			

LSD concentrations x incubation time _ (0.01)

Figure.2 Changes in pH during mycelium production using different concentrations of molasses

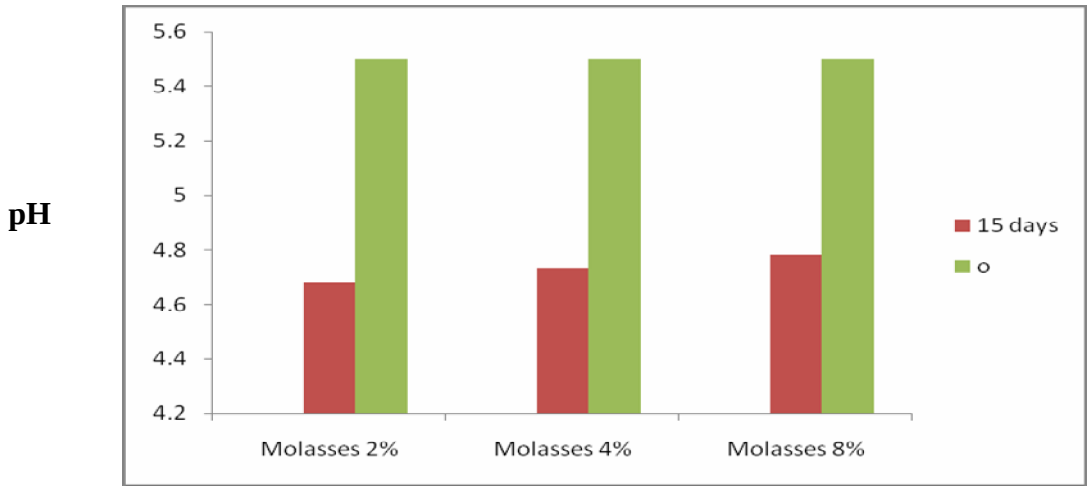

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Table.3 Exopolysaccharide production by *P. ostreatus* using different sugar types.

Types of sugar	Exopolysaccharide production g/l) at different incubation period.		Yield %	
	10 days	15 days	10 days	15 days
Glucose	1.04	1.07	5.2	5.35
Maltose	1.02	1.04	5.1	5.20
Lactose	0.80	1.00	4.0	5.00
Sucrose	1.00	1.01	5.0	5.05
Starch	0.80	0.90	4.0	4.50
Fructose	0.50	0.70	2.50	3.50
LSD (0.05)	0.26518			

LSD carbon sources x incubation time _ (0.05)

EEPS: Extracted exopolysaccharide

Table.4 Exopolysaccharide production by *P.ostreatus* using different molasses concentrations

Molasses	Exopolysaccharide production (g/l) after 15 days incubation period.	Yield%.
2%	4.01	20.05
4%	9.00	45.00
8%	14.05	70.25
LSD (0.01)	0.4618	

LSD concentration x incubation time _ (0.01)

The amount of exopolysaccharide at the beginning of experiment= 0.01 g/L

Table.5 Antimicrobial activity of exopolysaccharide of *P.ostreatus* in mushrooms complete medium containing different types of sugar types after 10 days and 15 days of cultivation using crude and diluted exopolysaccharide.

Carbon Source	Incubation Time (Days)	*Inhibition of microbial strains%*								
		Dilutions	*E.coli*	*B.subtilis*	*B.cereus*	*Pseudomonas aeruginosa*	*Salmonella*	*Staph aureus*	*Candida albicans*	*A.niger*
Glucose	10	Conc	54.16	61.64	58.22	16.12	25.58	33.33	35.51	16.66
		*Dilut	35.41	43.83	37.97	9.67	20.93	22.22	17.22	7.14
	15	Conc	55.31	63.15	59.25	17.24	26.82	36.84	36.27	17.02
		*Dilut	38.29	44.73	40.74	13.79	24.39	33.33	34.31	14.89
Maltose	10	Conc	43.75	61.64	53.16	9.67	19.76	35.18	29.90	23.80
		*Dilut	25	26.02	31.64	3.22	19.76	12.96	13.08	7.14
	15	Conc	44.68	61.84	58.02	10.34	20.73	36.84	30.39	25.59
		*Dilut	25.53	27.63	32.09	10.34	20.73	28.07	25.49	14.89
Lactose	10	Conc	14.58	9.58	32.91	0	2.32	14.81	10.28	4.76
		*Dilut	0	6.84	18.98	0	0	9.25	7.47	0
	15	Conc	14.89	10.52	33.33	3.44	2.43	15.78	10.78	6.38
		*Dilut	6.38	7.89	19.75	3.44	2.43	10.52	7.84	4.25
Sucrose	10	Conc	33.33	36.98	45.56	9.67	15.11	22.22	23.36	14.28
		*Dilut	12.50	19.17	22.78	0	8.13	12.96	9.34	7.14
	15	Conc	36.17	38.15	45.67	13.79	15.85	22.80	24.50	17.02
		*Dilut	17.02	19.73	23.45	6.89	13.41	15.78	16.66	12.76
Starch	10	Conc	20.83	20.54	26.58	12.90	9.30	12.96	17.75	7.14
		*Dilut	14.58	13.69	17.72	3.22	6.97	7.40	9.34	2.38
	15	Conc	21.27	25	29.62	13.79	10.97	14.03	18.62	8.51
		*Dilut	14.89	14.47	18.51	10.34	7.31	10.52	12.74	6.38
Fructose	10	Conc	6.25	9.58	12.65	6.45	5.81	7.40	6.54	7.14
		*Dilut	6.25	8.21	12.65	0	0	3.70	6.54	0
	15	Conc	8.51	10.52	13.58	6.89	6.09	10.52	6.80	6.38
		*Dilut	6.38	9.21	13.58	3.44	2.43	3.50	5.84	0

