Journal of Environmental Studies [JES]
An International Journal
https://jesj.journals.ekb.eg/
doi: 10.21608/JESJ.2022.164389.1031

Antimicrobial and Antioxidant Activities of Pleurotus Ostreatus as Edible Oyster Mushroom in Egypt

Youssef, M.S.[1*], Thanaa A. Maghraby[2], Hend Abo-Elsoaud A. Hussein[1]

[1] Botany and Microbiology Department, Faculty of Science, Sohag University, Sohag, Egypt.
[2] Botany and Microbiology Department, Faculty of Science, South Valley University, Qena, Egypt.

Citation: *Youssef, M.S., Maghraby T. A., Abo-Elsoaud H.A. (2022). Antimicrobial and Antioxidant Activities of Pleurotus Ostreatus as Edible Oyster Mushroom in Egypt, Vol. 28(1): 24-34.*

Article Information
Received 29 Sep. 2022,
Revised 02 Nov. 2022,
Accepted 08 Dec. 2022.
Published online
30 Decmber 2022

Abstract: The research was designed to study antimicrobial and antioxidant activities of *Pleurotus ostreatus* as one of the most common edible oyster mushroom in Egypt and around the world. Mushroom spawns cultivation, maturity growth and fruiting bodies harvesting, and different chemical analysis were performed. Antimicrobial activity of petroleum ether, methanol and acetone extracts of mushroom were tested against various pathogenic bacteria (5 gram +ve and 4 gram –ve species) and 5 pathogenic yeast species in addition to different antioxidant activities. Moisture content ranged between 84.3- 88.5% with 86.0% as average. Total carbohydrates was 101.97 g/kg, total proteins was 166.09g/kg, total lipids was 2.64 g/ kg and fiber was 42,60mg/kg, while ash ranged between 70- 80%. Minerals analysis showed that Zn was 90mg/100g, Mg 1175 mg/100g, Fe 17.9 mg/100g, Mn 1.5mg/100g and Ca 345mg/100g. Methanol extract, acetone extract followed by petroleum ether extract showed different degrees of inhibition against pathogenic microorganisms tested. On the other hand, antioxidant examination revealed that methanol extract of *Pleurotus ostreatus* antioxidant content was so high that ***β***-carotene-linoleic acid content was 11.9 mg/kg, total phenol was 1390.04 mg/kg and reducing power was 52.52 µM TE /mg as antioxidant agents.

Keywords: *Pleurotus ostreatus*, edible oyster mushroom, antimicrobial activity, antioxidant activity

Introduction

Mushrooms as macro fungi are usually large enough to be seen with the naked eye, and picked by hand, with distinctive fleshy and edible hypogenous or epigeous fruiting bodies. They have been widely used as delicious and nutritious foods. Some of them serve as food because of their nutrient contents while, some others have been used extensively in traditional medicine. Mushrooms can be consumed in different forms such as fresh, dried, powdered, canned, pickled, etc. Farming of different mushrooms has picked up a fast pace among contemporary entrepreneurs due to its low-cost input with high output, in addition to high nutritional and medicinal benefits (Syed *et al*., 2009, Bawadekji & Al-Ali, 2016).

Oyster mushrooms are one of functional foods, provide health benefits beyond the traditional nutrients they contain. *Pleurotus* genus is one of gilled mushrooms, containing more than 40 species called **"oyster mushrooms"**. *Pleurotus* genus comprises *P. ostreatus*, *P. citrinopileatus*, *P. cornucopea*, *P. cystidiosus*, *P. eryngii*, *P. flabellatus*, *P. florida*, *P. geesteranus*, *P. highbing*, *P. pulmonarius*, *P. sajorcaju*, *P. sapidus*, *P. ulmarium*, and others. Oyster mushrooms are healthy foods with low in calories and fat, while rich in proteins, vitamins minerals and chitin in addition to high amounts of ornithine and γ-amino butyric acid (GABA) **(**Akindahunsi & Oyetayo, 2006; Jayakumar *et al*., 2006; Alam *et al*., 2008**).**

In oriental culture for many years, mushrooms have been used as tea and nutritional food due to their special fragrance and texture **(**Manzi *et al*., 1999**).** The scientific research for new therapeutic alternatives, has investigated alot of kinds of mushrooms and has proved several therapeutic activities as anticarcinogenic, immune suppressor, anti-inflammatory, antioxidant, antibiotic, etc…. (Asfors & Ley, 1993; Longvah & Deosthale, 1998; Bawadekji & Al-Ali, 2016).

However, oxygen free radicals and other reactive oxygen types which in vivo are continuously produced, lead to cell death and tissue damage causing old and diseases, such as cancer, diabetes, atherosclerosis, and cirrhosis **(**Hu *et al*., 2006**)**. Antioxidant supplements, or foods containing antioxidants play an importat role in helping the human body to reduce oxidative damage **(**Cheung *et al*., 2003; Okafor *et al*., 2017**).**

* Corresponding author E-mail: hindelsod.2016@gmail.com

Edible mushrooms are considered rich sources of antioxidants as flavonoids, vitamin A, C, E, polyphenolic compounds and carotenoids because prevent damage of free radical and decrease risk of chronic diseases due to their capability to scavenge free radicals by single-electron transfer **(**Sies & Masumoto, 1997; Bawadekji *et al*., 2007; Sabino Ferrari, 2020**).** They have been widely used as food and food supplements for millennia. It is an important food item concerning human health, nutrition and disease prevention **(**Chang, 1996; Melinda-Fogarasi *et al*., 2021**).**

In recent times, there has been a renewed interest in traditional medicine and importance has been focused on the use of natural plant materials in the control of various infections and diseases. Mushrooms have been reported to contain dietary fibers, β-glucans, chitin, pectinous substances, natural antibiotics, phenolic compounds, flavonoids and several other secondary metabolites. Many recearchers reported the efficacy of various mushroom extracts against different pathogenic microorganisms (Jayakumar *et al*., 2006; Türkoğlu *et al*., 2007; Baeva *et al*., 2019).

