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Nutrition analysis, antimicrobial, and antioxidant activities of cultivated *Pleurotus floridanus* as an edible mushroom on different substrates

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Abstract: This research work introduces a study about mushroom cultivation, biochemical analysis, and antioxidant performance. The cultivation of edible oyster mushroom *Pleurotus floridanus* (EMPF) was carried out utilizing a hanging technique based on different substrate cultures including rice straw, wheat straw, a mixture of rice and wheat straw, a mixture of agricultural lime and wheat straw, a mixture of agricultural lime and rice straw, a mixture of quicklime and rice straw. After that, this research work analyzed the carbohydrate and protein contents in addition to investigating the antioxidant performance of EMPF as a species of oyster mushroom to improve awareness of the beneficial impact of EMPF in commercial markets. Based on the obtained results of biochemical analysis, the investigated EMPF (fresh) has 4.979 g/ 100 g carbohydrate using the traditional anthrone test. Additionally, the studied fresh EMPF has 2.413 g/ 100 g as a total protein content using the Folin-Ciocalteu test. Besides, FT-IR indicates the chemical existence of function groups of protein and carbohydrate in the investigated EMPF. The main contents of the cultivated EMPF are moisture followed by carbohydrates and protein in addition to traces from ash and metals. The antioxidant of the cultivated EMPF was evaluated via 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity values using different mushroom concentrations from 30 -150 g/L indicating that the optimum one is 90 g/L. The antimicrobial of the cultivated EMPF was tested and the results affirm the high performance of the EMPF as antibacterial or antifungal compared with familiar antibacterial or antifungal (Streptomycin or fluconazole, respectively). To conclude, this work introduces the antimicrobial and antioxidant of the studied EMPF.
Keywords: Antibacterial, Edible mushroom, Biochemical analysis, DPPH, Natural antioxidant.

1. Introduction

Recently, the commercial production of macrofungi in addition to their economic potential has been steadily going up globally. The biomedical application of natural foods derived from macrofungi is increasing because these fungi have considerable health benefits and are naturally abundant [1]. Macrofungi are fungi that can be easily seen without using a microscope such as mushroom which is edible hypogynous fruiting bodies. Some of the produced mushrooms serve as a favored food which could be attributed to the expected nutrient contents [2]. Mushroom substrates could be used in variable shapes such as fresh, powdered, dried, or pickled. Therefore, the high interest in mushroom studies comes from its low cost and health benefits [3, 4]. Currently, 200 types of mushrooms were reported and 100 of them were cultivated [5, 6]. Additionally, 60 mushroom kinds were commercially grown and 10 were produced for industrial purposes [5].

Oyster mushroom is a popular variety of mushroom having some health benefits. As well as supporting the immune system. *The pleurotus* genus includes 40 species named "oyster mushrooms" [7] and comprises *P. ulmarium, P. sajorcaju, P.ostreatus, P. cornucopea, P. citrinopileatus, P. cystidiosus, P. highbing, P. florida, P. pulmonarius, P. flabellatus, P. geesteranus, P. sapidus,* and *P. eryngi* [8]. These mushrooms could be used as edible, in addition to having several variable applications such as medicinal [9, 10], biotechnological [11], environmental [12, 13], and nutraceutical [3, 14, 15].

Mushrooms could be applied as an acceptable natural source of some essential proteins [16], vitamins [16], Fe [17], Ca [18], Zn [19], and other minerals [20]. Furthermore, mushrooms have various polyphenol compounds that have a considerable role in the antioxidant activity of natural foods [21] in addition to flavonoids [22] which could improve the reaction between free radicals and digested food [23]. So, biochemical analysis of the cultivated mushroom in the local environment will be essential.

Oxygen radicals and other reactive species naturally lead to tissue damage causing cancer [24]. Natural foods containing antioxidants could play a significant role in helping the human system to decrease oxidative damage [25]. Researchers in past research work have established the fact that mushroom has antioxidant characteristics because of having phenolics, glycosides, flavonoids, tocopherols, and polysaccharides [26]. These compounds in natural mushrooms have been identified by different physicochemical techniques such as high-performance liquid chromatography gas chromatography or various spectrophotometric assays [27]. Ferreira et al. have reported a scientific review on the antioxidant performance of wild mushrooms [28]. According to this report, the antioxidants of any cultivated mushroom could be studied to confirm the extent of antioxidant behavior which will be investigated in this study.

The bioactivity of the functionalized compounds can be extensively impacted by the mushroom variety, cultivation environment, and fruiting circumstances as well as the storage environment and cooking strategy [26, 29]. Therefore, cultivation of the EMPF was carried out in this work utilizing a hanging technique based on different substrate cultures including rice straw, wheat straw, a mixture of rice and wheat straw, a mixture of agricultural lime and wheat straw, a mixture of agricultural lime and rice straw, mixture of quicklime and rice straw. After that, this research work analyzed the carbohydrate and protein contents in addition to evaluating the antioxidant activity of the EMPF, aiming to raise public awareness about the benefits of oyster mushrooms.

