Utilization of Agro-Wastes to Produce Oyster Mushroom (*Pleurotus ostreatus*) with High Antioxidant and Antimicrobial Activities

Amal M. Abd El-Razek[1], Amel Ibrahim[2], Aisha Elattar[2] and Dalal Asker[1,3]

[1] Food Science and Technology Dept., Faculty of Agric., Alexandria University 21545, El-Shatby, Alexandria, Egypt.

[2] Dairy Science and Technology Dept., Faculty of Agric., Alexandria University 21545, El-Shatby, Alexandria, Egypt.

[3] Department of Materials Science & Engineering, University of Toronto, Toronto, ON, Canada.

Received: 1 August, 2019 ***Revised: 26 August, 2019*** ***Accepted: 25 September, 2019***

ABSTRACT

Oyster mushroom (*Pleurotus ostreatus*) is a good source of bioactive compounds that have numerous health benefits and medicinal properties. In this study, the effect of *P. ostreatus* cultivation in agro-waste substrates on its potential as a food additive with antioxidant and antimicrobial activities was investigated. Extracts of *P. ostreatus* cultivated on mixed rice and wheat straws (RS+WS) substrates showed higher total polyphenols (TPC), total flavonoids (TFC),α-tocopherol content, ferric reducing antioxidant power and antibacterial activities than those cultivated on rice straw (RS) alone. The HPLC analysis of their ethanolic extracts revealed 11 phenolic compounds including *p*-hydroxy benzoic acid, naringenin, kaempferol and apigenin as major compounds. The aqueous and ethanolic extracts of both cultivates showed significant antibacterial activity against *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus*, while no inhibitory effect on *Bacillus cereus* was observed. The results indicated that *P. ostreatus* cultivation is an effective bioconversion process that is capable to transfer agro-wastes into potentially valuable source of natural antioxidant and antimicrobial additives for further use in functional food products.

Keywords: Agro-waste, oyster mushrooms, antioxidants, antimicrobials, polyphenols, flavonoids.

INTRODUCTION

Mushrooms are macrofungi with a distinctive fruiting body that is large and visible to the naked eye. For centuries, edible mushrooms have been cultivated and used as food due to their attractive sensory properties, high levels of nutrients and relatively easy cultivation . Mushrooms contain 10-40% protein, 3-21% carbohydrates, 3-35% dietary fibers, 5% unsaturated fat and a variety of vitamins and minerals (Roncero-Ramos & Delgado-Andrade, 2017). Meanwhile, Chang (1996) mentioned that mushrooms have gained popularity as a functional food resource due to their content of several therapeutic compounds that have beneficial health effects.

The oyster mushroom ,*Pleurotus ostreatus*, is the second cultivated edible mushroom worldwide due to its high nutritional value (high protein, fiber, and carbohydrate contents), unique flavour and aromatic properties (Iwalokun *et al.*, 2007). The *P. ostreatus* also produces many health-promoting bioactives with antioxidative, antimicrobial, antitumor and anti-inflammatory properties (Iwalokun *et al.*, 2007, Parola *et al.*, 2017).The antioxidant effect of *P. ostreatus* and other related species has been correlated with their content of phenolic and flavonoids compounds (Iwalokun *et al.*, 2007, Karaman *et al.*, 2010, Gąsecka *et al.*, 2016). Other antioxidant compounds such as ascorbic acid, glycosides, tocopherols, polysaccharides, ergothioneine and carotenoids are also found in oyster mushroom (*Pleurotus* spp.), (Iwalokun *et al.*, 2007, Gąsecka *et al.*, 2016).The chemical structure of polyphenols is characterized by the presence of an aromatic ring with hydroxyl groups that exhibit a strong radical scavenger and free radical inhibitor activities (Frei & Higdon, 2003, Iwalokun *et al.*, 2007). The *in vitro* and *in vivo* studies have demonstrated that these bioactive compounds can delay, prevent or reverse oxidative damage to biomolecules as DNA, proteins and lipids, which prevents many chronic diseases including cancer, diabetes and neurodegenerative disorders (Frei & Higdon, 2003, Iwalokun *et al.*, 2007). The *P. ostreatus* extracts have broad-spectrum of antibacterial activity, which inhibits

the growth of both Gram positive and Gram negative bacteria.(Iwalokun *et al.*, 2007, Karaman *et al.*, 2010) . Increasing consumer awareness about the negative health impact of synthetic food additives as antioxidants and antimicrobial, has increased the demand for natural additives in functional food as foods that are fortified with biologically active ingredients, supplements and medicine. To meet this growing demand, natural bioactive compounds can be extracted from plants, food wastes and fruit fungi such as mushroom (Pandey *et al.*, 2018).

In developing countries, which face challenges due to poverty and shortage of resources and technology, mushroom can be a promising source to combat food insecurity and malnutrition (Pandey *et al.*, 2018). The *Pleurotus* spp. have many advantages including rapid growth in short time, low cost, minimal chance of disease manifestation, as well as easy adaptation and maintenance (Pandey *et al.*, 2018). They are usually found in tropical and subtropical rainforests where they can colonise and degrade lignocellulosic substrates. The *Pleurotus* spp. have thus been cultured on agro-wastes such as olive cake, pine needles, wheat straw and banana leaves (Koutrotsios *et al.*, 2018, Pandey *et al.*, 2018).

In Egypt, the estimated annual amount of agricultural wastes ranges from 30 to 35 million dry tonnes, of which about 12 million tons are utilized to produce feed and organic fertilizer (Elfeki *et al.*, 2017). The remaining plant wastes are incinerated or disposed in landfills resulting in environmental pollution and escalating rates of carbon dioxide and other gases that contribute to global warming (Sharma *et al.*, 2013).This huge amount of agro-wastes can be beneficially utilized in mushroom cultivation, a biotransformation of wastes into food and a remedy for pollution.

In previous work, Elattar *et al.*(2019) showed that the yield and overall acceptability of *P. ostreatus* were higher when grown on rice straw (RS) or rice and wheat straws (RS+WS) 1:1 (w/w) compared to those grown on other substrates such as saw dust and water hyacinth alone or mixed with wheat straw 1:1 (w/w). The main objective of the present study was to investigate the effect of *P. ostreatus* cultivation in two agro-waste substrates on its potential as a food additive with antioxidant and antimicrobial activities (Fig.1). Total polyphenols (TPC) and total flavonoids (TFC) contents were determined by colorimetric assays. Individual phenolic and flavonoid compounds were identified by HPLC. To determine the antimicrobial activities of mushroom extracts against both Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Salmonella typhimurium*) bacterial strains, two methods were used (agar well diffusion and macro-broth dilution methods).

