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Total Phenolics, Flavonoids, Tannin Contents and Antioxidant Properties of *Pleurotus ostreatus* Cultivated on Different Wastes and Sawdust

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Abstract: In this study, the usage possibilities of some agro-industrial wastes such as; peanut wastes, potatoes farm wastes, walnut and orange tree sawdust in *Pleurotus ostreatus* cultivation were investigated and total phenolic, flavonoid, condensed tannin content and antioxidant properties of these methanolic mushroom extracts were examined. For the determination of the total phenolic contents, the Folin-Ciocalteau procedure was used. The content of total flavonoid present in the methanolic extracts was measured using a spectrophotometric assay. Condensed tannins were determined according to the method by Julkunen-Tıtto. The antioxidant capacity was determined using ferric reducing antioxidant power (FRAP) and free radical scavenging activity of DPPH. The highest total phenolic content (2.672 ± 0.003 mg GAE/g) was found in mushroom cultivated on walnut sawdust. The highest condensed tannin (1.011 ± 0.088 CE mg/g) and ferric reducing antioxidant power (FRAP) (12.332 ± 0.017 µmol $FeSO4.7H_2O$/g) were observed in the same mushroom extract. The highest total flavonoid and free radical scavenging activity of DPPH were found in extract of mushroom cultivated on potatoes handle. Bioactive properties of *P. ostreatus* cultivated on walnut tree sawdust were generally exhibited remarkable results.

Keywords: Agro-Industrial Wastes, Antioxidant, Flavonoids, Phenolics, *Pleurotus ostreatus*

1. Introduction

As an edible white-rot fungus; *Pleurotus ostreatus* falls under the category of non- timber forest products (NTFP) and *Pleurotus* genus contains about 40 species [1]. Thanks to their enzyme systems; they can utilize lignocellulosic materials such as agricultural wastes [2]. *Pleurotus* mushroom is the third most cultivated edible mushroom worldwide after *Agaricus bisporus* [3]. Because of easy growing techniques and broad adaptability, *P. ostreatus* have an important role in using of recycling organic wastes [4]. Some industrial and agricultural wastes such as soybean, sorghum, peanut and wheat straw [5] leaves of hazelnut, waste paper [6] cotton straw, lentil straw, rice bran [7] etc. can be used as substrate for cultivation.

Mushrooms accumulate some metabolites such as terpenes and steroids, phenolic compounds, polyketides [8]. These metabolites influence odor, taste, appearance and oxidative stability of nutrients [9]. It was reported that some of them can have some of pharmacological and biochemical properties such as antioxidant, antimicrobial, antimutagenic, antithrombotic and anticarcinogenic activities [10-12]. These kind of natural

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bioactive compounds such as phenols and flavonoids become more important day to day since it has been announced carcinogenesis risk of synthetic antioxidants [13]. The phenolic configuration in mushrooms can be affected some factors such as composition of growth media for in vitro cultured species, mushroom strain/species, content of the substrates etc. [14]. Production of orange, peanut, walnut and potatoes are made in Turkey. Potatoes, orange, walnut and peanut production areas and amounts of production in 2015 are given Table 1 [15].

Table 1. Potatoes, orange, walnut and peanut production area and amount of production in 2015, Turkey

	Production area (decare)	Amount of production (ton)
Potatoes	1.540.801	4.763.060
Orange	542.984	1.816.798
Walnut	718.196	190.000
Peanut	377.729	147.537

In our country; sawdust of orange and walnut trees are generally used as firewood without re-cycled. Potatoes farm wastes and peanut wastes are used for fire, too. In this study, the possibility of using these wastes for *P. ostreatus* cultivation was investigated since this mushroom has high saprophytic ability and most of cellulosic wastes can grow its on [16].

The main objectives of the study were to investigate the usage possibilities of some agro-industrial wastes such as; peanut wastes, potatoes farm wastes, walnut and orange tree sawdust in *P. ostreatus* cultivation and to determinate the total phenolic, flavonoid, condensed tannin content and antioxidant properties of these mushrooms' methanolic extracts and compare them with each other.

2. Material and Methods

P. ostreatus spawn was purchased from a commercial firm located in Denizli province. Peanut wastes were obtained from one of peanut manufacturing in Osmaniye, orange tree sawdust from orange garden in Adana, potatoes farm wastes from a potatoes farm in Trabzon and walnut sawdust from workshop of Forest Industry Engineering, Karadeniz Technical University.