2. Materials and methods

2.1. Cultivation

At first, the pure mother culture was prepared by cutting small pieces of the gills side from fresh mushrooms under sterile conditions. The gills are grown in sterile media petri dishes Molded by Media PDA (potato dextrose agar) agar which consists of 200 g potato, 20 g agar, 20 g dextrose, and 1.5 g yeast extract. The used potatoes were cut into small pieces and saved in boiling water for 15 minutes followed by filtration. The obtained filtrate was taken and placed with the rest of the media components then, sterilized and poured into sterile Petri dishes. The pre-prepared mushroom parts were cultivated in the obtained dishes and incubated for 20 days at 25 ºC. After these 20 days, the mycelium spread all over the plate as shown in Fig. 1. On the other side, wheat grains were soaked in water to remove chaffy and damaged grains. The grains were saved in a vessel for 30 minutes to be softer. Then, the obtained grains were dried at room temperature to remove excess water. The grains were poured into bottles up to 75 % of their height. The obtained bottles were plugged tightly with non-absorbent cotton wool and were covered with aluminum foil. Then, it was autoclaved and sterilized for 2 hours followed by cooling and keeping them inside the culture room. These bottles were injected with pure culture mycelium which was prepared before and then were gently shaken on the 5^{th} and 10^{th} day to distribute the inoculum evenly in the bottles. The process of mushroom cultivation starts with the disinfection of the environment with chlorine to remove traditional pollutants. In this work, different cultivation substrates were used including rice straw, wheat straw, a mixture of rice and wheat straw, a mixture of agricultural lime and wheat straw, a mixture of agricultural lime and rice straw, mixture of quicklime and rice straw. The cultivation steps are displayed in Fig. 1 including the obtained digital photo from the experiments of cultivation. Before cultivation, the grain spawns were exposed to the air for 12-24 hours in a sterile place. The cultivation substrate was soaked in water for 6 hours then boilingit in water for 1 hour followed by filtration and cooling at room temperature for 24 hours. The humidity environment was found at 70 to 72% and the environment conditions temperature is from 20-28 ℃. Mushroom spawns were mixed with cultivation substrate in clear plastic bags, and sealed using adhesive, and the formed bags were holed in the edges of the bags to release excess water. Bags were filled with the cultivation substrate injected with spawn and stored in a dark place away from light, with humidity around 90%, and a temperature of 24 °C for a period of 15-21 days, which is called the incubation period. The prepared bags and the floor must be sprayed with a water sprayer constantly, in order to provide moisture during the incubation period, and the formation of cotton threads (mycelium). After the incubation period, the second stage started, and pale light was applied for 4-6 hours every day away from the sun. Then, the shock was applied by the low thermal stimulus for growth. Mushroom mycelium started to appear 15 days after the stimulation process so the moisture of the bags could be maintained, by constantly spraying the bags indirectly with water until the mushroom grains were ready for picking off, around four days after their appearance so that the first pick is harvested, as there is a period of two weeks between each picking and the other.

2.2. Determination of moisture and ash contents

Moisture analysis was done by drying 20 grams of fresh mushrooms in a hot air oven for 24 hours at 110 ºC. Then, the moisture content was estimated from the difference between the final weight after drying and the beginning weight. The estimation of total ash was carried out by calcination at 600 ºC. In detail, 4.9 g of the sample was put into the used crucible. This crucible was placed in a muffle furnace for 3 h at 600 ºC. The total ash was calculated by the following equation:

Ash content (g\100 g sample) = (weight of ash after calcination) x100 / (weight of sample)

2.3. Determination of protein and carbohydrates

Carbohydrates in the cultivated edible mushroom could be estimated by hydrolysis into simple sugars. After the hydrolysis step, glucose might be dehydrated to hydroxymethyl furfural which forms with an anthrone clear green color using wavelength corresponding to the highest absorption (λ_{max}) at 620 nm. The carbohydrate determination method was used to estimate the water-soluble carbohydrates and total carbohydrates. For water-soluble carbohydrates, 0.1 g of dry mushroom at 110 ºC was stirred in 10 ml distilled water and then put in a water bath at 100 ℃ for 2 h followed by traditional filtration. In contrast, the determination of total carbohydrates was carried out using 10 mL of 1.0 M HCl and 0.1 g of dry mushroom. The utilized **a**nthrone reagent was prepared by dissolving 2.0 g of anthrone in 8.0 ml absolute EtOH, 30 ml H_2O, and 100 ml of concentrated H_2SO_4 in the ice bath. The freshly prepared reagent was used for every experiment. The reaction between the anthrone reagent and the extract was done by mixing 0.5 ml of the previously prepared extract and 4.5 ml of anthrone reagent and the mixture was saved for 7 minutes in the water bath and left for cooling at room condition followed by UV-visible spectroscopy analysis to record the absorption at λ_{max} (620 nm). To design a standard curve of the relation between absorption and carbohydrate concentration, different glucose concentrations from 10 mg/l to 60 mg/l were applied and measured according to the method of [30].

