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Nutrition Value and Phytochemical Determination of Oyster Mushroom *(Pleurotus spp.)*

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Abstract

Oyster mushrooms, the common name for the species *pleurotus ostreatus*, are one of the most common types of an edible cultivated mushroom in the world that are necessary for a body health. The data obtained, from this research clearly show that cultivated *Pleurotus* spp. on Zea mays waste gave high level phytochemical compounds, carbohydrates, soluble proteins and amino acids indicating that Zea mays have been given mushroom more quality and more rich from these compounds than the other two wastes tested. It was observed that during this study, ethanolic extract from mycelia and fresh fruiting bodies have higher phytochemical contents than filtrate from liquid medium for all five *Pleurotus* spp. cultivated on the three agro- wastes.

Keywords: Bioactive compounds, Antioxidants, Flavonoids, Phenolic Compounds

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INTRODUCTION

The genus *Pleurotus* is commonly known as oyster mushroom. However, *Pleurotus* species can be grown in wild range of temperate and tropical areas as the wild mushroom. Nonetheless, *Pleurotus* species can be grown in wild range of temperate and tropical regions as the wild mushroom. It belongs to the family Tricholomataceae, order Agaricales, and class Basidiomycetes. There are 40 known species in this genus *P. ostreatus* and *P. sajor caju* are two species that have been extensively grown (Ahmed et al., 2009).

Furthermore, a large number of species in the *Pleurotus* genus have been used as sources of substances, including low-molecular-weight compounds (terpenoids, fatty acid esters, and polyphenols) and high-molecular-weight bioactive compounds (polysaccharides, peptides, and proteins) found in the fruiting bodies and mycelium that have anti-oxidative, anti-inflammatory, anti-neoplastic, anti-diabetic, anti-inflammatory, antibacterial, and immune-stimulatory qualities (Sarma et al., 2018; Golak-Siwulska et al., 2018).

Chemical composition of the fresh and dried mushroom *Pleurotus ostreatus* and their bioactive secondary metabolic products were studied by Mohamed and Farghaly 2014 they noticed the selenium content in *P. ostreatus* samples was measured by using ACAL –APR - 51-00 test methods and showed that the fresh sample had 58.24 mg/kg while the dry sample had 100.31 mg/kg. The ethanolic extracts of the *P. ostreatus* exhibit antioxidant activity by scavenging hydroxyl and superoxide radical and lipid peroxidation and inhibit the reducing power on ferric ions (Jayakumar et al., 2009).

Oyster mushrooms (*Pleurotus* spp.) are wood-inhabiting white-rot Basidio-mycetes with important biotechnological and environmental applications (Singh et al., 2012; Singh et al., 2013). They are highly adaptable to grow and fruit on a wide variety of forest and agro-industrial lignocellulosic substrates, because of their ability to synthesize the relevant hydrolytic and oxidative enzymes that convert the individual component of the substrate (cellulose, hemicellulose and lignin) into low-molecular weight compounds, which can be assimilated for fungi nutrition (Elisashvili et al., 2008).

Recently, mushrooms have been recognized as sources of antioxidants as they contain phenolic compounds and secondary metabolites that have a preventive effect against chronic diseases (Lbarmaic et al. 2009). Additionally, mushrooms are known to have therapeutic benefits; they are low in calories for diabetic and heart patients, lowering the blood cholesterol level, warding against cancer and also contain appreciable quantities of crude fibers on the total dietary fiber (TDF). Nowadays, mushrooms mainly are being considered as functional food because are a good source of proteins, amino acids (such as arginine, glutamine, and glutamic acid), vitamins (especially riboflavin (B2), niacin (B3) and folates (B9), minerals, fat lowering, sugars and sodium contents (Da Silva et al., 2012; Khan and Tania, 2012).The purpose of this study was focus in determination phytochemical compounds, carbohydrates, soluble proteins, amino acids, lipids, flavonoids and phenolic compounds of *Pleurotus* spp. which cultivated on the three agro-wastes (Zea mays, Sorghum bicolor Horse and Sorghum bicolor Giza 15).

MATERIALS and METHODS

Screening of some phytochemical compounds

Preparation of extracts

The dried samples were weighed and ground into powder prior to extraction. Then it was subjected with solvents, maintained at room temperature for 24 hours, sequentially. For the preparation of the extract, 1g dried powdered oyster mushroom was extracted successively with 50 ml ethanol (95%) in conical flask and then the contents were placed on a shaker for 24 hours at room temperature. The residual solvents were removed by evaporation at 40- 50 ºC for 30 minutes. The extracts were stored at 4^{o}C in sterile capped bottle for phytochemical, detection (Oseni et al., 2012).