2.1. Mushroom cultivation

Peanut wastes, potatoes farm wastes, walnut and orange tree sawdust moistened with water until 70-80% and sterilized in an autoclave at 121˚C for 1.5 h. After cooling the substrates to 20˚C, they were placed in nylon bags of 1 kg and inoculated by spreading spawn on the surface of the substrate with a weight percentage of about 3% of the wet weight of compost. Substrate condition was carried out in four replications. Each nylon bags were inoculated in mushroom growing laboratory (at 15-25˚C, 70-80% relative humidity). Harvesting was started in fifth week and the fruit bodies' stipe and cap were calculated and weighed.

2.2. Yield and biological efficiency

Mushroom yield was calculated as total fresh weight of mushrooms obtained from 3 or 4 flushes in the harvest period [17]. Biological efficiencies were defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrates [18].

2.3. Preparation of the extract

Approximately 5 g of mushroom samples in were placed into a falcon tube 50 mL 99% with additional methanol. The mixture was stirred continuously with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for a total of 24 hours. Particles were removed using filter paper. The final volume of the solution was adjusted by the level of methanol.

2.4. Determination of polyphenolic contents

The polyphenolic contents of the methanolic samples were evaluated three different ways; total phenolic contents (TPC), total flavonoids (TF) and total tannin (TT).

For the determination of the total phenolic contents, the Folin-Ciocalteau procedure was employed and gallic acid was used as standard [19]. Shortly, 20 µL of various concentrations of gallic acid and samples, 400 µL of 0.5 N Folin-Ciocalteu reagent and 680 µL of distilled water were mixed and vortexed. After 3 min incubation, 400 µL of Na_2CO_3 (10%) solution was added and vortexed. Then the mixture was incubated for 2 h at 20 °C with interrupted shaking. Absorbance measurement was carried out at 760 nm at the end of the incubation period. A standard curve was prepared using gallic acid as a standard with different concentrations of gallic acid, and the results were expressed as mg (GAE) per g methanolic extracts.

The concentration of total flavonoid present in the methanolic extracts was measured using a spectrophotometric assay. Briefly, 0.5 mL samples, 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL of 1 M $NH_4.CH_3COO$ were added to a test tube and incubated at room temperature for 40 min. Then the absorbance was measured against a blank at 415 nm. Quercetin was used for the standard calibration curve. The total flavonoid concentration was expressed as mg of quercetin equivalents per g sample [20]

Condensed tannins were determined according to the method by Julkunen-Tıtto [21]. For each sample, various concentrations of 25 µL mushroom extracts were mixed with 750 µl of 4% vanillin (prepared with MeOH) and then 375 µL of concentrated HCl was added. The well-mixed solution was incubated at room temperature in darkness for 20 min. The absorbance against the blank read at 500 nm. (+)-Catechin was used to make the standard curve (0.05–1 mg/ml). The results were expressed as mg catechin equivalent to (CE)/g sample.

2.5. Determination of antioxidant capacity

The antioxidant capacity was determined using ferric reducing antioxidant power and free radical scavenging activity of DPPH•.

2.5.1. Ferric reducing antioxidant assay (FRAP)

FRAP assay was tested to determine the total antioxidant capacity of the samples. This method is based on the reduction of tripyridyltriazine complex (Fe (TPTZ) $^{3+}$) to blue colored $Fe(TPTZ)^{2+}$ by antioxidants in acidic medium [22]. The preparation of working FRAP reagent was carried out by mixing 25 mL of 0.3 M acetate buffer pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution. The reaction mixture consisting of 1mL of the sample and 3 mL of freshly prepared FRAP reagent was incubated at 37 °C for 4 min. Then, the absorbance was determined at 593 nm against blank prepared with distilled water. A calibration curve prepared with an aqueous solution of ferrous sulfate $FeSO_4.7H_2O$ in the range of 100-1000 µM was used. Trolox was also tested under the same conditions as a standard antioxidant compound. FRAP values were expressed in wet weight of the samples as µmol of ferrous equivalent Fe (II) per g sample.

2.5.2. Scavenging of free radical (DPPH) assay

The DPPH assay was applied [23] to determine the radical scavenging capacity of the methanolic extracts of the mushroom. The simple method is based on scavenging the DPPH radicals with an antioxidant substance of the investigated solution. For each sample, six different concentrations of 0.75 mL of the extracts of the samples were mixed with 0.75 mL of 0.1 mM of DPPH in methanol, and the absorbance was read at 517 nm. The values were expressed as SC_{50} (mg sample per mL), the concentration of the samples causing 50% scavenging DPPH radicals.