The protein content was studied via the Folin-Ciocalteu test using water and NaOH aqueous solution to find the water-soluble protein and total protein, respectively. In detail, 0.03 g of the dried powder mushroom was boiled in 10 ml H_2O for 2 h. For total protein estimation, 0.03 g were boiled in 10 ml NaOH

(1 mol/l) for 2 h followed by filtration to obtain the sample solution. The alkaline reagent solution consists of 50 ml reagent A (2% Na_2CO_3 in 0.1 M NaOH) + 1 ml reagent B (0.5 g of $CuSO_4.5H_2O$ in 1%). Note that these reagents should be freshly prepared. The Foline reagent was diluted by H_2O (1:1 v/v) before using it. Five ml of the prepared alkaline reagent solution was mixed with 1 ml sample solution in a clean test tube followed by stirring and left for 10 min. Then, 0.5 ml of diluted foline phenol reagent 1:1 v/v was mixed well with the obtained solution from previous steps. After 30 min from mixing, the absorbance was measured at 750 nm which is the λ_{max} of the Foline reagent. To design a standard curve of the relation between absorption and protein concentration, different albumin concentrations from 10 mg/l to 60 mg/l were applied and measured by the similar previously mentioned steps [8, 16].

2.4. Determination of mineral analysis

The mineral analysis was studied in the dried mushroom after the formation of the total ash. In detail, 0.4 g of total ash was put in 2 ml Nitric acid followed by boiling. Then, hydrogen peroxide was added followed by cooling and filtration. The obtained volume was made up to 50 ml of deionized water. The pH of the obtained mixture was found around 2. Inductively coupled plasma (ICP) (ICP 2000 DV PerkinElmer) was employed to assert the mineral contents [19] in the obtained solution at a micro analytical center in Cairo University, Egypt.

2.5. FT-IR of the dried mushroom

The function or chemical groups could be investigated via FT-IR which is the best technique for this purpose. At the micro analytical Center at Cairo University, the FT-IR spectra of the dried EMPF in KBr pellets were tested using a Perkin-Elmer 1650 spectrometer.

2.6. DPPH radical scavenging activity

The DPPH radical scavenging activity was determined by the reaction between purchased DPPH and the fresh mushroom aqueous solution. Different concentrations of the cultivated EMPF (30 g/l, 60 g/l, 90 g/l, 120 g/l, and 150 g/l) were mixed with 200 µL DPPH solution (1 mmol/L), and the components were allowed to react for 30 min at room environment. The final solution was analyzed via measurement of the absorbance from UV-visible spectroscopy at 535 nm which is the λ_{max} using water as the blank [25, 26].

2.7. Antimicrobial testing of the cultivated mushroom extract

DMSO was used as a solvent to dissolve 15 g mushroom powder of EMPF. The antibacterial activity of the EMPF solution was evaluated by agar well diffusion method using the concentration of 0.3 g/mL against three pathogenic bacteria1 strains, two Gm (+ve) bacterial strains; *Bacillus Subtilis*, *Staphylococcus aureus* and one gm(-ve) bacteria strain; *Escherichia coli*. The utilized nutrient agar was prepared according to the previous study [8, 31]. The antifungal activity of the EMPF solution was tested by agar well diffusion versus three pathogenic yeast strains: *Candida albicans*, *C. glabrata*, and *C. stellatoidea*. The experiment was done in Petri plates having 15 cm^3 of PDA as reported before [8]. All found inhibition zones were measured manually by mm.

3. Results & Discussion

3.1. Effect of substrate on the produced yield:

In this study, the edible mushroom *P. florida* (EMPF) was cultivated in this work utilizing a hanging technique based on different substrate cultures. The applied methodology of cultivation was discussed in detail in the experimental part and Fig. 1.