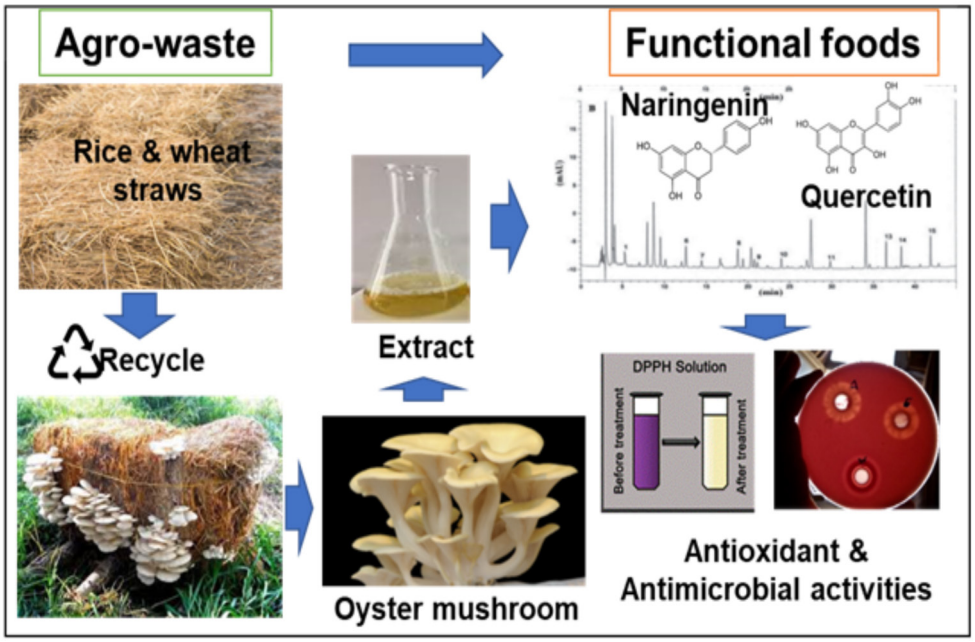

Fig. 1: Effect *of P. ostreatus* cultivation in two agro-waste substrates on its potential as a food additive with antioxidant and antimicrobial activities

MATERIALS AND METHODS

Materials:

Chemicals and reagents

Dimethyl sulfoxide (DMSO), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), $NaNO_2$, $AlCl_3$, Na_2CO_3, NaOH, HNO_3, H_2O_2, Folin–Ciocalteu phenol reagent, gallic, caffeic, catechol, *p*-hydroxy benzoic acid, caffeine, vanillic acid, syringic acid, vanillin, *p*-coumaric acid, benzoic acid, ferulic acid, ellagic, salicylic acid and cinnamic were purchased from Sigma-Aldrich (St. Louis, MO). Baird parker agar base, egg yolk tellurite emulsion, nutrient agar, violet red bile lactose agar and nutrient broth were obtained from Oxoid, England.

Methods:

Mushroom cultivation

The *P. ostreatus* was cultivated on rice straw (RS) or a 1:1 (w/w) mixture of rice and wheat straws (RS+WS), (Elattar *et al.*, 2019). Straws were cut into small pieces (2-3 inches long) and soaked in water at room temperature for 1-2 hr, then rinsed with clean water 2-3 times. Their moisture content was also adjusted to 65%. Then, $CaCO_3$ was added at a final concentration of 0.2% (w/w) of the total substrate. All substrates were filled into polyethylene bags and autoclaved at 121 °C for 80 min, then cooled down to room temperature and inoculated with 5 g spawn of *P. ostreatus* per 100 g of the substrate wet weight. The inoculated bags were incubated in darkness at 20–25 °C and 80-95% relative humidity for 3 weeks until the substrates were completely colonized with mycelium. Bags were punctured from the 4 sides to facilitate primordial initiation.

Preparation of mushroom extracts

Freshly harvested *P. ostreatus* samples were washed, sliced (2 mm thickness) and freeze-dried for 24 hr. Dried mushroom samples were ground into powder using a mechanical grinder (Moulinex, Germany). About 20 g of each of the ground samples were extracted with either 200 mL of cold water, 50% or 95% (v/v) ethanol by stirring for 2 hr at room temperature. After an overnight stand (about 18 hr) at room temperature, the samples were filtered using Whatman filter paper No. 1 (Sigma-Aldrich, Michigan, USA), and the residue was extracted with additional 200 mL of solvent as described previously. The combined extracts were evaporated under vacuum at 40°C using a rotary evaporator (BÜCHI 011 Rotavapor, Switzerland) to dryness (Reddy & Mathew, 2001). For determination of antioxidant activities and analysis of antioxidant components, the dried extracts were weighed, re-dissolved in 100 ethanol (95%) and stored at 4ºC until used. As for the antibacterial testing, the dried extracts were re-dissolved at concentration of 0.5g in 1 mL of dimethyl sulfoxide (DMSO) (0.1%, v/v in sterile water). Extracts were stored at -18°C until used.

Determination of bioactive components

The total polyphenol contents in the ethanolic (95%) extracts were determined according to the Folin-ciocalteu method (Singleton & Rossi, 1965). The concentration of total phenolic compounds was calculated based on standard curve of gallic acid (0.01- 0.05 mg/mL) and the results were expressed as mg gallic acid equivalent (GAE)/g.

The total flavonoid contents in the ethanolic (95%) extracts were determined using the aluminium chloride colorimetric assay (Zou *et al.*, 2004). In brief, 100 µL mushroom extract was mixed with 500 µL distilled water and 30 µL $NaNO_2$ solution (5%). After 5 min, 60 µL $AlCl_3.H_2O$ solution (10%) was added. After 6 min, 200 µL NaOH (1 M) and 110 µL distilled water were added to the mixture. The solution was mixed well, and the intensity of pink colour was measured at 510 nm using UV-vis Spectrophotometer. The total flavonoid concentration of each extract was calculated ,based on a standard curve of rutin (0.1-1.0 mg/mL), and was expressed as mg of rutin equivalents (RE) per g dry weight (dw) (Naegele**,** 2012). Ascorbic acid content in each mushroom sample was determined by titration with 2,6-dichlorophenol indophenol dye according to the AOAC (2010).

Crude fat was extracted using Soxhlet extraction method. Samples were saponified and vitamin E was extracted and determined according to the methods described by De Leenheer *et al.* (1988) and Podda *et al.* (1996). The extracted lipids (0.2 g) were saponified by adding 25 mL of 20% alcoholic potassium hydroxide and refluxing at 85 °C for 4 hr. The non saponified lipids were extracted twice with 50mL of diethyl ether. The combined ether layer was collected and washed with water, and then dried over anhydrous sodium sulphate. The solvent was then evaporated to dryness and the residuals were resuspended in HPLC grade meth-

anol and filtered with 0.2 µm PTFE filter before analysis. 20 µL were injected on top of the HPLC column Phenomenex, USA C18 (100 mm x 4.6 mm i.d.) operated at 35 ºC. Mobile phase consisting of acetonitrile: methanol (70:30) at a flow rate 0.7 mL/min was used in isocratic elution mode. The separated compounds were detected using VWD at 205 nm.(Puttaraju *et al.*, 2006)

Identification and quantification of individual phenolic compounds

To identify the phenolic compounds of mushroom extracts, HPLC 1260 Agilent Infinity (Agilent Technologies, Palo Alto, CA, USA) with a Variable Wavelength Detector (VWD) system was used according to Podda *et al.* (1996). Mushroom ethanolic (95%) extracts (10 µL) were injected into the HPLC system equipped with a reversed-phase column plusC18 (100 mm x 4.6 mm i.d.) operated at 30 ºC. The mobile phase used was ternary elution gradient mode with the following conditions: (A) HPLC grade water 0.2% H_2PO_4, (B) methanol, and (C) acetonitrile. The flow rate was set at 0.4mL/min, and the injection volume was 20 µL. Polyphenols were detected at 284nm using a Variable Wavelength Detector (VWD). For separation of flavonoids, column plus C18 (150 mm x 4.6 mm i.d.) operated at 35 ºC, mobile phase of HPLC grade water and 0.5% H_2PO_4 (50:50, v/v) with flow rate of 0.7 mL/min was used. The injection volume was 20µL and the separated compounds were detected using VWD at 273 nm. Fourteen commercial phenolic compounds were used as standards (gallic, caffeic, catechol, *p*-hydroxy benzoic acid, caffeine, vanillic acid, syringic acid, vanillin, *p*-coumaric acid, benzoic acid, ferulic acid, ellagic, salicylic acid and cinnamic acid).