Therefore, this research was designed to analyze the nutritional values and evaluate the antimicrobial potential and antioxidant activity of *Pleurotus ostreatus* as one species of oyster mushroom cultivated in Egypt, with a goal of increasing awareness of the beneficial effects of edible mushroom among the consumers.

Materials and Methods

1.a. Cultivation and harvesting of oyster mushroom

Fungal spawns of *Pleurotus ostreatus* was purchased from National Center for Agricultural Research**,** Cairo, Egypt. Paddy straw as a substrate used for the cultivation of oyster mushroom **(*Pleurotus ostreatous)*** was steam sterilized. Sterilized paddy straw was filled in the bags at a height of 5 inches and spawns were added on the sides as three to four layers. The sterile substrate was compressed and made airtight. The spawned bags were incubated in a dark room for 15-21 days. During incubation period, the mycelium growth appears in white color and the bags were transferred to the cropping room. Relative humidity in cropping room must be between 85-90% by spraying sterilized water daily. Fruiting bodies of mushrooms were developed after 15 -20 days of incubation and they were harvested at maturity **(**Dundar *et al*., 2008; Sharma, 2015**).**

1.b. Processing

Mushroom fruiting bodies lose their freshness and deteriorates rapidly at ambient temperature if not processed or refrigerated within a day. Pre-processing involves mushroom washing to remove any substrate debries or compost or adhering soil and blanching them for few minutes to inactivate the enzymes. To prevent mushrooms discoloration, they are treated with citric acid, salt or brine before canning or packaging. Then, processing and preservation methods are used according to Sharma (2015).

1.c. Drying

Moisture encourages the microbial growth and propagation; hence its removal will retard the microbial activity. In this study, oven drying was used for 24 hours at 60°C to have a very dried mushroom.

1.d. Grinding

It is processed to have the dried mushroom very fine to used it in the biological tests easily, so the mushroom was cut into small pieces after drying, crushed well using a mortal, and then smooth with clean sieve to obtain a fine powder that is easy to use in laboratory tests, or packed in clean transparent bags and stored in the freezer for later use at -8°C for period of not exceeding 6 months .

2. Determination of Chemical properties

2.a. Determination of moisture content

This was performed according to the method of Raghuramulu *et al*., 2003. The moisture content was calculated according to following equation:

Moisture (%) = (initial weight − final weight) × 100/initial weight of sample

2.b. Determination of total ash

Total ash was determined according to the method employed by Raghuramulu et al., 2003. Then total ash was calculated according to following equation:

Ash content (g/100 g sample) = weight of ash × 100/weight of sample taken.

2.c. Determination of total carbohydrates

2.c. 1. Extract preparation

The powder sample about 10gm was weighed, prepared and extracted using 100 ml of water with stirring for 24hrs. Then, filtered and used further.

2.c. 2. Estimation of total carbohydrates

1- Take 100mg of glucose in test tube. 2- Add to them, 5ml 2.5N HCl and boil on a water bath for 3hrs to hydrolyze. 3-Cool the mixture to room temperature. 4- add to them a sufficient quantity of solid sodium carbonate (Na_2CO_3) till effervescence ceases. 5-This indicates complete neutralization, then filter and make the volume up to 100ml. 6- Take out by pipette 0.2, 0.4, 0.6, 0.8, 1ml of working standards in different test tubes. 7-Then pipette out 0.2 ml of the sample solution and complete to 1ml with water. Set blank with all reagents without sample.8- Add 1ml phenol into each

tube. 9-To this, add 5ml 96% H_2SO_4 and shake well. 10-After 10 min, shake the contents in the different tubes and place them in a water bath at 25 - 30°C for 20min 11- Glucose was dehydrated to hydroxyl methyl furfural in hot acidic medium. 12-Then, green colored product was formed with phenol. 13-The color intensity was determined at 490 nm. 14-Finally total carbohydrates was calculated using the following formula as employed by Biswas *et al*., (2011).

$$\text{Amount of carbohydrate percent in 100 mg of the sample} = \frac{\text{mg of glucose}}{\text{volume of test sample}} \text{ X } 100$$

2.d. Determination of total proteins

To estimate the protein using Lowry's method, The –CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a blue colored complex as employed by Mahesha, (2012).

2.e. Determination of Lipid

Method of Folch *et al*., (1957) with slight modification was used for total lipid determination. Take 5 g of grinded mushroom and suspend in mixture of 50 ml chloroform : methanol (2 : 1 v/v), mix thoroughly and let to stand for 3 days. After that, solution was filtrated and centrifuged at 1000 rpm. Remove the methanol upper layer by Pasteur pipette and evaporate chloroform by heating. The remaining is the crude lipid. The total lipids of fresh mushroom was determined by taking 5 g of mushroom with 50 ml phosphate buffer and homogenized with a tissue homogenizer. Suspend 5 ml of homogenized substance with 50 ml mixture of chloroform:methanol (2:1 v/v) and lipid content was determined as mentioned above.

2.f. Determination of fiber

Take 10 g of dry and fat-free sample in a beaker and add 200 ml of boiling 0.255 N H_2SO_4. Boile the mixture for 30 minutes with keeping the volume constant by water addition at frequent intervals. Filter the mixture through a muslin cloth and wash the residue with hot water untill free from acid. Transfeert the material into a beaker, add 200 ml of boiling 0.313 N NaOH and boil for 30 minutes. After that, filter the mixture through a muslin cloth and wash the residue with hot water till free from alkali, followed by washing with some alcohol and ether. Then, transfeer the material into a crucible, dried overnight at 80 - 100°C and weighed (W_1). The crucible was heated in a muffle furnace at 600 °C for 5 - 6 hours, cooled and weighed again (W_2). The difference in the weights ($W_1 – W_2$) represents the weight of crude fiber as employed by Alam et al., 2008. Crude fiber (g/100g sample) was calculated according to the following Raghuramulu et al., 2003 formula:

Crude fiber (g/100g sample) = [100 – (moisture + fat)] x $(W_1 – W_2)/W_t$ of the sample.

3. Mineral analysis

Total ash was taken for the analysis of calcium (Ca), iron (Fe), manganese (Mn), magnesium (Mg), zinc (Zn), by flame and graphite method with atomic absorption spectrophotometer as employed by Perkin Elmer (1982).