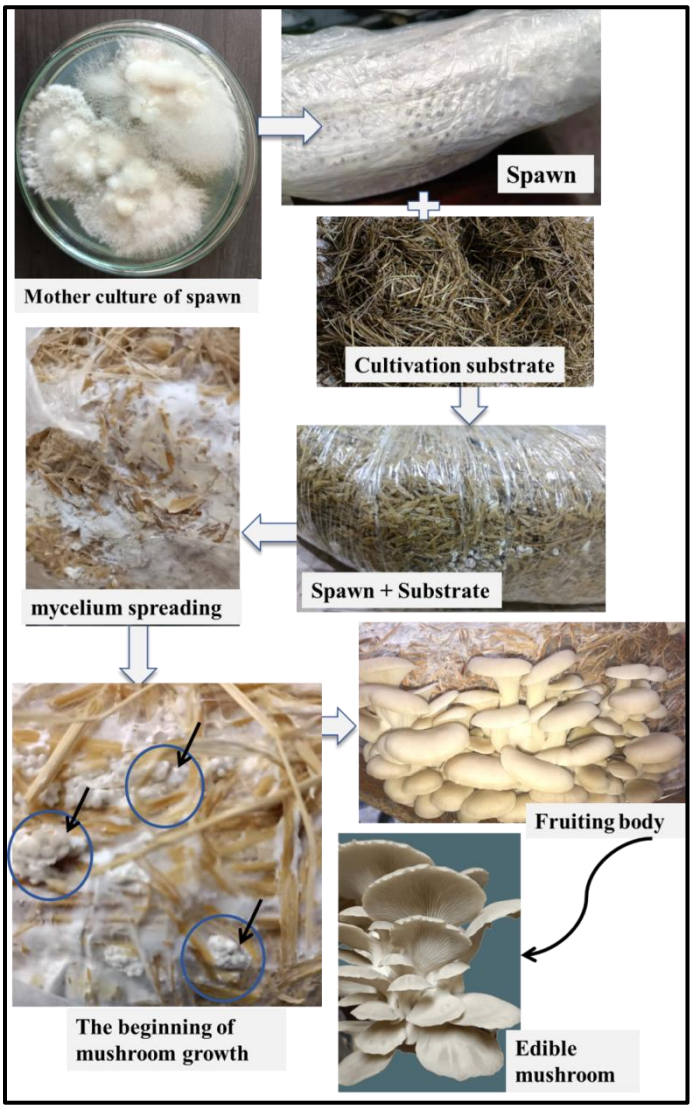

Figure 1. Cultivation steps of the studied oyster mushroom.

During the cultivation stage, the mycelium of spawn spreads 19 days from the initial time. The impact of cultivation substrate was studied and found that the main significant factor is the yield or the produced weight from *EMPF* [32]. The yield of the first crop was evaluated and displayed in Fig. 2. Based on rice straw, the observed first crop yield was found at 352.8 g /kg of dry substrate which is lower than what was observed in the case of wheat straw (566.0 g). Therefore, around 60 % enhancement in the production yield could be detected because of using wheat straw instead of rice straw. The reason for this improvement could be due to the particle size of the utilized straw which is

smaller in the case of wheat straw if compared with rice straw [33]. The smaller particles could play a significant role in increasing the surface area [34] and so, the period of incubation will be shorter. Thus, the spread of Mycelium is faster in the case of wheat straw if compared with rice straw, and therefore its production is higher in the first crop yield. After mixing wheat straw and rice straw, the first crop increased if compared with the pristine rice straw. Around 19 % improvements in the first crop yield because of supporting the rice straw substrate by 50 % wheat straw. The agriculture lime was mixed with wheat straw to form another cultivation substrate and the first crop yield was around 117.5 g which is less than the pure wheat straw. Besides, the agricultural lime was mixed with rice straw to form another cultivation substrate and the first crop yield was around 221.5 g which is less than the pure rice straw. The last studied cultivation substrate is a mixture of quicklime and wheat straw and the estimated first crop yield is 160 g. In general, the cultivation at mixture media is not flavored based on the first crop yield data. The yield of the total crops was evaluated and displayed in Fig. 3. Based on rice straw, the observed total crop yield was found at 1440.8 g which is better than what was found in the case of wheat straw (817.6 g). Thus, around 76 % enhancement in the production yield could be observed because of using rice straw instead of wheat straw. The reason for this improvement could be the biological stability of rice straw, and because rice straw is a derivative of the aquatic plant which is more resistant to mold than other straws [35, 36]. So, rice straw is better than wheat straw for producing EMPF by using all crops even, the first crop production was found to be higher in the case of wheat straw. After mixing wheat straw and rice straw, the total crop yield decreased if compared with the pristine rice or wheat straw. Around a 50 % decline in the total crop yield was observed because of mixing the rice straw substrate with 50 % wheat straw. The agricultural lime was mixed with wheat straw to form another cultivation substrate and the total crop yield was around 193.1 g which is less than the pure wheat or rice straw. Additionally, the agricultural lime was mixed with rice straw to form another cultivation substrate and the total crop yield was around 306 g which is less than the pure rice or wheat straw. The last studied cultivation substrate is a mixture of quicklime and wheat straw, and the estimated total crop yield is 520 g. To conclude, the best cultivation substrate for total crop yield is rice straw, and the first crop yield is the wheat straw in addition to the mixture media which is not preferred in either first or total crop yield.