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH scavenging activity was determined according to the modified method described by Brand-Williams *et al* (1995). Briefly, 0.25 mL of the ethanolic extracts (6.5 and 8.75 mg for mushrooms cultivated on RS and RS+WS, respectively) were added to 2.75 mL of 0.1 mmol DPPH in methanol solution. The mixture was shaken vigorously and left to stand in the dark for 30 min at room temperature, and the absorbance was then measured at 517 nm against a blank using the UV-vis Spectrophotometer (Pharmacia LKB, NOVA Spec II). The radical scavenging activity was calculated as a percentage of DPPH discoloration using the equation:

$$\text{Scavenging activity (\%)} = [(A_{Control} - A_{Sample})/A_{Control}] \times 100$$

Where A_{Sample} is the absorbance of tested sample and $A_{Control}$ is the absorbance of the control (DPPH solution). The IC_{50} value is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. L-ascorbic acid was used as standard control. The results of the assay were expressed as mg ascorbic acid equivalents (AAE)/g DW.

Ferric reducing power (FRP) assay

The reducing power of the mushroom extracts was determined using ferric reducing power method of Oyaizu (1986). In brief, 1.0 mL of the ethanolic extracts (26 and 35 mg for mushrooms cultivated on RS and RS+WS, respectively) were mixed with 2.5 mL of 1% potassium ferricyanide in 0.2 M phosphate buffer (pH 6.6), and the mixture was incubated at 50 °C for 20 min., 2.5 mL of 10% trichloroacetic acid were added to the mixture, which was then centrifuged at 1036 × g for 10 min. The upper layer of the solution (2.5mL) was mixed with distilled water (2.5 mL) and freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance of the solution was measured at 700 nm using the UV-vis Spectrophotometer. The standard (Potassium ferrocyanide) was also processed by the same procedure and the results were reported as mmol $K_4Fe(CN)_6$ equivalent/100 g dry weight.

Hydroxyl radical-scavenging activity

Hydroxyl radical scavenging activity of the 95% ethanolic extracts was measured according to the method of Halliwell *et al*. (1987) with a slight modification (Barros *et al.*, 2008). In brief, the assay was performed by adding, in sequence, 0.1 mL of EDTA (1 mM), 0.01 mL of $FeCl_3$ (10 mM), 0.1 mL of H_2O_2 (10 mM), 0.36 mL of deoxyribose (10 mM), 0.25 mL of the ethanolic extract (6.5 and 8.75 mg for mushrooms cultivated on RS and RS+WS, respectively) dissolved in 0.75 ml distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1mL of ascorbic acid (1 mM). This mixture was then incubated at 37 °C for 1 hr. A 1.0 mL portion of the incubated mixture was mixed with 1.0 mL of 10% TCA and 1.0 mL of 0.5% butylated hydroxyanisole (in 0.025 M NaOH containing 0.025%) to develop a pink chromogen that was measured at

532 nm using the UV-Vis Spectrophotometer. The hydroxyl radical-scavenging activity of the extract was reported as % inhibition of deoxyribose degradation and was calculated by the formula:

OH'-scavenged % = $[(A_{control} - A_{test})/A_{control}] \times 100$.

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of the tested sample.

Antibacterial activity

Antibacterial activities of mushroom extracts were tested against two Gram negative (*Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 14028) and two Gram-positive (*Staphylococcus aureus* ATCC 6538 and *Bacillus cereus* isolate) bacteria. The *B. cereus* isolate was isolated from Ultra-Heat milk and identified using 16S rRNA analysis. *Staph. aureus* and *B. cereus* were cultured on Baird parker agar and nutrient agar, respectively. Whereas, *E. coli* and *S. typhi*murium were both cultured on violet red bile lactose agar. The antimicrobial activity of mushroom extracts was assayed by 2 methods: agar-well diffusion assay (Magaldi *et al.*, 2004) and standard broth dilution method (Clinical and Laboratory Standards Institute,2009). All experiments were performed in triplicate.

Agar-well diffusion assay

In brief, an inoculum containing 10^8 CFU/ml of each of the bacterial cultures was added to an appropriate molten agar medium (20 mL) for each strain prior to its solidification, mixed and poured into a Petri dish. Then, 9 mm diameter wells were cut into the surface of the agar using the back of a sterile blue tip and 100 µL of each extract at concentration of 500 mg/mL was added to each well. Plates were incubated for 24 hr at 30 °C for *B. cereus* and at *37* °C for *Staph. aureus, E. coli* and *S. typhimurium*. Under the same conditions, tetracycline (250mg), clindamycin (150 mg), levofloxacin (250 mg), and Ampiflux (125 mg) were tested as the positive controls (standard antibiotics) for *B. cereus*, *Staph. aureus*, *E. coli* and *S. typhimurium*, respectively. The effect of dimethyl sulfoxide (DMSO) (0.1%) as a negative control on the growth of each strain was also tested. Inhibition zones around discs indicated the presence of antimicrobial activity, which was measured using a measuring ruler. The average diameter of the inhibition zone was measured in millimeter, and the activities were classified according to the following scale: inhibition zones less than 9 mm, inactive; 9–12 mm, moderately active; 13–18 mm, active; above 18 mm, very active. Each assay was carried out in triplicate (Magaldi *et al.*, 2004).

Minimal inhibitory concentrations assay

A serial of a 2-fold macro-broth dilution method was performed to determine the minimum inhibitory concentration (MIC) of mushroom extracts according to the standard method (Clinical and Laboratory Standards Institute, 2009). The stock solution of the tested extracts (500 mg/mL) were prepared, and 2-fold serial dilutions of extracts were performed. Concentrations of mushroom extracts ranged from 0.48 to 500 mg/mL were tested. To each test tube, 950 µL of nutrient broth, 50µL of bacterial suspension (10^8 CFU/mL), and 1000 µL of extract dilution were added. The positive controls contained 1950 µL of nutrient broth and 50 µL of each inoculum without extracts. The tubes were incubated for 24 hr at 30ºC for *B. cereus* and 37 ºC for the other 3 strains. Bacterial growth was determined by measuring turbidity at 600 nm using UV-Vis Spectrophotometer. MIC value (expressed as mg/mL) was determined for each extract at the lowest concentration where no turbidity was observed. All samples were tested in triplicates.

RESULTS AND DISCUSSION

Yield of mushroom extracts

Cold water or ethanol of 50% and 95% concentration were used to extract the bioactive compounds from *P. ostreatus* cultivated on RS or RS+WS substrates. Table (1) shows the effect of substrate and extraction solvent on the yield. The extract yield of mushroom grown on RS+WS substrate was higher than those grown on RS substrate alone in all the tested solvents. The yield ranged from 13.0 to17.5%, from 28 to 49% and from 31 to 60% for samples extracted with 95% ethanol, 50% ethanol and cold water, respectively (Table 1). In addition, the extraction yield of bioactive compounds decreased with increasing the ethanol concentration (Table 1). The yield of aqueous extracts was relatively higher than those of alcoholic extracts due to the high content of water soluble (polar) constituents in mushroom, such as proteins, polysaccharides and mineral salts (Puttaraju *et al.*, 2006). Moreover, Sudha *et al.* (2012)found that the extract yield of various mushrooms was relatively

higher in water than alcohols (ethanol or methanol) due to the extraction of high amount of polysaccharides such as glucan and soluble proteins. Also, the alcoholic extracts were very rich in phenolics and flavonoids.