4. Antimicrobial testing of *Pleurotus ostreatus* extracts

4.a. Preparation of oyster mushroom extracts

Three different solvents (petroleum ether, methanol and acetone) were used to fractionate the soluble compounds from the mushrooms in ascending polarity. According to the method of extraction employed by Cheung *et al*., (2003) & Melinda Fogarasi *et al*., (2021).

4.a. 1. Petroleum ether extract

After a was obtained using an electric mill (Restsch Ultra Centrifugal Mill and Sieving Machine, Haan, Germany). Twenty grams of dried fine powder (20 mesh) mushroom sample was extracted by stirring with 100 ml of petroleum ether at 25°C for 2h using Soxhlet apparatus and filtering through Whatman No. 4 filter paper. The residue was then re-extracted with additional 100 ml portions of petroleum ether, as described earlier. The combined petroleum ether extracts were then rotary evaporated at 40°C to dryness. The dried extract thus obtained was used directly or re-dissolved in petroleum ether to a concentration of 50 mg ml/1 and stored at 40°C prior to assay antimicrobial activity.

4.a. 2. Methanol extract

The residue of petroleum ether extract was re-extracted by stirring with 100 ml of methanol at 25°C for 2 h using Soxhlet apparatus and filtering through Whatman No. 4 filter paper. The residue was then extracted with additional 100 ml portions of methanol, as described earlier. The combined methanolic extracts were then rotary evaporated at 40°C to dryness. The dried extract thus obtained was used directly or redissolved in methanol to a concentration of 50 mg ml/1 and stored at 4°C prior to assay of antimicrobial activity.

4.a. 3. Acetone extract

The dried residue of methanolic extract was boiled at 100°C for 3 h with 100 ml acetone to obtain extract. The mixture was filtered through Whatman No. 4 filter paper. The residue was then extracted with additional 100 ml portions of acetone, as described earlier. The combined acetone extracts were then rotary evaporated at 40°C to dryness. The acetone extract was dried in a freeze drier. The dried extract thus obtained was used

directly or redissolved to a concentration of 50 mg ml/1 and stored at 4°C for assay of antimicrobial activity.

4.b. Disc-diffusion method

This method was used to measure the antimicrobial activity as employed by Janssen *et al*. (1987) & Ayoub (1990)**.** The mushroom extracts to be tested were added to filter paper disc reservoirs at a concentration of 50 µg/disc (Whatman No.3 filter paper, 0.5 cm diameter) for assay antibacterial activity and 100 µg/disc (Whatman No.3 filter paper, 0.9 cm diameter) for antifungal activity.

4.c. Assay of antibacterial activityOrganisms

The antibacterial spectrum of the different mushroom extracts was tested against nine pathogenic bacteria1 strains, five Gm+ve bacterial strains named; *Bacillus cereus*, *B. amyloliquefaciens*, *B. Subtilis*, *Staphylococcus aureus*, *S. epidermidis* and four gm-ve bacteria strains; *Escherichia coli*, *Pseudomonas. aeruginosa*, *Salmonella paratyphi* and *Proteus vulgaris.*

Nutrient agar medium

A basal medium (pH 7.2) of nutrient agar gm/l (Peptone, 10; lab-Lemco meat extract (Oxoid), 10; Sodium chloride, 5; Agar, 15-20) was used in this study. As described by Harrigan & Margaret Mccane, (1966). Mix agar with medium and heat to boiling solid medium was prepared in one-liter amounts and autoclaved at 121°C for 20 minutes. The different mushroom extracts were tested using 0.5 cm diameter filter paper of Whatman No 3, containing 50 µg of each extract. The discs were placed on nutrient agar plates seeded with 1ml of bacterial spores suspension containing ca.1×10^7 spores/ml. The plates were incubated at 35°C for 48 hours. Extract constituents diffused into the medium surrounding the disc and formed an inhibition zone of bacterial growth, which was measured with Vernier caliper and correlated with the degree of antibacterial activity.

4.d. Assay of antifungal activity

The assay for antifungal activity of each extract was evaluated by paper disc diffusion technique against five pathogenic yeast strains namely; *Candida albicans*, *C. glabrata*, *C. krusei*, *Pichia kudriavzavii* and *Rhodotorula Mucilaginosa*. The test was carried out in Petri plates containing 15 ml of potato dextrose agar **(**PDA medium**).** Mix agar with medium and heat to boiling. Solid medium was prepared in one-liter amounts and autoclaved at 121°C for 20 minutes. The mushroom extracts were tested using 0.9cm diameter filter paper disc of Whatman No 3, containing 100 µg of each extract. The discs were placed on the potato dextrose agar plates seeded with 1ml of yeast spores suspension containing ca.1×10^7 spores/ml. The plates were incubated at 37°C for 2 days. Extract constituents diffused into the medium surrounding the disc and formed an inhibition zone of yeast growth, which was measured with Vernier caliper and correlated with the degree of antifungal activity.

4.e. Antioxidant activity

4.e. 1.Determination of reducing power

Reducing power was carried out according to the FRAP assay (Benzie & Staring, 1996**)** as quick, sensitive and easy way for measuring the antioxidant capacity of different biological samples. The FRAP assay (ferric reducing antioxidant power) can detect antioxidant capacities as low as 0.2 mM Fe^{2+} equivalents. The antioxidant potential of samples is determined using a ferrous iron standard curve and results are expressed as Fe2+ equivalents (µM) or FRAP value. The assay measures the antioxidant potential in sample through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants present in the sample. Following the reduction of the ferric iron, a blue color develops that is read calorimetrically at 594 nm. A freshly prepared TPTZ reagent (300 mM acetate buffer (PH=3.6), 10 mM TPTZ in 40mM HCl, and 20 mM $FeCl_3$, in a ratio of 10:1:1 v/v/v, respectively). 190 ul from the freshly prepared TPTZ was incubated at room temp for 30 min in dark. At the end of the incubation period, the resulting blue colored was measured at 594nm, data were represented as means.