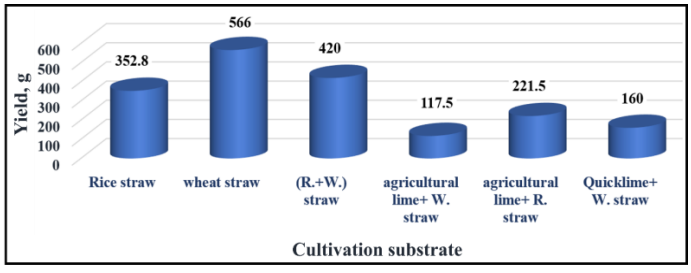

Figure 2. The mushroom yield in the first crop (grams) by the use of different cultivation substrates: rice straw, wheat straw, a mixture of rice and wheat straw, a mixture of agricultural lime and wheat straw, a mixture of agricultural lime and rice straw, mixture of quicklime and rice straw.

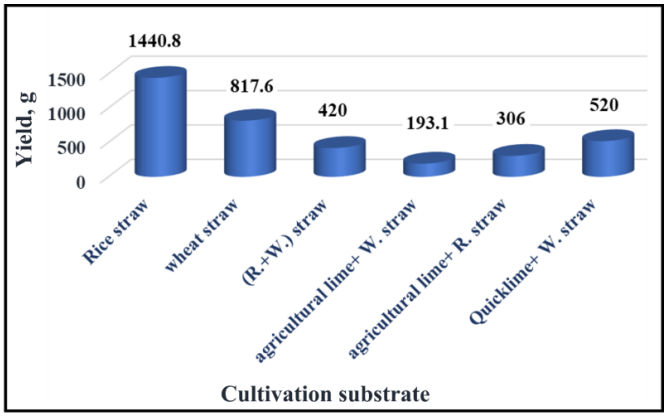

Figure 3. Total crop yield of mushroom (grams) by the use of different cultivation substrates: rice straw, wheat straw, a mixture of rice and wheat straw, mixture of agricultural lime and wheat straw, mixture of agricultural lime and rice straw, mixture of quicklime and rice straw.

3.2. Determination of carbohydrates and protein

In this work, carbohydrate contents were investigated in the cultivated EMPF by the traditional anthrone test [37, 38] which is a chemical evaluation to find the percentage of glucose in the tested samples by spectrophotometric method. The UV-visible spectroscopy was studied of some known concentrations of glucose and the standard curve using absorbance values was plotted. The estimated Pearson's correlation factor and adjusted R-squared for the standard curve were found at 0.9992 and 0.99799, respectively which indicates the successful standard curve to have linear behavior. After that, the unknown content of glucose in mushrooms was studied by the same methodology and shown by brown and orange dotted lines for water soluble sugars and total carbohydrate contents, respectively. The estimated values of soluble and total carbohydrates in the dried EMPF were found at 16.219 g/ 100 g and 39.359 g/ 100 g, respectively. Additionally, the calculated values of soluble and total carbohydrates in the fresh EMPF were found to be 4.979 g/ 100 g and 2.052 g/ 100 g, respectively. These values are higher than the value of *Pleurotus ostreatus* (2.872 g/100 g) and lower than *Agaricus bosporus* (9.074 g/100 g) and *Pleurotus eryngi* (5.51 g/100 g) [39]. The sugar composition of carbohydrates in mushrooms could be mainly mannose, glucose, galactose, and xylose [39-41]. The investigated EMPF has 4.979 g/ 100 g carbohydrate using the traditional anthrone test.

Protein contents were studied in the cultivated EMPF by the Folin-Ciocalteu method [42] by preparation of known protein concentrations ranging from 10 ppm to 60 ppm and applied to design the standard curve. The UV-visible spectroscopy curves of all known concentrations of protein were studied and the standard curve using absorbance values was plotted. The estimated Pearson's correlation factor for the standard curve was found at 0.98147 which suggests the linear characteristics of the designed standard curve. The unknown percentage of protein in mushrooms was studied by the same Folin-Ciocalteu technique. The determined values of soluble and total carbohydrates in the dried EMPF were found at 16.467 g/ 100 g and 19.074 g/ 100 g, respectively. In addition, the calculated values of soluble and total protein in the fresh EMPF were found at 2.083 g/ 100 g and 2.413 g/ 100 g, respectively. Therefore, 100 g of dry EMPF could provide 30 % of the Recommended Dietary Allowance

(RDA) for human beings [43, 44]. As reported, different factors could be the main reason for the variation in chemical composition including protein contents. These factors were related to the maturation stage and the nature of the studied mushroom. Thus, the cultivated EMPF meets the protein requirements according to the recommended essential protein profile for adults. To conclude, the studied EMPF has 19.074 g/ 100 g as a total protein content using the Folin-Ciocalteu test.