Qualitative determination of phytochemicals

Detection of alkaloids

About 50 mg of solvent free extract was stirred with 3ml of dilute hydrochloric acid and then filtrate was tested carefully with various alkaloids reagents as fallow; 1 ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube, the color changes were observed. A reddish brown precipitates confirm the test a positive (Wagner, 1993; Evans, 1997).

Detection of carbohydrates

One ml of extract was in addition to 1ml of Barfoed's reagent and heated on a boiling water bath for 2 minutes the color changes was noted and recorded. A red precipitate indicated presence of sugar. It is based on the reduction of copper acetate to copper oxide (Cu2O), which forms a brick-red precipitate (Barfoed, 1873).

Detection of Glycosides; Borntrager's test

First, 50 mg of the extract were hydrolyzed in hydrochloric acid conc. for 2 hr. Three ml of chloroform was added to 2 ml of oyster mushroom, extract and shaked, chloroform layer was separated, ammonia solution (10%) was added. The formation of pink color indicated the presence of glycosides (Evans, 1997).

Detection of phenols, Lead acetate test

The extract (50 mg) was dissolved in 5ml distilled water and 3 ml of 10% lead acetate solution was added, a bulky white precipitate indicated the presence of phenols (Lingaraoet al., 2012).

Detection of Tannins

The extract (50 mg) was dissolved in 3 ml of distilled water and few drops of neutral 5% ferric chloride solution were added, a dark green color indicated the presence of tannins (Mace Gorbach, 1963).

Detection of flavonoids

An aqueous solution of the extract was treated with sodium hydroxide solution the yellow flourescnce indicated the presence of flavonoid (Bello et al., 2011).

Detection of proteins

The extract (100 ml) in 10 ml distilled water, and filtered through filter paper. The filtrate (2ml) was treated with one drop of 2% copper sulphate solution and then added 1ml of ethanol (95%), followed by excess of potassium hydroxide pellets. The pink color appears in ethanol layer indicated presence of proteins (Gahan, 1984).

Detection of amino acids

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was in addition to 2ml of aqueous filtrate, a characteristic of purple color indicate of amino acids (Yasuma and Ichikawa, 1953).

Detection of terpenoids

2 ml of chloroform and concentrated sulphuric acid was added carefully to 0.5 ml of extract, formation of red brown color indicates the presence of terpenoids (Salkowski, 1904).

Quantitative determination of phytochemicals

Extraction (supernatant)

By the use of 1g of dry sample of mycelia or mushrooms were boiled in 10 ml distilled water for 1 hr. and then centrifuged. The supernatant was used as extract for carbohydrates, amino acids and proteins detection.

Quantification of carbohydrate

The anthrone-sulphuric acid method (Fales, 1951; Schlegel, 1956) was used for the determination of carbohydrates.
Anthrone- sulphuric acid reagent preparation; Anthrone 0.2 g, 30 ml distilled water, 8 ml absolute ethyl alcohol, and 100 ml concentrated H2SO4 (D=1.84) were respectively mixed in a conical flask under continuous cooling in an ice bath. This reagent should be always freshly prepared anthrone reagent. This was prepared by dissolving 0.2 g of anthrone in 100 ml of H2SO4, made by adding 500 ml of conc. acid to 200 ml of water. The reagent was permitted to stand for 30-40 min. with occasional shaking until it was perfectly clear (Trevelyan et al., 1950).

Quantification of free amino acids

Free amino acids were identified according to the method of (Moore and Stein, 1948). However, in this method traces of proline and hydroxyl proline were encountered.
Reagent preparation

This method makes up of the following reagents.
Ninhydrin reagent: 0.25 g ninhydrin dissolved in 100 ml ethanol.
Citrate buffer: 10.5 gm citric acid in 100 ml NaOH (2N) added drop wise to adjust pH 5.
Stannus Chloride reagent: 0.01g Stannus Chloride + 10 ml citrate buffer + 10 ml ninhydrin reagent.

Diluent solvent: Prepared by mixing equal volumes of distilled water and ethanol.

Estimation procedures

In clean empty test tube, add one ml of stannus chloride reagent to 0.5 ml of the extract. Boiling the test tubes in water bath for 20 min. and then cooling. Add 4 ml of diluent solvent and mixed rapidly. The extinction of violet color was recorded spectrophotometerically (Plant physiology laboratory) at wave length 570 nm against blank containing all the above reagents and distilled water instead of the extract of plant sample.
A calibration curve was constructed using glycine and the data were expressed as mg amino acid (glycine)/g D.W.