4.e. 2. Extraction

Fresh fungal material was milled by an electrical mill. Finely ground mushrooms (50 g) were extracted using methanol for 24 h. The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at -18°C until used in the tests. The extracts were dissolved in 5% dimethyl sulphoxide (DMSO)

4.e. 3. Reducing power

Standards and samples preparation: Trolox standard for FRAP assay Trolox stock solution of 2 mM in methanol was prepared, and the following dilutions were prepared at the concentrations of 1000, 800, 600, 400, 200, 100, 50 and 25 µM **(**Benzie & Staring, 1996, 1999**).**

4.f. Antioxidant activity by β-carotene-linoleic acid

The antioxidant activity of *Pleurotus ostreatus* extracts was evaluated using ***β***-carotene linoleate model system as described by Jayaprakasha *et al*., 2001**,** with some modifications. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the *β*-carotene using the following formula: $AA = 100[1-(A_0 - A_t)/($

$A^0_0 - A^0_t$)] where A_o and A^o_o are the absorbance values measured at zero time of the incubation for test sample and control, respectively. A_t and A^o_t are the absorbance measured in the test sample and control, respectively, after incubation for 60 min. The results were expressed in % basis of preventing bleaching of *β*-carotene.

4-g-Determination of total phenolics

The concentration of total phenols in the extracts was determined by using Folin–Ciocalteu reagent and external calibration with caffeic acid. Briefly, 0.2 ml of extract solution and 0.2 ml of Folin–Ciocalteu reagent were added and the contents mixed thoroughly. After 4 min, 1 ml of 15% Na_2CO_3 was added, and then the mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 760 nm using a HITACHI, U-2000 spectrophotometer. The concentration of the total phenolics was determined as mg of caffeic acid equivalent using an equation obtained from caffeic acid calibration curve. The estimation of phenolic compounds in the fractions was carried out in triplicate and the results were averaged as employed by Albugri & McElhenney (2013).

Results

The values of moisture content values of tested *Pleurotus ostreatus* mushroom were so high and fluctuated between 84.3 – 88.5 with mean value was 86.07% as shown in Table (1). Data in Table 2 revealed that total ash ranged between 70- 80% with mean value was 75%. Data in Table 3 of nutrients content analysis of *Pleurotus ostreatus* mushroom revealed that average total carbohydrates were 101970.53 mg/kg, while average total proteins was166090.29 mg/kg. Whereas lipid content of tested mushroom was 2643.17 mg/kg, whilst fiber value was 2643.17 mg/kg.

Table1: Moisture content of *Pleurotus ostreatus*

Initial weight	Final weight	Moisture content	Moisture (%)	Mean of Moisture (%)
178	27.9	150.1	84.3	86.07%
218	31.7	186.3	85.4	
561	65.4	495.6	88.5	

Table 2: Determination of total ash of *Pleurotus ostreatus*

crucible weight	Dry sample weight	Initial weight	Final weight	Average	Total ash (%)	Mean of total ash (%)
29	2.0	31	29.5	1.5	75	75
15	2.0	17	15.4	1.6	80	
33	2.0	35	33.6	1.4	70	

Table 3: Determinatin of total carbohydrates, total proteins, lipids and fiber

Nutrient	Average in mg/kg
Total carbohydrates	101970.53
Total proteins	166090.29
Lipids	2643.17
Fiber	42.60

Table 4: Analyzing of mineral element content (Zn, Mg, Fe, Ca and Mn) of *Pleurotus ostreatus*

Element content in 100 gm mushroom dry weight	Average of element content in mg/100 gm mushroom dry weight				
	Zn	Mg	Fe	Ca	Mn
Element mg/100 g	90	1175	17.9	1.5	345

The results of mineral analyzing in (Table,4) showed that Zn content was 90 mg/ 100g mushroom dry weight, while Mg content was 1175, whereas Fe content was 17.9, whilst Ca value was 1.5 and Mn value was 345.

Antimicrobial Activity

a-Antibacterial activity

Data in Table, 5 of antibacterial activity of methanol, petroleum ether and acetone extracts of Pleurotus ostreatus tested against 5 Gm+ve (Bacillus cereus, B. amyloliquefaciens, B. Subtilis; Staphylococcus aureus and S. epidermidis) and 4 Gm-ve (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Proteus vulgaris*) bacterial strains revealed that methanol extract had broad spectrum antibacterial activity inhibited 8 bacterial strains out of 9 tested with moderate bacterial inhibition zone between 10 - 19 mm. While, acetone extract ranked second order with intermediate spectrum antibacterial activity inhibited 5 bacterial strains out of 9 tested, with high bacterial inhibition zone more than 20mm on 4 out of 5 Gm+ve bacterial strains and moderate bacterial inhibition zone 15mm on Escherichia coli only out of 4 Gm-ve bacterial strains tested.

On the other hand, Petroleum ether extract had low spectrum antibacterial activity inhibited 2 bacterial strains only out of 9 tested with moderate bacterial inhibition zone (15 and 18 mm, respectively) on *Staphylococcus epidermidis* and *S. aureus* as Gm+ve bacterial strains.

So, according to antibacterial activity, methanol was highly active followed by acetone, while petroleum ether extract was the last active. Also, Gm+ve bacterial strains were more sensitive to extracts treatment than Gm-ve strains. On contrast, Gm-ve bacterial strains were more resistant.

Sensitivity of bacterial strain tested

S: Sensitive bacterial strain affected and inhibited by used extract.

