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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Do Different Substrates Affect Antioxidant Properties and Antimicrobial Activity of *Pleurotus ostreatus*? [*]

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Abstract: The aim of this study is to determine the effect of growing substrates on the antioxidant properties and antimicrobial activities of *Pleurotus ostreatus* (Jacq.) P. Kumm. In the study, growing substrates were prepared with five different tree sawdust (80%) and waste (20% Tea Waste or Wheat Bran) at different ratios (80% Sawdust + 20% TW, 80% Sawdust + 20% GW). The antimicrobial activities of methanol extracts from *P. ostreatus* grown on 19 different substrates were tested against microorganisms by agar well diffusion technique. The total phenolic content was measured by using the Folin-Ciocalteau procedure. The antioxidant capacity was determined by using ferric reducing antioxidant power (FRAP) and the free radical scavenging activity of DPPH. It was determined that the methanol extract of mushrooms obtained from the 80QS+20TW substrate was the most effective extract against all of the microorganisms investigated in this study. Significant differences ($P<0.01$) were found among extracts of mushrooms grown on different substrates. Total phenolic, FRAP and DPPH assay contents of methanol extracts from *P. ostreatus* varied between 1.016 to 4.772 mg GAE/g, 2.245 to 8.902 µmol $FeSO_4.7H_2O$/g, 4.650 to 22.922 mg/mL, respectively. The results of this study revealed that the substrate content affects the antioxidant properties and antimicrobial activities of *P. ostreatus*. In addition, it was observed that tea waste has a positive effect on antimicrobial activity when added to substrate.

Keywords: Antimicrobial, antioxidant, *Pleurotus ostreatus*, total phenolic.

Farklı Substratlar, *Pleurotus ostreatus*'un Antioksidan Özelliklerini ve Antimikrobiyal Aktivitesini Etkiler mi?

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Öz Bu çalışmanın amacı, yetiştirme substratlarının *Pleurotus ostreatus* (Jacq.) P. Kumm.'un antioksidan özellikleri ve antimikrobiyal aktiviteleri üzerindeki etkisini belirlemektir. Çalışmada beş farklı talaş türü (%80) ve atık (%20 Çay Atığı veya Buğday Kepeği) ile farklı oranlarda (%80 Talaş + %20 Çay atığı, %80 Talaş + 20% Buğday kepeği) yetiştirme substratları hazırlanmıştır. 19 farklı substrat üzerinde büyütülen *P. ostreatus*'tan elde edilen metanol ekstraktlarının antimikrobiyal aktiviteleri, agar kuyucuk difüzyon tekniği ile mikroorganizmalara karşı test edildi. Toplam fenolik içerik, Folin-Ciocalteau prosedürü kullanılarak ölçülmüştür. Antioksidan kapasitesi, ferrik indirgeyici antioksidan gücü (FRAP) ve DPPH'nin serbest radikal süpürücü aktivitesi kullanılarak belirlendi. 80QS+20TW substratından elde edilen mantarların metanol ekstraktının, bu çalışmada incelenen tüm mikrooganizmalara karşı en etkili ekstrakt olduğu belirlendi. Farklı substratlar üzerinde yetiştirilen mantar ekstraktlarının antioksidan içerikleri arasında önemli farklılıklar ($P<0.01$) bulundu. *P. ostreatus*'tan elde edilen metanol ekstraktlarının toplam fenolik, FRAP ve DPPH tahlil içerikleri, sırasıyla 1.016 ile 4.772 mg GAE/g, 2.245 ile 8.902 µmol $FeSO_4.7H_2O$/g, 4.650 ile 22.922 mg/mL arasında değişmiştir. Bu çalışmanın sonuçları, substrat içeriğinin, *P. ostreatus*'un antioksidan özelliklerini ve antimikrobiyal aktivitelerini etkilediğini ortaya koydu. Ayrıca çay atığı substrata eklendiğinde antimikrobiyal aktivite üzerinde olumlu bir etkiye sahip olduğu gözlenmiştir.

Anahtar kelimeler: Antimikrobiyal, antioksidan, *Pleurotus ostreatus*, toplam fenolik.