2.6. Statistical analysis

All assays were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 23.0). The obtained data were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

3. Results and Discussion

3.1. Yield and biological efficiency

P. ostreatus was cultivated on four different materials namely peanut wastes, potatoes farm wastes, walnut and orange tree sawdust. Yield (g/100g) and biological efficiency (%) are presented in Table 2.

Table 2. Yield (g/100g) and biological efficiency (%) of cultivated mushroom

Material	Yield (g/100g)	Biological Efficiency (%)
	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Potatoes farm wastes	11.4 ± 0.8[a]	40.8 ± 2.9[a]
Orange tree sawdust	16.9 ± 1.7[b]	60.1 ± 6.2[bc]
Walnut tree sawdust	18.4 ± 2.1[b]	65.2 ± 7.5[c]
Peanut wastes	14.7 ± 1.1[c]	52.4 ± 4.6[b]

[a] Means having the same superscript letter(s) are not significantly different ($p>0.05$) by Duncan's multiple range test.

Total yield (g/100g substrates) was calculated after harvest period and substrates, walnut tree sawdust produced highest yield (18.4 ± 2.1 g/100g substrates), whereas potatoes farm wastes produced the lowest (11.4 ± 0.8 g/100g substrates) and our results are comparable with other *P. ostreatus* cultivation studies with 2-41 g/100g substrates [24-26]. In previous studies, yield of *P. ostreatus* cultivated on different composts was reported from and biological efficiency was reported from 0-61% [27] to 48.9- 90.5% [28]. Differences of yield and biological efficiency can be results of different compost components [27, 29]. All of materials used in this study can be evaluated for *P. ostreatus* cultivation.

3.2. Polyphenolic contents

The total polyphenols (mg GAE/g), total flavonoids (mg QE/g) and condensed tannin contents (CE mg/g) of *P. ostreatus* cultivated on different medium are presented in Table 3.

In this study, the highest total phenolic content (2.672 ± 0.003 mg GAE/g) was determined in mushroom cultivated on walnut sawdust and the lowest one (1.073 ± 0.028 mg GAE/g) in mushroom cultivated on peanut wastes. These values are higher than some vegetables consumed frequently in Turkey such as *Chicory* and *Lepidium sativum* (1.091 and 1.261 mg GAE/g, respectively) [30] and higher than other wild mushroom's content such as

Pleurotus eryngii (0.634 ± 0.004 mg GAE/g) and *Cyttaria gunnii* (0.761 ± 0.004 mg GAE/g) [31].

High level of phenolic compounds in mushrooms have been attributed to antioxidant activity and they were recorded as natural substrates of oxidative enzymes in the literature [32, 33].

Table 3. Total polyphenols (mg GAE/g), total flavonoids (mg QE/g) and condensed tannin contents (CE mg/g) of *P. ostreatus* cultivated on different medium

Mushroom	Total Polyphenols (mg GAE/g)	Total Flavonoid (mg QE/g)	Condensed Tannin (CE mg/g)
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
P. ostreatus cultivated on potatoes farm wastes	1.389 ± 0.007[a]	0.134 ± 0.001[a]	0.694 ± 0.004[a]
P. ostreatus cultivated on orange tree sawdust	1.777 ± 0.024[b]	-	0.422 ± 0.018[b]
P. ostreatus cultivated on walnut tree sawdust	2.672 ± 0.003[c]	0.130 ± 0.006[a]	1.011 ± 0.088[c]
P. ostreatus cultivated on peanut wastes	1.073 ± 0.028[d]	-	0.447 ± 0.003[b]

[a] Means having the same superscript letter(s) are not significantly different (p>0.05) by Duncan's multiple range test.

The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The arrangement of hydroxyl groups and the other features characteristic in the chemical configuration of flavonoids are important for their free radical scavenging activities and antioxidant properties [34]. In this study, the highest total flavonoid content (0.134 ± 0.001 mg QE/g) was found in mushroom obtained from potatoes farm wastes and the lowest one (0.130 ± 0.006 mg QE/g) from walnut sawdust. These values are lower than some wild mushrooms such as *Pleurotus florida* (0.17 ± 0.02 mg QE/g), *Flammulina velutipes* (0.20 ± 0.05 mg QE/g) [35]. The flavonoid content in mushroom cultivated on peanut wastes and orange tree wastes couldn't determine.