3.3. FT-IR of the dried EMPF

The FT-IR analysis was studied for the prepared EMPF to describe the chemical or function groups and so, confirming the chemistry of the prepared mushroom. The FT-IR results are shown in Fig. 4 and have clear peaks at 3403.74 cm^{-1}, 2918.74 cm^{-1}, 1635.34 cm^{-1}, 1410.67 cm^{-1}, 1043.30 cm^{-1}, and 564.08 cm^{-1}. The peaks that appeared at 1635.34 cm^{-1} and 3403.74 cm^{-1} could be due to the stretching of C=O and O-H, respectively of the protein structure [45, 46]. The C-H stretching vibration and CH_2 bending were detected at 2918.74 cm^{-1} and 1410.67 cm^{-1} which could exist in carbohydrate structure [47]. The FT-IR band at 1043.30 cm^{-1} could be attributed to the C-C stretching in carbohydrate or protein structure. To conclude, FT-IR indicates the chemical existence of function groups of protein and carbohydrate in the investigated EMPF.

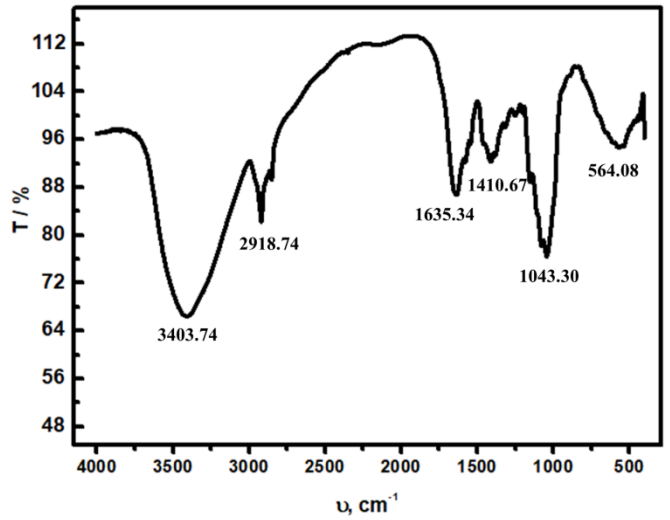

Figure 4. FT-IR of the dried cultivated mushroom.

3.4. Total analysis of the cultivated EMPF

After obtaining the cultivated EMPF, different chemical analyses were carried out and the discussed data were presented in Table 1. Results indicated that the moisture content of the fresh EMPF was 87.35 %. The water percentage could be considered as a high percentage if compared with the previous literature. For example, the previously reported moisture percentage was found at 87.37, 86.27, and 82.77 for *P. sajor-caju*, *P. ostreatus*, and *P. djmor* mushrooms [48]. The ash percentage was detected at 1.032 g/100 g. The ash content was estimated after saving the dried EMPF at a high temperature (600 ^{o}C). Therefore, the obtained ash content was small (1.032 %). For protein analysis, the protein concentration was found at 2.413 and 19.074 % for the dried and fresh EMPF, respectively. Whereas carbohydrate and fiber content of the evaluated EMPF was found at 4.979 and 4.223 %, respectively. The metallic content in the cultivated EMPF was estimated by the determination of iron, calcium, and zinc. The percentage of Fe, Ca, and Zn was found at 1.099 mg/100 g, 0.972 mg /100 g, and 0.609 mg/100 g. To conclude, the main content of the tested EMPF is water followed by carbohydrates and proteins in addition to traces of ash and metals.

Table 1. Estimated moisture, ash, protein, carbohydrate, fiber, and minerals in the EMPF

	Fresh Mushroom	Dried Mushroom
Moisture, g/100 g	87.35±1.05	0.00±0.0
Ash, g/100 g	1.032±0.01	8.16±0.17
Protein, g/100 g	2.413±0.09	19.074±0.85
Carbohydrate, g/100 g	4.979±0.26	39.359±0.98
Fiber, g/100 g	4.223±0.09	33.386±1.01
Fe, mg/100 g	1.099±0.19	8.686±0.23
Ca, mg/100 g	0.972±0.04	7.681±0.34
Zn, mg/100 g	0.609±0.07	4.820±0.26