Determination of total lipids

Total lipids contents were determined by the sulfophovanilin method (SPV) (Drevon and Schmitt, 1964)

Reagents;
a- concentrated sulphoric acid (36N)
b- phoshovanilin reagents (phoshoric acid, vanilin and ethanol)
Procedure
About 0.1 ml from extract was transferred to dry glass tube and 3ml conc H2SO4 was added and left in boiling water bath for 10 minutes after hydrolysis, 1.5 ml phoshovanilin reagent was added to 0.05 ml of hydrolyast, mixed well and incubated at 37°C for 10 minutes. The absorbance of characteristic pale pink color was measured at 530 nm.

Antioxidative properties of the broth medium, mycelia and fresh fruiting bodies:

Preparation of the ethanolic extract

Ten 10 g of fresh fruiting bodies and 10 g of mycelia were washed with tap water, rinsed with distilled water, drained, and chopped. Each of the samples was blended with 100 ml absolute ethanol using a homogenizer for 10 minutes and then filtration of the homogenate was carried out. The supernatants were freeze-dried and stored at -20º C until which used as extract.

Determination of free phenolic content

Free phenolic were determined according to Kofalvi and Nassuth (1995). 100 µl of the ethanol extract was diluted to 1 ml water and mixed with 0.5 ml 2 N Folin-Ciocalteu's reagent and 1.5 ml of 20% Na_2CO_3. After 20 min at room temperature, absorbance of samples was measured at 765 nm spectrophotometer. Phenolic concentration in the extract was determined from standard curve prepared with gallic acid and the data expressed as mg/g FW.

Determination of the total flavonoids

Total flavonoids were determined using spectrophotometric method as previously described by Zou et al. (2004). One milliliter of sample extract was combined with 0.3ml of 5% sodium nitrite ($NaNO_2$). The resulting solution was vortex mixed and permitted to stand at room temperature for 6 min. To this, 0.3 ml of 10% $AlCl_3$was added. After 5 min, 2 ml of Na OH (1N) was added and shaken to react. This was diluted by topping up to 5 ml mark. Each experiment was made in triplicates. Absorbance was measured at 510 nm. Quercetin was used as standard curve and flavonoids content were expressed as (mg/ml).

Determination of ascorbic acid

The ascorbic acid was determined according to Mushroom tissues (0.2 g) were ground with liquid nitrogen and suspended in 2 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 15 min. at 4 °C. Add 0.2 ml of tissue homogenate to 0.8 ml of 10% TCA. After vigorous shaking the tubes were stored in an ice bath for 5 min. and centrifuged at 3000 rpm for another 5 min. 0.5 ml of the extract was diluted to 2.0 ml using bi-distilled water, and 0.2 ml of diluted Folin's reagent (diluted 10-folds with bi-distilled water) was in addition to the extract, the tubes were vigorously shaken. After 10 min. the absorbance of the blue color developed was measured spectrophotometer at 760 nm. A standard curve was established by different concentrations of ascorbic acid (Jagota and Dani, 1982).

Statistical analysis

The data were statistically analyzed following the Randomized Complete Block Design (RCBD) with the arrangement of three replications and means were compared following Duncan's Multiple Range Test (DMRT) test at a 5% level of probability for interpretation of results (Gomez and Gomez, 1984).

RESULTS and DISCUSSION

The results in table (1) reveal that the highest carbohydrates content (24.6 ±0.05 mg/g) was obtained from filtrate on liquid medium cultivated with *P. ostreatus* on Zea mays while the lowest content (21.2 ±0.01mg/g) was obtained from *P. columbinus* on sorghum bicolor Giza 15. While, the highest carbohydrates content (35.5±0.02 mg/g) was estimated from ethanolic extract of mycelia on liquid medium of *P. floridanus* cultivated on Zea mays. On the other hand, the highest carbohydrates content (34.9±0.02mg/g) from ethanolic extract of fresh fruiting bodies was obtained from *P. sajor-caju* cultivated on Zea mays. These results are agreeing with (Egwin et al., 2011; Okwulehie et al., 2008).

On the other hand, the results in the current study were higher as compared to previous studies (Anjana et al., 2016). They recorded that, carbohydrate content in the *Pleurotus ostreatus* was 7.594 ± 0.59 mg/ml. On contrast, Alam et al. (2008) showed that the carbohydrate content of *P. florida*, *P. ostreatus*, and *P. sajor caju* were found to be 42.83, 37.8 and 39.82 g/100g respectively. Also, Dundar et al., 2008 reported that carbohydrate content of *P. eryngii*, *P. ostreatus* and *P. sajor-caju* were 39.85, 37.87 and 37.72 g/100g respectively. Similarly, the results of (Vishwakarma et al., 2017).