Bioactive components in mushrooms

Mushrooms have high levels of phenolic compounds, which are composed of one or more aromatic rings bearing one or more hydroxyl groups and comprise a large and diverse group of molecules, including flavonoids. Due to the presence of hydroxyl groups in phenolic compounds, they are more soluble in polar solvents such as methanol and ethanol as well as their aqueous mixtures. Because ethanol (95%) is not toxic, it was used for the extraction and assessment of total phenols (TP) and flavonoid (TF) contents, which were expressed as mg gallic acid (GAE/g DW) and rutin (RE/g DW) equivalents per gram of sample dry weight, respectively (Table 2). Mushroom grown on RS+WS contained higher amounts of total phenolic contents (TPC) and total flavonoid contents (TFC) than those grown on RS alone (Table 2). The TPC were 1.30±0.05 and 1.78±0.09 mg GAE/g DW for grown mushrooms on RS and RS+WS, respectively. These results are slightly similar to those reported previously, and ranged from 0.93 to 1.42 mg GAE/g DW based on the extraction solvent and species (Karaman *et al.*, 2010, Oke & Aslim, 2011). Higher TFC (2.53 and 2.83 mg RE/g DW) were measured in mushroom grown on RS and RS+WS, respectively (Table 2), which are close to those reported (1.2 to 2.9 µg/g) from different species of *Pleurotus* extract (Vieira *et al.*, 2013).The amounts of TPC and TFC in mushroom can vary with species, cultivation methods, substrates, drying methods, aging, extraction solvents and temperature(Choi *et al.*, 2006, Gąsecka *et al.*, 2016).

Ascorbic acid (vitamin C) is reported to interact directly with radicals such as hydrogen peroxide, peroxyl radicals, singlet oxygen, superoxide and hydroxyl radical, which prevents free radical damage. It also assists α-tocopherol in the inhibition of lipid peroxidation by recycling the tocopherol radical (Beyer, 1992).Relatively high concentrations of vitamin C (6.3 and 5.2 mg/100 g DW) were found in the cultivated mushroom on RS and RS+WS, respectively. In general, there are contradictory results about *P. ostreatus* content of vitamin C that ranges from 0 (not detected) to 20 (high) mg/100g DW (Mattila *et al.*, 2001). Previous reports indicated that vitamin C content in mushroom varies depending on species, age, climate, storage, drying and handling (Tsai *et al.*, 2006).Importantly, significantly high amount of α-tocopherol (1.1 and 27.9 mg/100g DW) was detected in the mushroom grown on RS-WS compared to that grown on RS alone.

Elattar *et al.* (2019) showed that mushroom cultivated on RS-WS was higher in its fat content compared with that grown on RS. The α-tocopherol is a fat soluble vitamin, thereby its concentration depends on the fat content in mushroom sample. Fresh *P. ostreatus* mushroom contained 40.46–55.16 mg/100 gm total tocopherols (Jaworska *et al.*, 2015). While, Jayakumar *et al*. (2009) reported the presence of α-tocopherol in an amount of 30.1 mg /100 g dry weight. The α-tocopherol is the most biologically active form of vitamin E, and capable of protecting human from degenerative diseases like cardiovascular diseases or cancer. It prevents lipid peroxidation due to its high antioxidant activity and ability to deactivate the reactive oxygen species. Together, these results suggest that the mushroom grown on agro-waste such as RS and its supplement with WS (1:1) are good sources of several bioactives compounds such as polyphenols, flavonoids and vitamins (Table 2).

Table 1: Effect of different substrates and solvents on yield of *P. ostreatus* extracts

Solvent	Extraction Yield (%, w/w)	
	RS	RS+WS
95% ethanol	13±0.03	17.5±0.07
50% ethanol	28±0.05	49±0.02
Cold water	31±0.06	60±0.06

Values represent the mean ± standard error of 3 replicates. RS and RS+WS, *P. ostreatus* was cultivated on rice straw and a mixed rice and wheat straws (1:1, w/w) substrates, respectively.

Phenolic profiles

Polyphenols are the major antioxidant components that were reported in extracts of several mushroom species including *P. ostreatus* (Gąsecka *et al.*, 2016).Fourteen standard phenolic compounds were used for identification of phenolics, among which the phenolic acids and flavonoids were identified in ethanolic extracts of both cultivated mushrooms on RS and RS+WS substrate using HPLC analy-

Table 2: Bioactive compounds contents of *P. ostreatus* cultivated on agro-wastes.

Mushroom sample	Total Phenols (mg GAE*/g)	Total flavonoid (mg RE*/g)	Ascorbic acid (mg/100g)	α-tocopherol (mg/100 g)
RS	1.30±0.05	2.53±0.07	6.3±0.0	1.1
RS+WS	1.78±0.09	2.83±0.02	5.2±0.0	27.9

Values represent the mean ± standard error of 3 replicates. RS and RS+WS, P. ostreatus was cultivated on rice straw or mixed rice and wheat straws (1:1, w/w) substrates, respectively. *GAE, gallic acid equivalent. RE, rutin equivalent.

sis (Table 3). Nine phenolic compounds including, naringenin (68.7 and 56.6 µg/g DW), apigenin (39.8 and 16.9 µg/g DW), *p*-hydroxy benzoic acid (15.3 and 9.4 µg/g DW), kaempferol (11.3 and 6.5 µg/g DW) and quercetin (6.4 and 5.4 µg/g DW) as the major compounds, in addition to, caffeic acid, cinnamic acid, gallic acid and *o*-coumaric acid were detected in both of the ethanolic extracts (Table 3). On the other hand, ellagic acid was only detected in mushroom grown on RS, while *p*-coumaric acid was detected in mushroom grown on RS+WS (Table 3). Low amounts of gallic acid were detected in both of the extracts of cultivated mushrooms (2.1 and 0.5 µg/g DW) (Table 3). Similarly, low amount of gallic acid was reported (7 and 13 µg/g DW) by Palacios *et al.* (2011).The *p*-Hydroxy benzoic acid was the most abundant phenolic acid in both of the cultivated mushroom. Previously, *p*-coumaric acid was only identified in *P. ostreatus* at 11.15 and 76.91µg/g DW (Gąsecka *et al.*, 2016). High amounts of naringenin, a flavonoid, was found in a range of 56.6 and 68.7µg/g DW in both of the cultivated mushrooms (Table 3). Previous studies showed a very low level of naringenin that ranged

Table 3: Phenolic acid and flavonoid composition of the ethanolic extracts of oyster mushrooms cultivated on agro-wastes.

Compound	Concentration (µg/G)		Activity	References
	RS	RS+WS		
Phenolic acid				
Caffeic acid	0.8	0.6	antibacterial&antioxidant	Liu *et al.*, 2014
Cinnamic acid	0.7	0.4	antimicrobial	Liao *et al.*, 2012
Ellagic acid	0.6	0.0	antimicrobial & anti-biofilm formation	De *et al.*, 2018 and Fontaine *et al.*, 2017
Gallic acid	2.1	0.5	bacteriostatic & antioxidant	Kim, 2007
o-Coumaric acid	0.9	0.3	radical scavenging	Velkov *et al.*, 2007
p-Coumaric acid	0.0	0.2	antioxidant & antimicrobial	Ekinci-Akdemir *et al.*, 2017 and Lou *et al.*, 2012
p-Hydroxy benzoic acid	15.3	9.4	LDL oxidation inhibitor, Antifungal &antimicrobial	Chong *et al.*, 2009; Heleno *et al.*, 2013 and Hur *et al.*, 2003
Flavonoids				
Apigenin	39.8	16.9	anti-fungal	Cushnie and Lamb, 2005 and Leopoldini *et al.*, 2004
Kaempferol	11.3	6.5	antioxidant	Calderon-Montano *et al.*, 2011; Park *et al.*, 2006 and Zeng *et al.*, 2019
Naringenin	68.7	56.6	antibacterial & antioxidative	Sajitha Lulu *et al.*, 2016 and Cavia Saiz *et al.*, 2010
Quercetin	6.4	5.4	antioxidant & anti-biofilms	Bu *et al.*, 2011; Ouyang *et al.*, 2016; Sajitha Lulu *et al.*, 2016; Velkov *et al.*, 2007 and Zeng *et al.*, 2019

RS and RS+WS, *P. ostreatus* was cultivated on rice straw or mixed rice and wheat straws (1:1, w/w) substrates, respectively.

from 0.15±0.03 to 10 µg/g DW (Yen *et al.*, 1993, Naczk & Shahidi, 2004). Other flavonoids were also detected in large amounts such as apigenin (39.8 and 16.9 µg/g DW), quercetin (6.4 and 5.4 µg/g DW) in the mushroom (Table 3).