3.5. Antioxidant investigation of the cultivated EMPF

The antioxidant analysis was tested via the traditional 1, 1-Diphenyl-2-picrylhydrazyl or DPPH radical methodology [49-51]. The EMPF impact on the DPPH could be used to predict antioxidant behavior. The antioxidant analysis by the DPPH technique is reported in recent literature which could be due to its simplicity and short time [52]. In this method, the analysis of the remaining DPPH could be analyzed via UV-visible spectroscopy by studying the spectroscopy analysis of pure DPPH, EMPF solution (30 g/l), and a mixture of DPPH with EMPF samples. As seen, the DPPH curve has an absorbance of around 0.445 at the characteristics wavelength (λ_{max}) of DPPH. Interestingly, this absorbance was sharply decreased after mixing the EMPF with the DPPH solution which indicates the antioxidant characteristics of the studied EMPF. This analysis was confirmed via another concentration from the studied EMPF which is 90 g/l and the found data has the same conclusion that the cultivated EMPF has an acceptable antioxidant characteristic.

After that, the effect of EMPF contents (30 g/l, 60 g/l, 90 g/l, 120 g/l, and 150 g/l) was studied as shown in Fig. 5A. All investigated EMPF concentrations have low absorbance at 535 nm (λ_{max} of DPPH) if compared with pure DPPH. The lowest absorbance values were found at 90 g/l which indicates that this EMPF concentration is the best one if compared with the studied concentrations from 30 -150 g/l. The scavenging activity values after estimation as shown in the experimental part were drawn against the studied mushroom concentrations from 30 -150 g/l (Fig. 5B). The first trend is a direct increase of scavenging activity as mushroom contents went up and this trend was seen from 30 g/l to 90 g/l. After 90 g/l, the trend becomes negative as more mushroom concentration decreases the scavenging activity. This active scavenging activity could be due to the small content of phenolic compounds, polysaccharides, and steroids [26, 53, 54].

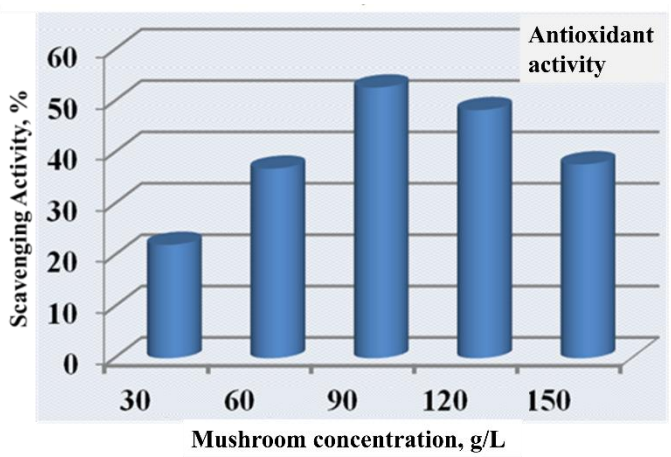

Figure 5. Scavenging activity of fresh mushroom solutions having different concentrations from 30-150 g/l.

3.6. Antimicrobial investigation of the cultivated EMPF

The antibacterial activity of the EMPF was evaluated versus three pathogenic bacteria1 strains, one GM (-ve) bacteria strain; *Escherichia coli*, and two Gm (+ve) bacterial strains; *Bacillus Subtilis* and *Staphylococcus aureus* as shown in Fig. **6A**, **6B**, and **9C** using DMSO as a solvent. From these measurements, the DMSO solvent has low activity towards all tested bacteria. Interestingly, the cultivated EMPF has a higher inhibition zone (18, 17, and 19 mm against *Escherichia coli*, *Bacillus Subtilis*, and *Staphylococcus aureus*, respectively). Additionally, this inhibition zone was compared with a familiar antibiotic (Streptomycin). The measurements confirmed that the EMPF extract achieved 60 %, 57 %, and 63 % of the tested Streptomycin versus *Escherichia coli*, *Bacillus Subtilis*, and *Staphylococcus aureus*, respectively. Therefore, the EMPF is a promising antibacterial agent against *Escherichia coli*, *Bacillus Subtilis*, and *Staphylococcus aureus*. Besides, the antifungal activity of the EMPF was evaluated versus three pathogenic yeast strains: *Candida albicans, Candida glabrata*, and *Candida stellatoidea* as shown in Fig. **6D**, **6E**, and **6F** using DMSO as a solvent. The inhibition zone was measured for DMSO solvent in addition to EMPF extract and antibiotics (Streptomycin). The DMSO solvent has no activity towards all tested yeast strains. Interestingly, the cultivated EMPF has a considerable inhibition zone (14, 11, and 13 mm against *Candida albicans, C. glabrata*, and *C. stellatoidea*, respectively). Additionally, this inhibition zone was compared with familiar antifungal (Fluconazole). The found data affirmed that the EMPF extract has 127 %, 92 %, and 118 % of the tested fluconazole versus *Candida albicans, Candida glabrata,* and *Candida stellatoidea*, respectively. Therefore, the EMPF is a promising antifungal agent against *Candida albicans*, *Candida glabrata*, and *Candida stellatoidea*.