Table 5: Comparison of antibacterial activity of methanol, petroleum ether and acetone extracts of *Pleurotus ostreatus* against tested pathogenic Gm+ve and Gm-ve bacteria**.**

G+ve bacteria	Methanol Extract (Bro.)					Petroleum ether Extract (L)					Acetone Extract (Int.)				
	Result	Inhibition Zone (mm.)				Result	Inhibition Zone (mm)				Result	Inhibition Zone (mm)			
		1	2	3	Average		1	2	3	Average		1	2	3	Average
B. amyloliquefaciens	S	15	13	17	15	R	-ve	-ve	-ve	-ve	S	28	30	33	30H
B. cerus	S	13	12	15	13M	R	-ve	-ve	-ve	-ve	S	20	22	21	20H
B. subtilis	S	14	18	12	14M	R	-ve	-ve	-ve	-ve	S	23	25	28	25H
Staph. epidermidis	S	26	25	27	26H	S	10	10	15	13M	R	-ve	-ve	-ve	-ve
Staph . aureus	S	18	20	16	18M	S	15	15	20	18M	S	21	20	23	20H
G-ve bacteria	Methanol Extract					Petroleum ether Extract					Acetone Extract				
	Result	Zone (mm.)				Result	Inhibition Zone (mm)				Result	Inhibition Zone (mm)			
		1	2	3	Average		1	2	3	Average		1	2	3	Average
E. coli	S	9	7	10	9 W	R	-ve	-ve	-ve	-ve	S	15	18	13	15 M
Sal . paratyphi	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve
Pr. Vulgaris	S	13	12	15	13M	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve
Ps. Auruginosa	S	16	14	18	16M	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve

Table,6: Comparison of antifungal activity of methanol, petroleum ether and acetone extracts of *Pleurotus ostreatus* against tested pathogenic yeast strains

Yeast Speices	Methanol Extract (Int.)					Petroleum Extrac (NF)					Acetone Extract (Int.)				
	Res.	Zone (mm.)				Res.	Zone (mm.)				Res.	Zone (mm.)			
		1	2	3	Average		1	2	3	Average		1	2	3	Average
Candida albicans	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve
Candida glabrata	S	27	29	26	27 H	R	-ve	-ve	-ve	-ve	S	27	25	24	27 H
Candida krusei	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve
Pichia Kudriavzavii	S	15	14	17	15 M	R	-ve	-ve	-ve	-ve	S	15	15	14	15 M
Rhodotorula mucilaginosa	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve	R	-ve	-ve	-ve	-ve

R: resistant bacterial strain not inhibited by used extract.

Antibacterial activity of tested extract

H: Highly active antibacterial extract inhibited bacterial strain with inhibition zone more than 19 mm

M: Moderate active antibacterial extract inhibited bacterial strain with inhibition zone between 10 - 19 mm

W: Week active antibacterial extract inhibited bacterial strain with inhibition zone less than10 mm

Antibacterial spectrum of extract

Broad spectrum (Bro.): Extract which inhibited more than 5 bacterial strains out of 9 tested.

Intermediate spectrum (Int.) : Extract which inhibited 3 - 5 bacterial strains out of 9 tested.

Low spectrum (L): Extract which inhibited 1 -2 bacterial strains out of 9 tested.

b-Antifungal activity

Data in Table, 6 of antifungal activity of methanol, petroleum ether and acetone extracts of Pleurotus ostreatus tested against 5 yeast strains (*Candida albicans*, *C. glabrata*, *C. Krusei*, *Pichia Kudriavzavii* and *Rhodotorula mucilaginosa*) revealed that both of methanol and acetone extracts had intermediate spectrum antifungal activity that inhibited 2 yeast strains only (*Candida glabrata* and *Pichia Kudriavzavii*) out of 5 tested, with High 27mm, and moderate 15 mm antifungal inhibition zones for each, respectively.

On the other hand, petroleum ether extract was non effective spectrum antifungal activity against any yeast strains tested. So, according to antifungal activity, methanol and acetone extracts had intermediate spectrum antifungal activity, while petroleum ether extract was the last active without any effect against any strains out of 5 tested.

Sensitivity of yeast strain tested

S: Sensitive yeast strain affected and inhibited by used extract.

R: resistant yeast strain not inhibited by used extract.

Antifungal activity of tested extract

H: Highly active antifungal extract inhibited yeast strain with inhibition zone more than 20 mm

M: Moderate active antibacterial extract inhibited yeast strain with inhibition zone between 15 - 20 mm

W: Week active antifungal extract inhibited yeast strain with inhibition zone less than15 mm

Antifungal spectrum of extract

Broad spectrum (Bro.): Extract which inhibited more than 3 yeast strains out of 5 tested.

Intermediate spectrum (Int.): Extract which inhibited 2 - 3 yeast strains out of 5 tested.

Low spectrum (L): Extract which inhibited one yeast strain out of 5 tested.

Non efictivef (NF): Extract do inhibit any yeast strain out of 5 tested.

Antioxidant

Data in Table, 7 revealed a significantly higher total antioxidant capacity in methanol extract of Pleurotus ostreatus tested that reducing power was 52.52 µM, β-carotene-linoleic acid was 11.9 mg/ kg and total phenol was 390.04 mg/kg as antioxidant compounds.

Table, 7: Antioxidant capicity of *Pleurotus ostreatus* methanol extract

Antioxidant parameter	Result	Unit
1-*β*-carotene-linoleic acid	11.9	mg/kg
2-Total phenol	1390.04	mg/kg
3- Reducing Power	52.52	µM TE /mg

Discussion

This study aimed to evaluate the antimicrobial and antioxidant capacity of *Pleurotus ostreatus* as edible oyster mushroom. These oyster mushrooms are well known for their nutritional value and therapeutic properties. Oyster mushrooms **(***Pleurotus* genus**)** are edible and nutritious, ranked second among the commercially cultivated mushrooms in the world **(**Chang, 1999**)** and possess important bio-active compounds **(**Paul et al., 2017**).** They were widely used to prevent various diseases such as hypercholesterolemia, hypertension, cancer and other diseases. These biological activities are principally due to their chemical composition **(**Gunde-Cimeman, 1999, Bawadekji et al., 2017**).**

The values of moisture contents of tested *Pleurotus ostreatus* mushroom were so high and fluctuated between 84.3 – 88.5 with mean value was 86.07%. Chemical analysis of ash and nutrients of tested *Pleurotus ostreatus* revealed that total ash ranged between 70- 80% with mean value was 75%. While nutrients content analyzing of *Pleurotus ostreatus* mushroom revealed that average total carbohydrates was 101970.53mg/kg, whilst, average total proteins was166090.29mg/kg. Whereas lipid content of tested mushroom was 2643.17 mg/kg, whereas fiber value was 2643.17mg/kg.