Antioxidant activity

The antioxidant activity of various mushroom extracts may be due to the reduction of hydroperoxide, inactivation of free radicals, binding with metal ions, presence of phytochemicals (i.e. phenolic compounds), or a combination of these possibilities (Choi *et al.*, 2005).Many studies have shown a high correlation between antioxidative activities and phenolic content (Fernandez-Panchon *et al.*, 2008). Phenolic compounds display extensive free radical-scavenging activities as hydrogen donors, electron-donating agents (reducing agents), and metal ion chelating properties. Therefore, the antioxidant activity of the 95% ethanolic extracts of both cultivated mushrooms was evaluated by three different methods; namely, DPPH free radical scavenging, ferric reducing power (FRP) and hydroxyl scavenging activity assays, the results are shown in Table (4).

Scavenging of DPPH free radicals is a widely used method to evaluate antioxidant activity of specific compounds or extracts in a relatively short time compared with other methods. The DPPH is a stable, free radical that accepts an electron or hydrogen radical producing a purple colour in alcohols but becomes pale when it reacts with antioxidant molecules (Chorvathova *et al.*, 1993). The scavenging effects of the ethanolic extracts of cultivated mushrooms on DPPH radical are shown in Table (4).

For both mushrooms grown on RS and RS+WS, the scavenging effect on DPPH radicals increased with increasing the concentration of mushroom extract (not shown here). The ethanolic extracts of *P. ostreatus* grown on RS+WS had a slightly higher scavenging ability (51.40%; inhibition %) than that grown on RS (50.87%) (Table 4). These activities imply the presence of antioxidant components that can react rapidly with DPPH radicals. The IC_{50} values were found to be 48.39 (RS) and 47.62 (RS+WS) mg/ml for 50% inhibition of DPPH free radicals (Table 4). These results are in agreement with several studies, which detected a potent antioxidant activity against DPPH radical in ethanolic extracts of *Pleurotus* spp.(Fernandez-Panchon *et al.*, 2008).

The efficacy of certain antioxidants is known to be associated with their reducing power. Therefore, transformation of Fe^{3+} to $Fe^{2+}$was measured according to the method of Oyaizu (1986).Ethanolic extracts from mushrooms samples grown on RS+WS showed a higher reducing power (316.90 mmol $K_4Fe(CN)_6$ equivalent/100 g DW) than that grown on RS (193.66 mmol $K_4Fe(CN)_6$ equivalent/100 g DW) as shown in Table (4). Thus, this result indicated the presence of reductone, which could react with free radicals to stabilize and block radical chain reactions.

The hydroxyl radical is the most reactive oxygen species that can induce oxidative damage to DNA, lipids and proteins (Choi *et al.*, 2006). For RS and RS+WS cultivated mushrooms extracts, the hydroxyl radical-scavenging effects were 40.74 and 45.50%, respectively (Table 4). These results together indicate that the cultivated mushrooms on RS and RS+WS substrates contain significant

Table 4: Antioxidant activity of *P. ostreatus* cultivated on agro-wastes.

Assay	RS	RS+WS
DPPH scavenging assay		
Scavenging activity (%)	50.87±1.0	51.40±2.0
Ascorbic acid equivalent (mg/g)	1.55±0.03	1.58±0.03
IC_{50} (mg/ml)	48.39±0.9	47.62±1.8
FRAP* assay		
mmol $K_4Fe(CN)_6$ equivalent/100 g	193.66±3.6	316.90±3.6
OH′-scavenging activity		
OH′-scavenged (%)	40.74±0.5	45.50±0.7

Values represent the mean ± standard error of 3 replicates. RS and RS+WS, *P. ostreatus* was cultivated on rice straw or mixed rice and wheat straws (1:1, w/w) substrates, respectively. *FRAP= ferric reducing antioxidant power.

amounts of antioxidant compounds such as polyphenols and flavonoids, which are rich in the alcoholic extract of mushrooms (Venkatakrishnan *et al.*, 2010).In addition, their alcoholic extracts can be used as food additives with a free radical inhibitor or scavenger activity for inactivating free radicals. The greater numbers of hydroxyl groups in the phenolics may result in higher antioxidant activity(Fernandez-Panchon *et al.*, 2008). Natural phenolics are able to provide antioxidative function through intercepting singlet oxygen, decomposing primary products of oxidation, preventing continued hydrogen abstraction from substances, etc. (Gunde-Cimerman, 1999).

Antibacterial activity of mushrooms extracts

Many studies have reported that mushrooms are potential sources for antimicrobial against different classes of pathogens including pathogenic bacteria, fungi and virus (Masri *et al.*, 2017). Most of the identified 70 species of *Pleurotus* spp. have medicinal effects such as antimicrobial properties.

The prepared extracts were evaluated for potential antimicrobial activity against four foodborne bacterial pathogens using two methods (agar-well diffusion and macro-broth dilution methods). The antimicrobial activity of the water and ethanolic (50 and 95%) extracts of mushroom cultivated on RS and RS+WS substrates were tested using the agar-well diffusion plate and expressed as zone of inhibition (mm ± SD). Preliminary results showed that a very weak antibacterial activity was obtained when 95% ethanolic extracts were tested (not shown). Ethanolic extract (50%) exhibited high antibacterial activity against Gram-negative bacteria and caused inhibition halo ranging from 16 to 23 mm for the *Salmonella typhimurium* (19.5±0.7 mm) and the highest for *E. coli* (23.25±1.8 mm) strains (Table 5). While, the mean inhibition zone of growth was (16.75±0.35) against the tested Gram-positive bacteria (*Staph aureus*) (Table 5).The lowest antibacterial activities were obtained with ethanol RS extracts, which produced 11.5±0.7 mm zone of inhibition against *Staph aureus* (Table 4). On the other hands, the aqueous extract of mushroom cultivated on RS showed the lowest activity against *S. typhimurium* (12±0.0 mm, inhibition zone diameter). However, all mushroom extracts showed no inhibition against the Gram-positive bacteria *B. cereus* (Table 5). Importantly, extracts of mushroom cultivated on RS+WS had more inhibition zone diameter against *E. coli* than when using levofloxacin as a standard drug. The aqueous extracts of mushrooms showed higher activity than hot water extracts (data not shown here), which agrees with previous works (Akyuz & Kirbag, 2009). Furthermore, Venturini *et al.*(2008) reported that the methanol extract of *P. ostreatus* possessed antimicrobial activity only against *Clostridium perfringens* (Gram-positive bacteria) and not against other tested pathogenic bacterial strains including *B. Cereus*.

Table 5: Antimicrobial activity of mushrooms extracts against selected Gram-negative and Gram-positive pathogenic bacteria

Tested bacteria	RS extracts		RS+WS extracts		Standard
	Ethanol	Water	Ethanol	Water	Drugs*
Gram-negative bacteria					
S. typhimurium	18.5±0.7	12±0.0	19.5±0.7	15.5±0.7	30±0.0
E. coli	18.5±0.7	15.5±0.7	23.25±1.8	18.5±0.7	16±0.0
Gram-positive bacteria					
Staph. aureus	11.5±0.7	15.5±0.7	16.75±0.35	17.75±0.4	54±0.0
B. cereus	N.D.	N.D.	N.D.	N.D.	29±0.0

Antimicrobial activity was determined in the extracts using agar-well diffusion and expressed as zone of inhibition in mm ± SD. RS and RS+WS, *P. ostreatus* was cultivated on rice straw or mixed rice and wheat straws (1:1, w/w) substrates, respectively. N.D., Not detected. * standard drugs (250 mg/mL) are listed in Materials and Methods. The mean zone of inhibition was determined from three independent results (n) = 3.