All, these results of carbohydrate content were higher than the results in the current study. However, it was observed that during this study, ethanolic extract from mycelia and fresh fruiting bodies have higher carbohydrate contents than filtrate from liquid medium for all five *Pleurotus* spp. cultivated on the three agro-wastes. Although, Okwulehie et al. (2008) reported high crude protein and carbohydrate contents in *P. ostreatus* cultivated on different substrates.

Table 1. Determination of total carbohydrates of filtrate from liquid medium, ethanolic extract from mycelia cultivated on liquid medium and ethanolic extracte from fruiting bodies

Type of mushroom / Type of waste		Filtrate from liquid medium (mg/g)	Ethanolic extracted from mycelia (mg/g)	Ethanolic extracted from fresh fruiting bodies (mg/g)
P. ostreatus	**Zea Mays**	**24.6 ±0.05**	33.7 ±0.05	31.7 ±0.03
	Sorghum bicolor Horse	22.7 ±0.06	32.5 ±0.06	32.6 ±0.01
	Sorghum bicolor Giza 15	23.6 ±0.08	30.2 ±0.03	32.4 ±0.04
P. columbinus	**Zea Mays**	**21.4 ±0.02**	28.4 ±0.04	33.8 ±0.05
	Sorghum bicolor Horse	22.6 ±0.03	33.2 ±0.02	34.3±0.03
	Sorghum bicolor Giza 15	21.2 ±0.01	29.3 ±0.03	31.3 ±0.02
P. pulmonarius	**Zea Mays**	22.5 ±0.05	28.7 ±0.02	32.8 ±0.01
	Sorghum bicolor Horse	23.8 ±0.04	29.3 ±0.01	33.4 ±0.01
	Sorghum bicolor Giza 15	24.9 ±0.03	28.9 ±0.04	31.4 ±0.03
P. sajor-caju	**Zea Mays**	21.9 ±0.01	29.7 ±0.03	34.9±0.02
	Sorghum bicolor Horse	21.8 ±0.02	32.6 ±0.02	**33.9 ±0.04**
	Sorghum bicolor Giza 15	22.6 ±0.03	32.5 ±0.01	29.5 ±0.03
P. floridanus	**Zea Mays**	23.3 ±0.04	35.5 ±0.02	32.5 ±0.04
	Sorghum bicolor Horse	23.7±0.03	34.9 ±0.03	31.7±0.05
	Sorghum bicolor Giza 15	**24.4 ±0.02**	**35.3±0.05**	33.2 ±0.02

Soluble protein

The results obtained in table (2) indicate that the highest amount of soluble protein determined from filtrate on liquid medium was found to be (18.8±0.05mg/g) of *P. ostreatus* cultivated on Zea mays. While, the highest amount of soluble protein obtained from dried mycelium cultivated on liquid medium was found to be (25.4 ±0.04mg/g) from *P. columbinus* cultivated on Zea mays. But, in case of dried fruiting bodies, the highest amount (28.8 ±0.02 mg/g) of soluble protein was obtained from *P. sajor-caju* cultivated on Zea mays. The lowest amount of soluble protein content was found to be (14.2 ±0.02 mg/g) obtained from filtrate on liquid medium for *P. floridanus* cultivated on sorghum bicolor Giza15. Li et al. (2017) reported protein levels of 27.4–34.8% from *P. sajorcaju* fruit bodies grown on wheat straw supplemented with raw/detoxified mahua cake (Gupta et al., 2013), the values obtained in Jin et al. (2018) are comparable to the reported values of 20.5–26.1% protein from *P. ostreatus* cultivated on cotton seed hull mixed with perilla stalk. According to Koutrotsios et al. (2014) the crude protein content was highly variable (14.64–31.36%) for *P. ostreatus*, which was produced on nine cultivation substrates. Gupta et al. (2013) reported that the protein content of mushrooms varies with the type of substrate, as a result of the differences in nutrient supply.