Also, the results of mineral analyzing showed that Zn content was 90 mg/ 100g mushroom dry weight, while Mg content was 1175, whereas Fe content was 17.9, whilst Mn value was1.5 and Ca value was345. The previously obtained results were in full harmony with those reported by different researchers in different places around the world **(**Schillaci et al., 2013, Komura et al., 2014, Bawadekji et al., 2017, Okafor et al., 2017, Baeva et al., 2019**).** Many investigations from different regions of the world confirmed that the *Pleurotus* mushrooms having high nutrition, also contain various bioactive compounds including terpenoids, steroids, phenols, alkaloids, lectinsand nucleotides which have been isolated and identified from the fruit body, mycelium and culture broth of mushrooms and are shown to have promising biological effects **(**Alam et al., 2008, Attia et al., 2016, Paul et al., 2017, Bello et al., 2017, Bawadekji et al., 2017**).**

Antimicrobial Activity

a- Antibacterial activity

The antimicrobial activities of different extracts of *Pleurotus ostreatus* as oyster mushroom species were estimated from their ability to inhibit different pathogenic bacteria and yeast species. Data of antibacterial activity of methanol, petroleum ether and acetone extracts of *Pleurotus ostreatus* tested against 5 Gm+ve (*Bacillus cereus, B. amyloliquefaciens, B. Subtilis*; *Staphylococcus aureus* and *S. epidermidis*) and 4 Gm-ve (*Escherichia coli, Pseudomonas. aeruginosa, Salmonella paratyphi* and *Proteus vulgaris*) bacterial strains revealed that methanol extract had broad and wide spectrum antibacterial activity inhibited 8 bacterial strains out of 9 tested with moderate bacterial inhibition zone between 10 - 19 mm. While, acetone extract ranked second order with intermediate spectrum antibacterial activity inhibited 5 bacterial strains out of 9 tested, with high bacterial inhibition zone more than 20mm on 4 out of 5 Gm+ve bacterial strains and moderate bacterial inhibition zone 15mm on *Escherichia coli* only out of 4 Gm-ve bacterial strains tested. On the other hand, Petroleum ether extract had low spectrum antibacterial activity inhibited 2 bacterial strains only out of 9 tested with moderate bacterial inhibition zone (15 and 18 mm, respectively) on *Staphylococcus epidermidis* and *S. aureus* as Gm+ve bacterial strains.

So, according to antibacterial activity, methanol was highly active followed by acetone, while petroleum ether extract was the last active. Also, Gm+ve bacterial strains were more sensitive to extracts treatment than Gm-ve strains. On contrast, Gm-ve bacterial strains were more resistant depending on their cell wall structure. These results were in full agreement with those obtained by Jayakumar et al., 2009, Vamanu, 2012, Pauliuc and Botâu, 2013, Younis et al., 2015, Okafor et al., 2017, Bawadekji et al., 2017, Özcan and

Ertan, 2018, Baeva et al., 2019**.** Also, gram negative bacterial strains such as *E. coli* and *Shigella dysenteriae* were highly resistant to different extracts of *P. ostreatus* and *P. sajor-caju* as reported by Okafor et al. 2017 during their study on antimicrobial activity of oyster mushroom which in full harmony with our results that gram negative bacterial strains tested were more resistant .

b- Antifungal activity

The antifungal activity of methanol, petroleum ether and acetone extracts of *Pleurotus ostreatus* were assayed against 5 yeast pathogenic species namely; *Candida albicans*, *C. glabrata*, *C. krusei*, *Pichia kudriavzavii* and *Rhodotorula Mucilaginosa***.** Data of antifungal activity of different tested extracts of *Pleurotus ostreatus* revealed that both of methanol and acetone extracts had intermediate spectrum antifungal activity that inhibited 2 yeast strains only out of 5 tested, *C. glabrata* with high antifungal inhibition zone 27mm and *Pichia kudriavzavii* with moderate 15mm antifungal inhibition zone. Other three yeast strains tested; *Candida albicans*, *C. krusei*, and *Rhodotorula mucilaginosa* were not affected with different extracts used. Petroleum ether extract was no effective spectrum antifungal activity against any yeast strains tested.

So, according to antifungal activity, methanol and acetone extracts had intermediate spectrum antifungal activity, while petroleum ether extract was the last active without any effect against any strains out of 5 tested. Also, yeast as eukaryotic organisms were highly resistant. These results were in full agreement with those obtained by Bawadekji et al., 2017 during their study on antimicrobial activity of methanol, chloroform and hexane extracts of oyster mushroom *P. ostreatus* had no effect on *Candida albicans* which in full harmony with our results that 3 pathogenic strains out of 5 tested namely; *Candida albicans*, *C. krusei*, and *Rhodotorula mucilaginosa* were not affected with different extracts tested, methanol, acetone and petroleum ether. Also, Bawadekji et al., 2017 mentioned that technique of alcohol based solvent extraction may affect the efficacy of the obtained extract. As well as, Iwalokun, et al., 2007 reported that the variations in the antimicrobial activities of mushroom may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients in edible mushrooms.

Antioxidant Capacity

The antioxidant capacity is a way of depicting the effect of reducing components in the mushroom extract. Total antioxidant activity can be attributed to the presence of phytochemicals. Bioactive compounds in edible mushroom play a vital role in promoting health (Okafor et al., 2017**).** Mushrooms are found to be rich source of these antioxidants with immense antiradical activity **(**Valentão et al., 2005**).** A significantly higher total antioxidant capacity in methanol extract of *Pleurotus ostreatus* tested was 52.52 µM as reducing power and contained 11.9mg/kg as *β*-carotene-linoleic acid and 1390.04 mg/kg total phenol as antioxidants. Edible mushrooms are rich sources of antioxidants such as vitamin A, C, E, carotenoids, polyphenolic compounds and flavonoids**,** which prevent free radical damage and reducing risk of chronic diseases due to their ability to scavenge free radicals by single-electron transfer **(**Sies, 1997, Bawadekji et al., 2017**)**. They have been widely used as food and food supplements for millennia. It is an important food item concerning human health, nutrition and disease prevention **(**Chang, 1996, Ferrari et al., 2020). Oboh & Shodehinde, 2009 and Hamzah et al., 2013, Alispahić et al., 2015, Melinda Fogarasi, 2021 reported that antioxidant components might be used directly in enhancement of antioxidant defenses through dietary supplementation to reduce the level of oxidative stress. These antioxidants play a vital role in medicinal properties of mushrooms. Therefore, these mushrooms can be harnessed in the management of oxidative stress induced diseases.