***Dilut:** Diluted 1:1 .

LSD carbon sources x incubation time x Concentration x strains _ (0.05) = 0.7693

Table.6 Antioxidant activity of crude exopolysaccharide of *P.ostreatus* cultivated in media containing different carbon sources

Exopolysaccharide obtained from different types of sugars	Antioxidant activity of Exopolysaccharide [DPPH assay scavenging %]			
	25 µl	50 µl	100 µl	150 µl
Glucose EEPS	27.34%	31.51%	41.41%	53.65%
Maltose EEPS	24.22%	29.69%	38.28%	46.61%
2% Molasses EPS	21.09%	27.60%	35.94%	41.93%
Glucose CEPS	31.25%	35.68%	46.09%	59.90%
LSD (0.01)	0.9057			

LSD carbon sources x concentrations _ (0.01)

EEPS: *Extracted e*xopolysaccharide obtained from different types of sugars

CEPS: *Crud exopolysaccharide obtained from medium supplemented with glucose as carbon source.*

fungal species may have the ability to utilize a particular carbon source for vegetative growth but may be unable to use it for production of specialized structure (Garraway MO and Evans, 1984)

It is interesting to note that little difference was seen in the inhibition activity of the extracts from the media containing fructose and lactose. The biomass in the growth medium containing maltose was lower than that of fructose; however the bioactivity was found to be similar. Bioactivity per gram was therefore increased when maltose was utilized as the carbon source. It is possible that fructose as a simple sugar interferes with product formation and maltose as a slow releasing carbon sources supported production of bioactive compounds rather than growth of the fungus. Also, with the exception of the medium containing glucose, none of the other cultures totally inhibited spore germination of test organism. Sucrose did not support growth of the fungus and consequently had no effect on production of bioactive compounds. The same result has also been reported that sucrose is a very poor carbon sources for growth of *Polorteus oysterous*, compared to fructose and glucose (Moore,1969).

The crude concentrated exopolysaccharide filtrate of mushrooms under study showed a wide range of antibacterial and antifungal activity (Table.5). They exhibited moderate to good antibacterial activity against the bacteria pathogens tested. The filtrate of *Pleurotus ostreatus* is very effective against *E. coli* and *S.aureus*. This report is similar to the findings of Ishikawa *et al* (2001*),* who showed that the mycelial culture filtrate of *Lentinula edodes* inhibited the growth of *B. subtilis*. The observed inhibitory effect of *Pleurotus ostreatus* on both gram negative and gram positive bacteria is in line with the report of Komemushi *et al (*1995, 1996) who studied the antimicrobial substances in *L. edodes*. Imtiaj and Lee (2007) also worked on the antibacterial and antifungal activities of Korean wild mushrooms and found that several filtrates of wild mushrooms inhibited the growth of many pathogenic bacteria such as *P. aeruginosa* and *S. aureus.* The metabolite showed

antagonistic effect against all tested pathogens and slightly affected *P. aeruginosa*, with in parallel with the results of Adebayo et al (2012) .The ability of the metabolite to inhibit all tested organisms with slightly inhibition to *P. aeruginosa*, suggests that this product contained potential antibacterial agents against infections from these pathogens.

The obtained results are in agreement with the results reported by Ramseur et al (2003) who investigated that crude extract of African mushroom, *P.tuberregium* had antibacterial activity against several food pathogens such as *Staphylococcus aureus Salmonella typhi* and *E.coli.*

Also, Gbolagade et al (2007) reported that *P. tuber-regium* extract had powerful medicinal importance by inhibiting the growth of *Bacillus cereus, E. coli, K. pneumoniae, S. aureus, Proteus vulgaris,* and *P. aeruginosa*.

The antimicrobial activity of mushroom extracts has been reported earlier by several researchers (Beattie el.,2010, Rosa et al., 2003). The highest zone size (30mm) against *S. aureus* suggested that the metabolite could be used in the treatment of infections commonly associated with the organisms (Bala et al., 2011) and confirmed the traditional use of *P.ostreatus* extract in treatment of skin diseases (*Öztürk et al., 2011*).

Also, In the present study the extracts of mushroom crude exopolysaccharide was able to decolorize DPPH and the free radical scavenging potentials of the extract was found to be on the high side (Table.6). It appears that the extracts from the *P. ostreatus* evaluated in this study possess hydrogen donating capabilities to act as antioxidant.(Gezer et al., 2006).

Finally, this study has revealed the antimicrobial activity of the edible mushrooms under study and can be suggested that the bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harnessed as potential antibacterial and fungi toxicants. Further extensive studies are recommended for this mushroom and other mushrooms types to actually identify the bioactive components responsible for their antimicrobial activities.

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