Table 2. Determination of soluble protein from filtrate on liquid medium, dried mycelium and dried fruiting bodies (mg/g)

Type of mushroom / Type of waste		Filtrate from liquid medium (mg/g)	Ethanolic extracted from mycelia (mg/g)	Dried fruiting bodies. mg/g)
P. ostreatus	**Z M**	**18.8 ±0.05**	24.3 ±0.05	28.6 ±0.03
	S H	17.9 ±0.06	27.1 ±0.06	26.5 ±0.01
	S G	16.9 ±0.08	25.2 ±0.03	27.4 ±0.04
P. columbinus	**ZM**	**18.4 ±0.02**	25.4 ±0.04	25.8 ±0.05
	S H	19.3 ±0.03	24.2 ±0.02	27.3±0.03
	S G	18.6 ±0.01	25.3 ±0.03	25.3 ±0.02
P. pulmonarius	**Z M**	17.5 ±0.05	22.7 ±0.02	26.8 ±0.01
	S H	16.8 ±0.04	21.3 ±0.01	27.4 ±0.01
	S G	17.9 ±0.03	24.9 ±0.04	28.1 ±0.03
P. sajor-caju	**Z M**	18.9 ±0.01	25.7 ±0.03	28.8±0.02
	S H	17.8 ±0.02	24.6 ±0.02	**27.9 ±0.04**
	S G	16.6 ±0.03	25.5 ±0.01	28.5 ±0.03
P. floridanus	**Z M**	17.3 ±0.04	21.5 ±0.02	27.5 ±0.04
	S H	18.7±0.03	23.9 ±0.03	27.6±0.05
	S G	**14.4 ±0.02**	**21.3±0.05**	26.2 ±0.02

Data illustrated in table (3) show that the highest content of amino acids (22.2 ±0.04 mg/g) from filtrate of liquid medium was obtained from *P. floridanus* cultivated on the Zea mays. In case of dried mycelium of liquid medium the highest content of amino acids (22.8 ±0.09 mg/g) was obtained from *P. columbinus* cultivated on Zea mays bagasse. Also, the highest content of amino acids (22.9 ±0.11 mg/g) from dried fruiting bodies was estimated from *P. floridanus* cultivated on Zea mays. The lowest amount of amino acids content was found to be (18.6 ±0.02 mg/g) obtained from filtrate on liquid medium from *P. pulmonarius* cultivated on Sorghum bicolor Giza15. It was observed that all extracts obtained from filtrates of liquid medium, dried mycelia and dried mushroom, for all the five *Pleurotus* spp. were grown on the three agro-wastes in this study have high amino acids contents. While, carbohydrates content was higher than amino acid content (mg/g) of the mushrooms. Chirinang and Intarapichet, (2009) found that, both mushrooms had comparable amounts of total amino acids (21.11 mg/g and 20.12 mg/g) of fresh weight, for *P. ostreatus* and *P. sajor-caju*, respectively. Similar results were obtained by Mattila et al., (2002) and Mendez et al., (2005) for *P. ostreatus*. These results were lower than that of the results in the current study.

Table 3. Determination of amino acids of filtrate from liquid medium, dried mycelia and dried fruiting bodies.

Type of mushroom / Type of waste		Filtrate from liquid medium (mg/g)	Dried mycelia (mg/g)	Dried fruiting bodies (mg/g)
P. ostreatus	**Z M**	19.6 ±0.05	21.6 ±0.12	21.8 ±0.08
	S H	20.7 ±0.03	20.9 ±0.23	**21.7 ±0.12**
	S G	**18.8 ±0.06**	21. 9±0.12	20.7 ±0.17
P. columbinus	**ZM**	**19.6 ±0.07**	20.8 ±0.07	21.4 ±0.18
	S H	18.6 ±0.06	22.8 ±0.09	20.6 ±0.08
	S G	19.6 ±0.03	20.9 ±0.14	21.7 ±0.07
P. pulmonarius	**Z M**	18.6±0.02	21.2 ±0.17	20.8 ±0.15
	S H	19.3 ±0.04	21.5±0.18	21.6±0.11
	S G	20.7 ±0.05	20.2 ±0.12	**20.3 ±0.10**
P. sajor-caju	**Z M**	20.8 ±0.03	20.8±0.023	21.7±0.08
	S H	19.5 ±0.02	22.2 ±0.27	216 ±0.07
	S G	20.5 ±0.01	**20.9 ±0.34**	21.6±0.1
P. floridanus	**Z M**	22.2 ±0.04	22.2 ±0.26	22.9 ±0.11
	S H	19.4 ±0.06	**22.7 ±0.08**	**22.7 ±0.07**
	S G	21.3 ±0.05	22.3 ±0.23	22.4 ±0.09