Conclusion

Oyster mushroom *Pleurotus ostreatus* is considered as a valuable mushroom, not only for its nutritional value, but also for its significant antibacterial, antifungal properties and bioactive compounds which have positive effect on human health. It is easy to cultivate on agro-wastes and constitute an important source of these compounds. *P. ostreatus* can be considered as medicinal mushroom. Obtained results may also confirm using *P. ostreatus* as an alternative source of antibacterial and antifungal agent. Oyster mushrooms can also be used as functional foods since they have significant antioxidant activity for enhancement of the immune system against oxidative damage. They can be used as easily accessible source of natural antioxidants and as food supplement and in pharmaceutical industry considering the lingering threat of multi-drug resistance.

References

Akindahunsi, A.A., Oyetayo F.L. (2006) Nutrient and antinutrient distribution of edible mushroom, *Pleurotus tuberregium* (Fries) singer. LWT Food Sci Tech 39, 548–53.

Alam, N., Amin, R., Khan, A., Ara, I., Shim, M.J., Lee, M.W., Lee, T. S. (2008) Nutritional analysis of cultivated mushroom in Bangladesh-*Pleurotus ostreatus*, *Pleurotus sajor-caju*, *pleurotus florida*

and *Clocybe indica*. Microbiology, 36(4): 228-232.

Albugri, D.A., McElhenney, W.H. (2013) Extraction of total phenolic and flavonoids from edible wild and cultivated medicinal mushroom as affected by different solvents. J Nat Prod Plant Resour 3:3742

Alispahić, A., Šapčanin, A., Salihović, M., Ramić, E., Dedić, A., Pazalja, M. (2015) Phenolic content and antioxidant activity of mushroom extracts from Bosnian market. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, 44: 5-8 Print ISSN: 0367-4444,Online ISSN: 2232-7266

Asfors, K.E., Ley, K. (1993): Sulfated polysaccharides in inflammation. Journal of Laboratory and Clinical Medicine, 121: 201-202.

Attia, W.Y., Bawadekji, A., Al-Ali, K.H. (2016) Antioxidant and immunomodulating activities of mycelia extract from the edible mushroom *Pleurotus ostreatus*. South Asian Journal of Experimental Biology, 6(3): 83 – 91.

Ayoub, S.M. (1990) Antibacterial and antifungal activities of some Libyan aromatic plants. Planta Medica,56: 644.

Baeva, E., Bleha, R., Lavrova, E. (2019) Polysaccharides from Basidiocarps of cultivating mushroom *Pleurotus ostreatus*: Isolation and structural characterization. Molecules, 24, 130-137.

Bawadekji, A., Al-Ali, M. (2016) Antioxidant and immune modulating activities of mycelial extract from the edible mushroom *Pleurotus ostreatus*. South Asian Journal of Experimental Biology, 6(3): 83-91.

Bawadekji, A., Mridha, M.A.U., Al Ali, M., Jamith Basha, W. (2017) Antimicrobial activities of oyster mushroom *Pleurotus ostreatus* (Jacq.ex. Fr.) Kummer. Journal of Applied Biology Science, 7(10):227-231.

Bello, M., Oluwamukomi, M.O., and Enujiugha, V.N. (2017) Anti-diabetic activity of three species of oyster mushrooms. Annals Food Science and Technology, 18(2): 247-254.

Benzie, I.F.F., Strain, J.J. (1996) The Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power : The FRAP assay: Analytical Biochemistry .293 : 70-76.

Benzie, I.F.F., Strain, J.J. (1999) Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 299, 15-27

Biswas, A.K., Kumar, V., Bhosle, S., Sahoo, J., Chatli, M.K. (2011) Dietary fibers as functional ingredients in meat products and their role in human health. International Journal of Livestock Production 2 (4):45-54.

Chang, R. (1996) Functional properties of mushrooms. Nutrition Review, 54:91-93.

Chang, S.T. (1999) World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on Lentinus edodes (Berk.) Sing. in China. International Journal of Medicinal Mushrooms 1, 291-2300.

Cheung, L.M., Cheung, P.C.K. and Ooi,V.E.C. (2003) Antioxidant activity and total phenolics of edible mushroom extracts. Journal of Food Chemistry, 81, 249–255.

Dundar A, Acay H, Yildiz, A. (2008) Yield performances and nutritional contents of three oyster mushroom species cultivated on wheat stalk. African Journal of Biotechnology 7:3497–3501.

Folch, J., Lees, M., Sloane-Stanely, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. Journal Biological Chemistery, 226:497-509.

Gunde-Cimerman, N. (1999) Medicinal value of the genus Pleurotus (Fr.) P. Karst. (Agaricales, Basidiomycetes). International Journal of Medicinal Mushroom,1(1):69-80.

Hamzah, R.U., Egwin, E.C., Kabiru, A.Y. and Muazu, M.B. (2013) Phytochemical and in vitro antioxidant properties of the methanolic extract of fruits of *Bighia sapida*, *Vitellaria paradoxa* and *Vitex doniana*. Oxidants and Antioxidants in Medical Science, 2930: 217- 223.

Harrigan, A., Margaret Mccane, A. (1966) Laboratory methods in microbiology. Bibliography :P 401-430.

Hu SH, Liang Z.C., Chia Y.C., Lien J.L., Chen K.S., Lee M.Y., Wang J.C. (2006) Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. J. Agr. Food Chem. 22, 2103–2110.

Iwalokun, B.A., Usen, U.A., Otunba, A.A., and Olukoya, D.K. (2007) Comparative phytochemical evalution, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. African Journal of Biotechnology, 6(15): 1732.