Minimal inhibitory concentrations

The antibacterial activity of extracts of mushroom cultivated on RS+WS against *E. coli*, *Staph. aureus* and *S. typhimurium* were slightly higher

than those cultivated on RS. Aqueous extract of mushroom cultivated on RS showed high antibacterial activity against *S. aureus* with minimal inhibitory concentrations (MIC) value of 125 mg/mL, while ethanol extract of cultivated mushroom on RS did not show any antibacterial activity against *Staph. aureus* and *B. cereus* (Table 6). These results indicated that both aqueous and ethanolic extracts exhibit different antimicrobial activities against Gram-positive and Gram-negative bacteria. These differences in their antimicrobial activities might be due to a variation in the tested pathogenic strain, extraction solvent and the extracted antimicrobial components in each extract (Akyuz & Kirbag, 2009, Manjunathan & Kaviyarasan, 2010). These variations may also be due to the highly diversified groups of bioactive compounds present in mushrooms and also their high content of proteins that exhibit antibacterial activity(Younis *et al.*, 2015). For example, methanol and ethanol extracts of two strains of *P. ostreatus* (EVFB1 and EVFB4) showed weak antibacterial activity against both Gram-positive (*Bacillus cereus* CMGB 215) and Gram-negative (*Escherichia coli* CBAB 2, and *Listeria innocua* CMGB 218) bacterial strains (Vamanu *et al.*, 2011). However, other research showed *P. ostreatus* extract to have high antimicrobial activities against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria due to the presence of 3-(2-aminophenylthio)-3-hydroxypropanoic acid, which was identified as the antibacterial active compound (Younis *et al.*, 2015).

Certain phenolic compounds were reported to be associated with antioxidant activity such as radical scavenging activity (Velkov *et al.*, 2007; Vaz *et al.*, 2011). As shown in Table (3), both extracts contain polyphenols and flavonoids components that exhibit not only antioxidant activity but also antimicrobial activity (e.g., gallic acid, caffeic acid, *p*-coumaric acid and quercetin). Therefore, the antioxidants and antibacterial activities of both extracts are linked to the activity of individual phenolic and flavonoids compounds. Extracts of mushroom cultured on RS+WS presented higher antimicrobial activity due to their high contents of TPC, TFC and α-tocopherol. These results thus suggest that the aqueous and ethanolic extracts of both cultivated mushrooms on agro-waste can be utilized as natural antimicrobials.

Table 6. Minimum inhibitory concentrations (mg/ mL) of mushrooms extracts against selected Gram negative and Gram-positive pathogenic bacteria.

Tested bacteria	RS extracts		RS+WS extracts	
	Ethanol 50%	Water	Ethanol 50%	Water
Gram-negative bacteria				
S. Typhimurium	31.25	N.D.	15.625	31.25
E. coli	15.625	62.5	7.81	15.625
Gram-positive bacteria				
Staph. aureus	N.D.	125	31.25	15.625
B. cereus	N.D.	N.D.	N.D.	N.D.

Antimicrobial activity was determined in the extracts using a serial two-fold macro-broth dilution method and expressed as MIC and was determined from three independent results (n) = 3. RS and RS+WS, *P. ostreatus* was cultivated on rice straw or mixed rice and wheat straws (1:1, w/w) substrates, respectively. Extracts were added at from stock solutions (500 mg/mL), and then two-fold serial dilutions of extracts were performed. N.D., Not detected. All extracts were found to be ineffective in inhibition of *B. cereus* growth up to 500 mg/mL.

CONCLUSION

The *P. ostreatus* cultivation is an effective bioconversion process that is capable of transfering agro-wastes, such as rice and wheat straws, into potentially valuable bioactive extracts with great potential as natural antioxidant and antimicrobial additives for improving the shelf-life of food products during storage by preventing proteins and lipids oxidation as well as microbial growth.

ACKNOWLEDGMENTS

The authors gratefully thankful to Prof. Dr. Ahmed Rafik El-Mahdy (Alexandria University) and Dr. Tarek Awad (University of Toronto) for fruitful discussions, and to Dr. Tarek Awad and Miss Alaa Awad (University of Toronto) for critically proof reading the manuscript. This study is a part of project entitled: Vigorous mushroom fermented milk summit proceeding. Funded by the Academy of Scientific Research and Technology, Egypt. Project ID 22.

REFERENCES

Akyuz, M. & Kirbag, S. **2009.** Antimicrobial activity of *Pleurotus eryngii* var. *ferulae* grown on various agro-wastes. EurAsian Journal of BioSciences, **3:** 58-63.

AOAC **2010**. Official Methods of Analysis, 18th edn. Association of Official Analytical Chemists, Washington D. C., USA.

Barros, L., Falcão, S., Baptista, P., Freire, C., Vilas-Boas, M. & Ferreira, I.C. **2008.** Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chemistry, **111:** 61-66.

Beyer, R.E. **1992.** An analysis of the role of coenzyme Q in free radical generation and as an antioxidant. Biochemistry and Cell Biology, **70**: 390-403.

Brand-Williams, W., Cuvelier, M.-E. & Berset, C. **1995.** Use of a free radical method to evaluate antioxidant activity. LWT-Food Sciences and Technology, **28:** 25-30.

Bu, T., Mi, Y., Zeng, W. & Zhang, C. **2011.** Protective effect of quercetin on cadmium□induced oxidative toxicity on germ cells in male mice. Anat. Rec.: Advances in Integrative Anatomy and Evolutionary Biology, **294**: 520-526.

Calderon-Montano, M., Burgos-Morón, E., Pérez-Guerrero, C. & López-Lázaro, M. **2011.** A review on the dietary flavonoid kaempferol. Mini-Reviews in Medicinal Chemistry, **11:**298-344.

Cavia-Saiz, M., Busto, M.D., Pilar-Izquierdo, M.C., Ortega, N., Perez-Mateos, M. & Muñiz, P. **2010.** Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: a comparative study. Journal of the Sciences of Food and Agriculture, **90:** 1238-1244.

Chang, R. **1996.** Functional properties of edible mushrooms. Nutrition Reviews, **54**, S91-93.

Choi, Y., Lee, S., Chun, J., Lee, H. & Lee, J. **2006.** Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chemistry, **99:** 381-387.

Choi, Y.M., Ku, J.B., Chang, H.B. & Lee, J. **2005.** Antioxidant activities and total phenolics of ethanol extracts from several edible mushrooms produced in Korea. Food Sciences Nutrition,**14**: 700-703.

Chong, K.P., Rossall, S. & Atong, M. **2009.** In vitro antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against *Ganoderma boninense*. Journal Agriculture Sciences, **1:** 15.

Chorvathova, V., Bobek, P., Ginter, E. & Klvanova, J. **1993.** Effect of the oyster fungus on glycaemia and cholesterolaemia in rats with insulin-dependent diabetes. Physiology Research, **42:** 175-175.

Clinical and Laboratory Standards Institute. **2009**. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard: Nineteenth Informational Supplement M100-S19. CLSI, Wayne, PA, USA.

Cushnie, T.T. & Lamb, A.J. **2005.** Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, **26**: 343-356.

De Leenheer, A.P., Nelis, H.J., Lambert, W.E. & Bauwens, R.M. **1988.** Chromatography of fat-soluble vitamins in clinical chemistry. Journal of Chromatography B: Biomedical Sciences and Applications, **429**: 3-58.