Table (4) showed that the highest content of lipids from filtrate on liquid medium was found to be (2.9 ±0.02 mg/g) of *P. ostreatus* cultivated on Zea mays. In case of dried mycelia on liquid medium, the highest content of lipids (2.80±0.02 mg/g) was obtained from *P. ostreatus* cultivated on Zea mays. Also, the highest content of lipids (2.7 ±0.03 mg/g) from dried fruiting bodies was estimated from *P. columbinus* cultivated on Sorghum bicolor Horse. The lowest amount of amino acids content was found to be (2.4±0.05mg/g) which obtained from filtrate on liquid medium of *P. columbinus* cultivated on Sorghum bicolor Horse. While, these results were lower than that of the study of Anjana et al. (2016) who reported that the content of lipid extracted of wild edible Nigerian species *Pleurotus ostreatus* was (4.89 ± 0.13%). Also, the lipid content in different species of *Pleurotus* species variably ranges from 0.2 to 8 g per 100 g dried fruit bodies, which have been reported from different studies (Hossain et al., 2007). However, Naraian and Dixit, (2017) found that the analyses showed maximum level of lipid (0.61g) in the fruiting of *Pleurotus sajor-caju* mushroom. Also, the results of Vishwakarma et al. (2017) were found to be (0.42±0.05 - 0.65±0.08%) of lipid content. In a similar observation Alam et al., (2008) determine the lipid content of *P. florida, P. ostreatus* and *P. sajor caju*, were found it to be 0.54, 0.68, 0.57 g/100g, respectively. These findings were lower than that of our study.

Table 4. Determination of lipids of filtrate on liquid medium, dried mycelia and dried fruiting bodies.

Type of mushroom / Type of waste		Filtrate from liquid medium (mg/g)	Dried mycelia (mg/g)	Dried fruiting bodies (mg/g)
P. ostreatus	**Z M**	2.9±.02	2.8±0.02	2.6 ±0.02
	S H	2.6 ±0.02	2.7 ±0.03	**2.5 ±0.05**
	S G	2.7 ±0.02	2.8 ±0.01	2.5 ±0.04
P. columbinus	**ZM**	2.6 ±0.02	2.5 ±0.04	2.4 ±0.03
	S H	2.8 ±0.03	2.4 ±0.01	**2.5 ±0.02**
	S G	2.7 ±0.02	2.5 ±0.02	2.4 ±0.05
P. pulmonarius	**Z M**	2.6 ±0.02	2.6 ±0.03	2.5 ±0.06
	S H	2.5 ±0.02	**2.7 ±0.01**	2.7 ±0.04
	S G	2.7 ±0.01	**2.8 ±0.03**	2.5 ±0.03
P. sajor-caju	**Z M**	2.6 ±0.02	2.6 ±0.02	2.5 ±0.04
	S H	2.7 ±0.02	**2.5 ±0.03**	2.6 ±0.02
	S G	2.6±0.02	2.5 ±0.04	2.5 ±0.03
P. floridanus	**Z M**	2.4 ±0.02	2.7±0.02	2.4 ±0.04
	S H	2.5 ±0.01	2.6 ±0.02	2.6 ±0.02
	S G	**2.7 ±0.02**	**2.6±0.02**	**2.7 ±0.03**

Data presented in Table (5) indicate that the highest flavonoid content (1.48 ±0.07mg/g) was obtained from filtrate in liquid medium of *P. columbinus* cultivated on Zea mays While, the highest flavonoid content (1.53 ±0.08 mg/g) was obtained from ethanolic extracte of mycelia from *P. pulmonarius* cultivated on Zea mays. On the other hand, the highest flavonoid content (1.62 ±0.04 mg/g) was estimated from ethanolic extracte of fresh fruiting bodies from *P. pulmonarius* cultivated on Zea mays. The lowest amount of flavonoid content was found to be (1.21±0.06mg/g) which obtained from filtrate of liquid medium of *P. floridanus* cultivated on Sorghum bicolor Giza 15.

Hamzah et al., (2014) reported that the high flavonoid content in *Pleurotus ostreatus* was found to be higher than that found in an edible mushroom (2.84±0.12mg/g). Obodai et al., (2014) recorded that the total flavonoid contents in the mushroom extracts varied from 0.20 to 2.03 µg of RE/g of dry weight of extracts with a grand mean of 0.85 µg of RE/g of dry weight of extracts.

Table 5. Determination of flavonoid from filtrate in liquid medium, ethanolic extract from mycelium and ethanolic extracted from fresh fruiting bodies.