Janssen, A.M., Scheffer, J.J.C. and Svendsen, A.B. (1987) Antimicrobial activity of essential oils: A 1976- 1986 literature review. Aspects of the test methods, Planta Medica, 395-398.

Jayakumar T., Ramesh, E., Geraldine, P. (2006) Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus* on CCl4-induced liver injury in

rats. Journal of Food Chemistry and Toxicology, 44, 1989–96.

Jayakumar T., Thomas, P.A. and Geraldine, P. (2009) In-vitro antioxidant activities of ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. Innovative Food Science and Emerging Technology, 10(2): 228-234

Jayaprakasha, G.K., Sigh, R.P., Sakariah, K.K. (2001) Antioxidant activity of grape seed (Vitis vinifera) extract on peroxidation models in vitro. Journal of Food Chemistery,73:285-290.

Komura, D.L., Ruthes, A.C., Carbonero, E.R., Gorin, P.A., Iacomini, M. (2014) Water-soluble polysaccharides from *Pleurotus ostreatus* var. florida mycelia biomass. International Journal of Biological Macromolecules, 70: 354-359.

Longvah, T., Deosthale Y.G. (1998) Compositional and nutritional studies on edible wild mushroom from northeast India. Food Chem. 63: 331-334.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein Measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275

Mahesha H. B. (2012): Estimation of Protein by Lowry's method, 1-2, available from :https://www.researchgate.net/publication/337864741.

Manzi, P., Gambelli L, Marconi, S., Vivanti, V., Pizzoferrato, L. (1999) Nutrients in edible mushroom: An inter-species comparative study. Journal of Food Chemistery, 65: 477-482.

Maria Socaciu, L., Claudiu Sâlâgean, D., Ranga, F., Anca Fârcas, C., Sonia Socaci, A., Carmen Socaciu, Dorin Tibulcâ, Szabolcs Fogarasi, Cristina Semeniue, A. (2021) Comparison of different extraction solvents for characterization of antioxidant potential and polyphenolic composition in *Boletus edulis* and *Cantharellus cibarius* mushrooms from Romania. Molecules Journal, 26(24): 7508, https://doi.org/10.3390/ molecules 26247508

Oboh, G., Shodehinde, S.A. (2009) Distribution of nutrients, polyphenols and antioxidant activities in the pilei and stipes of some consumed edible mushrooms in Nigeria. Bulletin of the Chemical Society of Ethiopia 23(3):391- 398

Okafor D.C., Onuegbu, N.C., Odimegwu, N.E., Ibeabuchi, J.C., Njoku, N.E., Agunwa, I. M., Ofoedu, C.E. and Njoku, C.C. (2017) Antioxidant and antimicrobial activities of oyster mushroom. American Journal of Food Science and Technology, 5(2): 64-69.

Özcan1, Ö., Ertan, F. (2018) Beta-glucan content, antioxidant and antimicrobial activities of some edible mushroom species. Food Science and Technology 6(2): 47-55

Paul, C., Roy, T., Das, N. (2017) Potentiality of oyster mushroom (Pleurotus spp.) in medicine. A review, Annals of Food Processing and Preservation, 2(2): 1014.

Pauliuc, I., Botau, D. (2013) Antibacterial activity of *Pleurotus ostreatus* gemmotherapic extract. Journal of Horticulture, Forestry and Biotechnology, 17(1): 242-245.

Perkin-Elmer, A. S. (1982) Analytical methods of atomic absorption spectrophotometer. Perkin Elmer Corp., USA

Raghuramulu, N., Madhavan, N.K., Kalyanasundaram, S. (2003) A Manual of Laboratory Techniques, pp. 56-58. National Institute of Nutrition. Indian Council of Medical Research, Hyderabad, India.

SabinoFerrari, A.B., de Oliveira, G.A. , Mannochio Russo, H., de Carvalho Bertozo, L. , de Silva Bolzani, V., Cunha Zied, D., Farias Ximenes, V., Luiza Zeraik, M. (2020) *Pleurotu ostreatus* and *agaricus subrufescens* investigation of chemical composition and antioxidant properties of these mushrooms cultivated with different handmade and commercial supplements. International Journal of Food Science and Technology, 56(1):1-9.

Schillaci, D., Arizza, V., Gargano,M.L., and Venturella, G. (2013) Antibacterial activity of Mediterranean oyster mushrooms, species of genus *Pleurotus* (higher basidiomycetes). International Journal of Medical Mushroom, 15(6): 591- 594.

Sharma, K. (2015) Mushroom: Cultivation and processing. International Journal of Food Processing Technology, 5: 9-12.

Sies , H. and Masumoto, H. (1997): Ebselen as a glutathione peroxidase mimic and as a reactant with peroxynitrite. Advances in Pharmacology 38 ,229-246.

Syed, A.A., Kadam J.A., Mane V.P., Patil S.S., Baig M.M.V. (2009) Biological efficiency and nutritional content of Pleurotus florida cultivated on different Agro-waste. Nature and Science, 7 (1), 44 – 49.

Türkoğlu, A., Duru, M.E. and Nazime Mercan, N. (2007) Antioxidant and antimicrobial activity of Russula delica Fr: An edidle wild mushroom. Eurasian Journal of Analytical Chemistry,2(1): 55-66

Valentão, P., Lopes, G., Valente, M., Barbosa, P., Andrade, P.B., Silva, B.M. and Seabra, R.M. (2005) Quantization of nine organic acids in wild

mushrooms. Journal of Agricultural and Food Chemistry, 53(9): 3626-3630.

Vamanu, E. (2012): In Vitro Antimicrobial and antioxidant activities of ethanolic extract of lyophilized mycelium of *Pleurotus ostreatus* PQMZ91109. Molecules, 17(4): 3653-3671.

Younis, A.M., Wu, F.S., El-Shikh, H H. (2015) Antimicrobial activity of extracts of the oyster culinary medicinal mushroom *Pleurotus ostreatus* (Higher Basidiomycetes) and identification of a new antimicrobial compound. International Journal of Medicinal Mushroom, 17(6): 579-585.