De, R., Sarkar, A., Ghosh, P., Ganguly, M., Karmakar, B.C., Saha, D.R., Halder, A., Chowdhury, A. & Mukhopadhyay, A.K. **2018.** Antimicrobial activity of ellagic acid against *Helicobacter pylori* isolates from India and during infections in mice. Journal of Antimicrobial Chemotherapy, **73:** 1595-1603.

Ekinci-Akdemir, F., Gülçin, I., Gürsul, C., Alwasel, S. & Bayir, Y. **2017.** Effect of p-coumaric acid against oxidative stress induced by cisplatin in brain tissue of rats. Journal of Applied Pharmaceutical Science, **27**:1560-1564.

Elattar, A., Hassan, S.M. & Sh, A. **2019.** Evaluation of Oyster Mushroom (*Pleurotus ostreatus*) Cultivation Using Different Organic Substrates. Alexandria Science Exchange Journal, **40**: 427-440.

Elfeki, M., Elbestawy, E. & Tkadlec, E. **2017**. Bioconversion of Egypt's Agricultural Wastes into Biogas and Compost.Polish Journal of Environmental Studies, **26**:1-10.

Fernandez-Panchon, M., Villano, D., Troncoso, A. & Garcia-Parrilla, M. **2008.** Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. Critical Reviews in Food Science and Nutrition, **48:** 649-671.

Fontaine, B.M., Nelson, K., Lyles, J.T., Jariwala, P.B., García-Rodriguez, J.M., Quave, C.L. & Weinert, E.E. **2017.** Identification of ellagic acid rhamnoside as a bioactive component of a complex botanical extract with anti-biofilm activity. Frontiers in Microbiology, **8:** 496.

Frei, B. & Higdon, J.V. **2003.** Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. The Journal of nutrition, **133:** 3275S-3284S.

Gąsecka, M., Mleczek, M., Siwulski, M. & Niedzielski, P. **2016.** Phenolic composition and antioxidant properties of *Pleurotus ostreatus* and *Pleurotus eryngii* enriched with selenium and zinc. European Food Research and Technology, **242**: 723-732.

Gunde-Cimerman, N. **1999.** Medicinal value of the genus *Pleurotus* (Fr.) P. Karst.(agaricales sl, Basidiomycetes). International Journal of Medicinal Mushrooms, **1:** 60-80.

Halliwell, B., Gutteridge, J. M. C., & Arnoma, O. L. **1987**. The deoxyribose method: A simple test tube assay for the determination of rate constant for reaction of hydroxyl radical. Analytical Biochemistry, **165**: 215−219.

Heleno, S.A., Ferreira, I.C., Esteves, A.P., Ćirić, A., Glamočlija, J., Martins, A., Soković, M. & Queiroz, M.J.R. **2013.** Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. Food Chemistry Toxicology, **58:** 95-100.

Hur, J.M., Park, J.G., Yang, K.H., Park, J.C., Park, J.R., Chun, S.S., Choi, J.S. & Choi, J.W. **2003.** Effect of methanol extract of *Zanthoxylum piperitum* leaves and of its compound, protocatechuic acid, on hepatic drug metabolizing enzymes and lipid peroxidation in rats. Bioscience, Biotechnology, and Biochemistry, **67:** 945-950.

Iwalokun, B., Usen, U., Otunba, A. & Olukoya, D. **2007.** Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. African Journal of Biotechnology, **6**:1732-1739.

Jaworska, G., Pogoń, K., Bernaś, E. & Duda Chodak, A. **2015.** Nutraceuticals and antioxidant activity of prepared for consumption commercial mushrooms *Agaricus bisporus* and *Pleurotus ostreatus*. Journal of Food Quality, **38**: 111-122.

Jayakumar, T., Thomas, P. & Geraldine, P. **2009.** In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. Innovative Food Science and Emerging Technologies,**10**: 228-234.

Karaman, M., Jovin, E., Malbaša, R., Matavuly, M. & Popović, M. **2010.** Medicinal and edible lignicolous fungi as natural sources of antioxidative and antibacterial agents. Phytotherapy Research, **24:** 1473-1481.

Kim, Y.-J. **2007.** Antimelanogenic and antioxidant properties of gallic acid. Biological and Pharmaceutical Bulletin, **30:** 1052-1055.

Koutrotsios, G., Kalogeropoulos, N., Kaliora, A.C. & Zervakis, G.I. **2018.** Toward an increased functionality in Oyster (*Pleurotus*) mushrooms produced on grape marc or olive mill wastes serving as sources of bioactive compounds. Journal of Agriculture and Food Chemistry, **66:** 5971-5983.

Leopoldini, M., Pitarch, I.P., Russo, N. & Toscano, M. **2004.** Structure, conformation, and electronic properties of apigenin, luteolin, and taxifolin antioxidants. A first principle theoretical study. Journal of Physical Chemistry A, **108:** 92-96.

Liao, J.-C., Deng, J.-S., Chiu, C.-S., Hou, W.-C., Huang, S.-S., Shie, P.-H. & Huang, G.-J. **2012.** Anti-inflammatory activities of *Cinnamomum cassia* constituents *in vitro* and *in vivo*. Evidence-Based Complementary and Alternative Medicine, **2012**: 429320.

Liu, M., Song, S., Li, H., Jiang, X., Yin, P., Wan, C., Liu, X., Liu, F. & Xu, J. **2014.** The protective

effect of caffeic acid against inflammation injury of primary bovine mammary epithelial cells induced by lipopolysaccharide. Journal of Dairy Sciences, **97:** 2856-2865.

Lou, Z., Wang, H., Rao, S., Sun, J., Ma, C. & Li, J. **2012.** p-Coumaric acid kills bacteria through dual damage mechanisms. Food Control, **25**, 550-554.

Magaldi, S., Mata-Essayag, S., De Capriles, C.H., Perez, C., Colella, M., Olaizola, C. & Ontiveros, Y. **2004.** Well diffusion for antifungal susceptibility testing. International Journal of Infectious Diseases, **8:** 39-45.

Manjunathan, J. & Kaviyarasan, V. **2010.** Solvent based effectiveness of antibacterial activity of edible mushroom *Lentinus tuberregium* (Fr.). International Journal of PharmTech Research, **2**: 1910-1912.

Masri, H.J.M.H.J., Maftoun, P.M.P., Malek, R.A., Boumehira, A.Z., Pareek, A., Hanapi, S.Z., Ling, O.M. & El Enshasy, H. **2017.** The edible mushroom *Pleurotus* spp.: ii. medicinal values. International Journal of biotechnology for wellness industries, **6:** 1-11.

Mattila, P., Könkö, K., Eurola, M., Pihlava, J.-M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M. & Piironen, V. **2001.** Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. Journal of Agriculture and Food Chemistry, **49:** 2343-2348.

Naczk, M. & Shahidi, F. **2004.** Extraction and analysis of phenolics in food. Journal of Chromatography A,**1054**: 95-111.

Naegele, E. **2012.** Qualitative & quantitative determination of phenolic antioxidant compounds in red wine and fruit juice with the Agilent 1290 Infinity 2D-LC Solution. Agilent Application Note, Publication Number - 5991-0426 EN.

Oke, F. & Aslim, B. **2011.** Protective effect of two edible mushrooms against oxidative cell damage and their phenolic composition. Food Chemistry, **128:** 613-619.

Ouyang, J., Sun, F., Feng, W., Sun, Y., Qiu, X., Xiong, L., Liu, Y. & Chen, Y. **2016.** Quercetin is an effective inhibitor of quorum sensing, biofilm formation and virulence factors in *Pseudomonas aeruginosa*. Journal of Applied Microbiology,**120**: 966-974.

Oyaizu, M. **1986.** Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, **44**: 307-315.