Type of mushroom / Type of waste		Filtrate from liquid medium (mg/g)	Ethanolic extracte from mycelia (mg/g)	Ethanolic extracted from fresh fruiting bodies (mg/g)
P. ostreatus	**Z M**	1. 35±0.012	1.29±0.07	1.44 ±0.03
	S H	1.26 ±0.013	1.41 ±.09	1.42 ±0.04
	S G	1.37±0.024	1.32 ±0.03	1.53 ±0.02
P. columbinus	**ZM**	1.48 ±0.07	1.34 ±0.05	1.44 ±0.04
	S H	1.40 ±0.08	1.43 ±0.07	1.55 ±0.02
	S G	**1.41 ±0.04**	1.32 ±0.06	1.56 ±0.03
P. pulmonarius	**Z M**	1.39 ±0.12	1.53 ±0.08	1.62±0.04
	S H	1.23 ±0.09	1.44 ±0.02	1.47 ±0.05
	S G	1.36 ±0.07	1.54 ±0.05	1.55±0.07
P. sajor-caju	**Z M**	1.23 ±0.06	1.35 ±0.07	1.43 ±0.01
	S H	1.37±0.03	1.27 ±0.04	1.47±0.04
	S G	1.46 ±0.04	**1.48 ±0.05**	1.38 ±0.03
P. floridanus	**Z M**	1.45 ±0.06	1.38 ±0.04	**1.59 ±0.04**
	S H	1.41±0.07	1.46 ±0.02	1.55 ±0.02
	S G	1.21 ±0.06	**1.28±0.04**	1.64 ±0.03

Data recorded in table (6) clear that the highest ascorbic acid content from filtrate of liquid medium (0.080 ±0.05mg/g) was obtained from *P. ostreatus* cultivated on Zea mays. Also, the highest ascorbic acid content of ethanolic extract from mycelia (0.075±0.04mg/g) was obtained also from *P. columbinus* cultivated on Zea mays. On the other hand, the highest ascorbic acid content from ethanolic extracted of fresh fruiting bodies (0.085 ±0.02mg/g) was estimated from *P. floridanus* on Zea mays. The lowest amount of flavonoid content was found to be (1.21±0.06mg/g) obtained from filtrate of liquid medium from *P. sajor-caju* cultivated on Sorghum bicolor Giza 15. Matured fruit-bodies from *Andropogon* had higher, vitamins in mg/100g ascorbic acid (86.53 ± 6.72) and the matured fruit-bodies from Khaya was ascorbic acid (45.33 ± 2.31) (Okwulehie et al., 2014). However, Sharma and Gautam, (2015) recorded that the bioactive compounds evaluated are fatty acids, amino acids, tocopherol content, carotenoids (*β*-carotene, lycopene), flavonoids, ascorbic acid, and anthocyanidins.

Table 6. Determination of ascorbic acid of filtrate from liquid medium, ethanolic extract of mycelia and ethanolic extracted of fresh fruiting bodies.

Type of mushroom / Type of waste		Filtrate from liquid medium (mg/g)	Ethanolic extract from mycelia (mg/g)	Ethanolic extracted from fresh fruiting bodies (mg/g)
P. ostreatus	**Z M**	0.080 ±0.05	0.060 ±0.01	**0.050 ±0.02**
	S H	0.070 ±0.03	0.050 ±0.02	0.060 ±0.04
	S G	0.060 ±0.03	0.065 ±0.03	**0.080 ±0.06**
P. columbinus	**ZM**	0.055±0.02	0.075 ±0.04	0.025 ±0.02
	S H	0.070 ±0.01	0.070 ±0.03	0.030 ±0.01
	S G	0.060 ±0.02	0.080 ±0.02	0.040 ±0.03
P. pulmonarius	**Z M**	0.070 ±0.03	0.060 ±0.01	0.055 ±0.05
	S H	0.060 ±0.01	0.050 ±0.04	0.068±0.01
	S G	0.050 ±0.04	0.060 ±0.04	0.043±0.04
P. sajor-caju	**Z M**	0.070 ±0.05	**0.050 ±0.05**	0.050 ±0.03
	S H	**0.078 ±0.02**	**0.072 ±0.02**	0.060 ±0.01
	S G	0.060 ±0.03	0.050 ±0.02	0.052 ±0.04
P. floridanus	**Z M**	0.070 ±0.01	0.060 ±0.04	0.085 ±0.02
	S H	0.070 ±0.03	0.050 ±0.02	**0.098 ±0.01**
	S G	0.060±0.01	0.060 ±0.01	0.066 ±0.02