As it is known that tannins are polyphenolic compounds responsible for several bioactivities such as antitumor, antimicrobial and antioxidative activities [36]. In this study; the highest condensed tannin content (1.011 ± 0.088 CE mg/g) was seen in mushroom cultivated on walnut sawdust. The lowest condensed tannin content was observed (0.422 ± 0.018 CE mg/g) in mushroom cultivated on orange tree wastes. Our values are higher than some reported wild mushrooms such as *Lentinus ciliatus* (0.343 ± 0.030 CE mg/g), *Schizophyllum commune* (0.280 ± 0.024 CE mg/g), *Hygrocybe conica* (0.251 ± 0.011 CE mg/g) and *Pleurotus ostreatus* (cultivated) (0.326 ± 0.025 CE mg/g) [37]. The antioxidant activity of *P. ostreatus* cultivated on different medium is presented in Table 4.

Table 4. The antioxidant activity of P. *ostreatus* cultivated on different medium

Mushroom	FRAP (μmol $FeSO_4.7H_2O$/g)	DPPH-SC_{50} (mg/mL)
	$\bar{X}$ ± SD	$\bar{X}$ ± SD
P. ostreatus cultivated on potatoes farm wastes	4.826 ± 0.001[a]	15.473 ± 0.001[a]
P. ostreatus cultivated on orange tree sawdust	6.976 ± 0.012[b]	7.641 ± 0.499[b]
P. ostreatus cultivated on walnut tree sawdust	12.332 ± 0.017[c]	4.937 ± 0.001[c]
P. ostreatus cultivated on peanut wastes	4.096 ± 0.037[d]	8.596 ± 0.002[d]

[a] Means having the same superscript letter(s) are not significantly different (p>0.05) by Duncan's multiple range test.

The FRAP assay actually measures the ability of antioxidants to reduce ferric iron [38]. According to the Table 4; the highest FRAP activity (12.332 ± 0.017 µmol $FeSO_4.7H_2O$/g) was found in mushroom cultivated on walnut sawdust and the lowest (4.826 ± 0.001 µmol $FeSO_4.7H_2O$/g) one in mushroom cultivated on potatoes farm wastes. The FRAP activities of *P. ostreatus* was expressed as 2.385,71(µmol/g) by Keleş et al [39].

DPPH method characterizes the antioxidant capacity of extracts against oxidation caused by free radicals. [40]. In this study, the highest DPPH activity (15.473 ± 0.001 mg/mL) was found in mushroom obtained from potatoes farm wastes. The lowest one (4.937 ± 0.001mg/mL) obtained from walnut sawdust. DPPH activity of studied mushrooms' methanolic extracts are is generally higher than *Lactarius deterrimus* (5.85 ± 0.51 mg/mL) and lower than *Boletus edulis* (21.90 ± 0.92 mg/mL) and *Xerocomus chrysenteron* (27.42 ± 1.23 mg/mL) [41].

4. Conclusion

In this study the usage possibilities of some agro-industrial wastes such as; peanut wastes, potatoes farm wastes, walnut and orange tree sawdust in *Pleurotus ostretatus* cultivation were investigated and the total phenolic, flavonoid, condensed tannin contents and antioxidant properties of these mushrooms' methanolic extracts were examined.

The results of this study indicated that peanut wastes, potatoes handle, walnut sawdust and orange tree sawdust can be used as substrate for *Pleurotus ostreatus* cultivation. In many respects; bioactive properties of *P. ostreatus* cultivated on especially walnut tree sawdust were generally exhibited remarkable results (the highest yield and biological efficiency, the highest total phenolic content, the highest condensed tannin and the highest FRAP activity) compared to the bioactive properties of mushrooms cultivated on the other wastes types. So; walnut tree sawdust and its habitat can be investigated by further analysis. On the other hand; it has not been determined flavonoid content in mushroom cultivated on peanut wastes and orange tree wastes.

Total phenolic, flavonoid and antioxidant properties of mushrooms cultivated on different medium were found significantly different ($p<0.05$) by Duncan's multiple range test. Bioactive properties of mushrooms highly depend on mushroom species, growing conditions, extraction process and substrate medium. To obtain better results from mushroom extracts, different substrate types and different extraction methods can be tested.

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