Palacios, I., Lozano, M., Moro, C., D'arrigo, M., Rostagno, M., Martínez, J., García-Lafuente, A., Guillamón, E. & Villares, A. **2011.** Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chemistry,**128:** 674-678.

Pandey, V.V., Kumari, A., Kumar, M., Saxena, J., Kainthola, C. & Pandey, A. **2018.** Mushroom cultivation: Substantial key to food security. Journal of Applied and Natural Science,**10**: 1325-1331.

Park, J.S., Rho, H.S., Kim, D.H. & Chang, I.S. **2006.** Enzymatic preparation of kaempferol from green tea seed and its antioxidant activity. Journal of Agriculture and Food Chemistry, **54:** 2951-2956.

Parola, S., Chiodaroli, L., Orlandi, V., Vannin, C. & Panno, L. **2017.** *Lentinula edodes* and *Pleurotus ostreatus*: functional food with antioxidant-antimicrobial activity and an important source of Vitamin D and medicinal compounds. Functional Foods in Health and Disease, **7:** 773-794.

Podda, M., Weber, C., Traber, M.G. & Packer, L. **1996.** Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols, and ubiquinones. Journal of Lipid Research, **37:** 893-901.

Puttaraju, N.G., Venkateshaiah, S.U., Dharmesh, S.M., Urs, S.M.N. & Somasundaram, R. **2006.** Antioxidant activity of indigenous edible mushrooms. Journal of Agriculture and Food Chemistry, **54:** 9764-9772.

Reddy, C. A. & Mathew, Z **. 2001.** Bioremediation Potential of White Rot Fungi. In: Fungi in Bioremediation, G.M. Gadd, (Ed.), Cambridge University Press, London, pp: 52-78.

Roncero-Ramos, I. & Delgado-Andrade, C. **2017.** The beneficial role of edible mushrooms in human health. Current Opinion in Food Science, **14**: 122-128.

Sajitha Lulu, S., Thabitha, A., Vino, S., Mohana Priya, A. & Rout, M. **2016.** Naringenin and quercetin–potential anti-HCV agents for NS2 protease targets. Natural Product Research, **30:** 464-468.

Sharma, S., Yadav, R.K.P. & Pokhrel, C.P. **2013.** Growth and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates. Journal of New Biology Reports, **2:** 03-08.

Singleton, V.L. & Rossi, J.A. **1965.** Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture,**16**: 144-158.

Sudha, G., Vadivukkarasi, S., Shree, R.B.I. & Lakshmanan, P. **2012.** Antioxidant activity of various extracts from an edible mushroom *Pleurotus eous*. Food Science and Biotechenology, **21**: 661-668.

Tsai, S.-Y., Huang, S.-J. & Mau, J.-L. **2006.** Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. Food Chemistry, **98**: 670-677.

Vamanu, E., Ene, M., Vamanu, A., Smarandache, D., Sârbu, I., Popa, O., Băbeanu, N., Nita, S. & Veaceslav, B. **2011.** Antioxidant and antibacterial properties of the extracts from *Pleurotus ostreatus* EVFB1 and EVFB4. Romanian Biotechnological Letters,**16.** 41.

Vaz, J.A., Barros, L., Martins, A., Santos-Buelga, C., Vasconcelos, M.H. & Ferreira, I.C. **2011.** Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. Food Chemistry,**126**: 610-616.

Velkov, Z., Balabanova, E. & Tadjer, A. **2007.** Radical scavenging activity prediction of *o*-coumaric acid thioamide. Journal of Molecular Structure: Theochem, **821**: 133-138.

Venkatakrishnan, V., Shenbhagaraman, R., Kaviyarasan, V., Gunasundari, D., Radhika, K., Dandapani, R. & Jagadish, L.K. **2010.** Antioxidant and antiproliferative effect of *Pleurotus ostreatus*. Journal of Phytology, **2**: 022-028.

Venturini, M., Rivera, C., Gonzalez, C. & Blanco, D. **2008.** Antimicrobial activity of extracts of edible wild and cultivated mushrooms against foodborne bacterial strains. Journal of Food Protection, **71**: 1701-1706.

Vieira, P.A.F., Gontijo, D.C., Vieira, B.C., Fontes, E.A., de Assunção, L.S., Leite, J.P.V., Oliveira, M.G.d.A. & Kasuya, M.C.M. **2013.** Antioxidant activities, total phenolics and metal contents in *Pleurotus ostreatus* mushrooms enriched with iron, zinc or lithium. LWT-Food Science and Technology, **54**: 421-425.

Yen, G.C., Duh, P.D. & Tsai, C.L. **1993.** Relationship between antioxidant activity and maturity of peanut hulls. Journal of Agricultural and Food Chemistry, **4:**67-70.

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 Mushrooms, **17:**579-590.

Zeng, Y., Nikitkova, A., Abdelsalam, H., Li, J. & Xiao, J. **2019.** Activity of quercetin and kaemferol against *Streptococcus* mutans biofilm. Archives of Oral Biology, **98:** 9-16.

Zou, Y., Lu, Y. & Wei, D. **2004.** Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. Journal of Agriculture and Food Chemistry, **52:** 5032-5039.

استخدام المخلفات الزراعية لإنتاج فطر المحار (*Pleurotus ostreatus*) عالي الأنشطة المضادة للأكسدة والميكروبات

أمل محمد عبد الرازق[١]، أمل إبراهيم[٢]، عيشة العطار[٢]، دلال عسكر[٣،١]

١- قسم علوم وتقنية الأغذية -كلية الزراعة (الشاطبى)- جامعة الإسكندرية

٢- قسم علوم وتقنية الألبان -كلية الزراعة (الشاطبى)- جامعة الإسكندرية

٣- قسم علوم المواد والهندسة – جامعة تورنتو- كندا

يعتبر المشروم المحارى (*Pleurotus ostreatus*) مصدرًا جيدًا للمركبات النشطة حيويا والتي لها فوائد صحية وطبية عديدة. في هذه الدراسة ، تم بحث تأثير زراعة المشروم المحارى علي بيئات من المخلفات الزراعية المختلفه بما في ذلك قش الأرز (RS) وخليط قش الأرز مع تبن القمح (RS + WS) بهدف معرفة تأثيرها علي إنتاج مضافات غذائية ذات خصائص حيوية كمضادات للأكسدة والميكروبات. وقد أظهرت مستخلصات المشروم المحارى المزروعة على خليط قش الأرز وتبن القمح احتواءها علي كميه عالية من البوليفينولات الكلية (TPC)،الفلافونويدات (TFC) والـ α-tocopherol، وتميزت أيضا بنشاط اعلى كمضادات أكسدة ومضادات بكتيرية عن تلك المزروعة على قش الأرز بمفرده. وقد كشف تحليل HPLC لمستخلصات الإيثانول عن وجود أحد عشر مركبًا فينوليًا منها حمض بارا-هيدروكسي البنزويك، نارينجين، كيمبفيرول وأبيجينين كمركبات رئيسية. كما أظهرت النتائج ان المستخلصات المائية والإيثانولية للمشروم المزروع في كلتا البيئتين تميزت بنشاطها العالي ضد بكتيريا مثل *Staphylococcus aureus* ، *Escherichia coli* ، *Salmonella typhimurium* ، في حين لم يلاحظ أي تأثير مثبط على *Bacillus cereus*. أشارت النتائج إلى أن زراعة *P. ostreatus* تعتبر عملية فعالة للتحويل الحيوي ومن ثم فهي مفيدة في الاستفادة من المخلفات الزراعية كمصدر لإنتاج مضادات أكسدة طبيعية ومضادات للميكروبات والتى يمكن الاستفادة منها مستقبلاً فى المنتجات الغذائية الوظيفية.