Data in Table (7) showed that the highest amount was found to be (2.60±0.06 mg/g) of phenolic compounds that determined from filtrate on liquid medium of *P. floridanus* cultivated on Zea mays. The highest phenolic content was found to be (2.7 ±0.04mg/g) from ethanolic extract of mycelia of *P. ostreatus* cultivated on Zea mays. On the other hand, the highest phenolic compounds content was found to be (2.70 ±0.04 mg/g) obtained from ethanolic extracted of fresh fruiting bodies of *P. pulmonarius* cultivated on Zea mays. The lowest content (2.11±0.04 mg/g) of phenolic compound was obtained from ethanolic extract from mycelia for *P. columbinus* cultivated on Sorghum bicolor Giza15. Although, Zea Mays mushrooms are very rich in phenolic compounds which are the source of their major antioxidant machineries. While, Kim et al., (2008) showed that the total phenolic content for both *Pleurotus eryngii* and *Pleurotus ostreatus* were 0.03 mg/g and 0.09 mg/g of dry weight, which are lower than the values for the *Pleurotus* spp. in the present study. Also, the results observed by Jayakumar et al., (2009) for *Pleurotus ostreatus* gave 0.71 mg/g of dry weight. In another study, Shirmila and Radhamany, (2013) reported that the total phenolic contents (5.5 mg/g) which was higher than that of the present study. The antioxidant characteristics of certain species result from a higher presence of phenolic compounds, β-carotene, lycopene, ascorbic acid, anthocyanidins, and tocopherol content within them (Sharma and Gautam, 2015).

Table 7. Determination of phenolic compounds of filtrate from liquid medium, ethanolic extract from mycelium and ethanolic extracted from fresh fruiting bodies.

Type of mushroom / Type of waste		Filtrate from liquid medium (mg/g)	Ethanolic extract from mycelia (mg/g)	Ethanolic extracted from fresh fruiting bodies (mg/g)
P. ostreatus	**Z M**	2.3 ±0.1	2.7 ±0.04	2.5 ±0.04
	S H	2.4 ±0.2	**2.4 ±0.01**	2.4 ±0.03
	S G	2.5 ±0.09	2.2 ±0.03	2.6 ±0.03
P. columbinus	**ZM**	2.2 ±0.08	2.2 ±0.02	2.5 ±0.02
	S H	2.4 ±0.03	2.2 ±0.01	2.4 ±0.05
	S G	2.3 ±00.3	2.5 ±0.03	2.6 ±0.04
P. pulmonarius	**Z M**	**2.2 ±0.01**	2.4 ±0.02	2.7 ±0.04
	S H	2.8 ±0.02	2.5 ±0.04	2.5 ±0.04
	S G	2.5±0.04	2.3 ±0.03	**2.6 ±0.05**
P. sajor-caju	**Z M**	2.2 ±0.16	2.4 ±0.04	2.6 ±0.03
	S H	2.3 ±0.04	2.6 ±0.02	2.5 ±0.04
	S G	2.5 ±0.06	2.3 ±0.01	2.5 ±0.05
P. floridanus	**Z M**	2.6 ±0.16	2.5 ±0.03	2.4 ±0.02
	S H	2.6 ±0.02	**2.4±0.04**	2.5 ±0.03
	S G	**2.5 ±0.05**	2.3 ±0.05	2.3 ±0.04

In general, during this study, it was observed that, ethanolic extract from mycelia and fresh or dry fruiting bodies have higher phytochemical compounds than filtrate from liquid medium for all five *Pleurotus* spp. cultivated on the three agro- wastes. T

hese results are agreeing with the results of Morris et al., (2017) who reported that, fruiting body and mycelia for *Pleurotus* spp. contain secondary metabolites and their isolated showed strong versatile health-promoting and therapeutic effects. In addition, It was observed that the submerged liquid fermentation in liquid medium (SmF) was suitable for the growth of all *pleurotus* species, (*P. ostreatus, P. columbinus, P. pulmonarius, P. sajor-caju* and *P. floridans*) when, cultivated on each of the agro-wastes used in this study (Zea mays, Sorghum bicolor H0rse and Sorghum bicolor Giza15). Our results were in harmony with (Friel and McLoughlin, (2000) and Yang and Liau, (1998).

They found that submerged liquid fermentation (SLF) give rise to possibility of high mycelial production in a compact space and shorter time with lesser chances of contamination. On the contrary, other studies found that SSF is the best culture system to study differences between aerial hyphae and those that penetrate in the solid matrix (Hölker et al., 2005).

CONCLUSION

Data showed that carbohydrates, soluble protein, amino acids, lipids, ascorbic acid, phenolic compounds and ethanolic extract were obtained in a higher contents from *Pleurotus* spp. cultivated on Zea mays waste these indicted that Zea mays was the best waste than the other two which have been giving mushroom more quality and more rich from these compounds were tested in this article.

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