4. Conclusion

This work studied mushroom cultivation, biochemical analysis, and antioxidant activity. The cultivation of edible mushroom *Pleurotus floridanus* (EMPF) was done using different substrate cultures. Then, the work analyzes carbohydrates and proteins in addition to studying the antioxidant performance of EMPF to enhance awareness of the beneficial impact of EMPF in Egyptian markets. The fresh EMPF has 0.04979 wt% carbohydrate and 2.413 wt% protein using anthrone and Folin-Ciocalteu tests, respectively. Besides, the major contents of the cultivated EMPF are water followed by carbohydrates and protein in addition to traces from ash and metals. The optimum concentration of the studied EMPF was found at 90 g/L using DPPH scavenging activity. The cultivated EMPF extract was tested as an antibacterial agent and the found data confirmed that the EMPF extract achieved 60 %, 57 %, and 63 % from the familiar antibacterial (Streptomycin) versus *Escherichia coli*, *Bacillus Subtilis*, and *Staphylococcus aureus*, respectively. Additionally, the antifungal activity was compared with familiar antifungal (Fluconazole). The found data affirmed that the EMPF extract has 127 %, 92 %, and 118 % of the tested fluconazole versus *Candida albicans*, *Candida glabrata*, and *Candida stellatoidea*, respectively. Therefore, this work presented the cultivation of EMPF at different cultivation substrates and its biochemical analysis as well as its antioxidant, antibacterial, and antifungal activity.

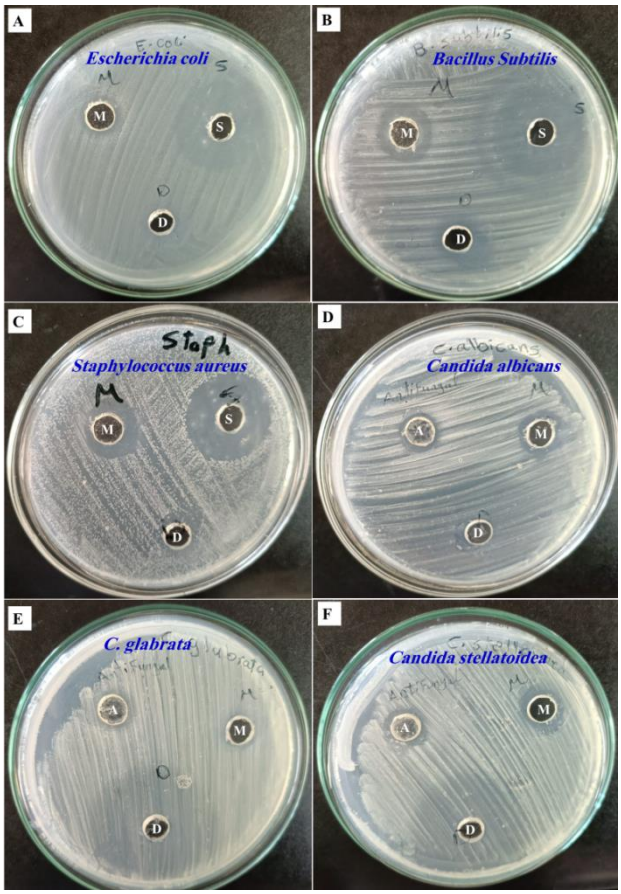

Figure 6. Inhibition zones of one gm (-ve) bacteria strain; Escherichia coli: (A), two Gm (+ve) bacterial strains called; Bacillus Subtilis: (B), Staphylococcus aureus: (C), three pathogenic yeast strains called; Candida albicans: (D), C. glabrata: (E), and Candida stellatoidea: (F). D=DMSO, M = Mushroom extract, S = Streptomycin, A = Antifungal (Fluconazole).

CRediT authorship contribution statement:

Author Contributions: "Conceptualization, M.S.Y and S.I.A.; methodology, S.I.A.; software, S.I.A., M.M.A.; validation, M.S.Y, S.I.A., and M.M.A.; formal analysis, S.I.A., and M.M.A.; investigation, M.S.Y, S.I.A.; resources, M.S.Y, S.I.A.;

data curation, M.S.Y, S.I.A., and M.M.A.; writing—original draft preparation, M.S.Y, S.I.A., and M.M.A.; writing—review and editing, M.S.Y, S.I.A.; visualization, M.S.Y, M.M.A.; supervision, M.S.Y, M.M.A.; project administration, M.S.Y, M.M.A.; funding acquisition, M.S.Y, S.I.A. All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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