[*] *This study was produced from* Sibel AVCI's *the master thesis.*

INTRODUCTION

In many parts of the world, people are struggling with hunger due to food starvation. On the other hand, more than half of the agricultural production, shell, cob, husk, straw, stalks, food industry wastes, etc. are thrown into the environment as waste material. Every year, 600 million tons of agricultural and forest industry waste are emerging in the world. When these wastes are reused for mushroom cultivation, approximately 360 million tons of mushroom can be produced. *Agaricus bisporus* (J.E. Lange) Imbach, *Pleurotus ostreatus* (Jacq.) P. Kumm. and *Lentinula edodes* (Berk.) Pegler have the highest share in species distribution in mushroom production (Eren & Pekşen, 2014). The diversity of the waste material used in the mushroom growing environment depends on the regions and countries. Growing substrates containing different materials affect not only the yield and quality of the mushroom but also the nutrient content (Ponmurugan et al., 2007). Due to its low calorie and fat level as well as its high protein, vitamin, chitin, and mineral content, it is reported to be healthy food (Akindahunsi & Oyetayo, 2006; Kalač, 2009; Sabaratnam et al., 2011). Agricultural (for example, wheat, rice, corn stalk, corn straw, banana straw, banana leaves, etc.) and industrial lignocellulosic wastes generated after the processing of plants grown for industrial purposes can be used as a substrate source for the cultivation of these mushrooms (Kumla et al., 2020). During the production of black tea, solid wastes consisting of garbage, fiber, and dust arise. The waste material rate in black tea should be around 3–5%. However, this rate exceeds 17% due to the fact that tea leaves are not collected in accordance with the standards and too much nitrogen fertilizer is applied to the soil (Kacar, 1987). For human health, antioxidants play an important role due to their ability to scavenge free radicals in the bodies (Arbaayah & UmiKalsom, 2013; Kumari & Atri, 2014). Mushrooms are also a important source of some secondary metabolites, including phenolic compounds, ergotionein, ascorbic acid, carotenoids, polyketides, terpenes, and steroids (García-Lafuente et al., 2011). Although studies on the nutritional and antioxidant properties of cultivated mushrooms have increased, there is still a lack of information about their chemical composition, which is necessary to evaluate their functional ingredient potential. The nutritional and chemical composition of mushrooms varies depending on species, edible wild mushroom ecosystems and soil, substrates used in cultivation and techniques of cultivation, fruiting conditions, and age of the fresh mushroom sample (Kalac & Svoboda 2001; Akyüz & Kırbağ, 2010; Yıldız et al., 2017; Sevindik et al., 2018). *Pleurotus* species are widely cultivated because they have short life cycles, their production is cheap and requires low technology, and their nutrient content has medicinal properties (Baysal et al., 2003). The main aim of this study is to determine the antioxidant properties and antimicrobial activities of *P. ostreatus* grown on substrates prepared with different tree sawdust (poplar, oak, beech, linden, and alder sawdust) and on five tree logs.

MATERIAL AND METHOD

Materials: *P. ostreatus* mycelia was obtained commercially from company (Denizli, Turkiye). The chemical substances and analytical solutions in experiments were purchased commercially. The tree logs (oak, beech, poplar, linden, and alder) and sawdusts used in the study were obtained from Rize Forest Management (Rize, Turkiye). Tea waste used as an additive material was obtained from the Tea Factory in Rize. Microorganisms used in antimicrobial activity were purchased commercially from Refik Saydam Institute of Hygiene and Public Health (Ankara, Turkiye).

Substrate preparation: In this study, sawdusts from different tree species (poplar (*Populus* L.), beech (*Fagus orientalis* L.), oak (*Quercus* sp. L.), linden (*Tilia rubra* subsp. *caucasica* (Rupr.) V. Engl.), and alder (*Alnus glutinosa* subsp. *barbata* (C.A. Mey.) Yalt.) were used as a basal component for substrate preparation. Tea waste and wheat bran were used as supplements in substrate preparation. For each sawdust of different tree species, 3 substrate formulations were evaluated: (1) (100%) sawdust, (2) 80% sawdust: 20% wheat bran (80S:20WB) and (3) 80% sawdust: 20% tea waste (80S:20TW). The tea wastes supplied from the tea production factory and formed after the tea production process contained dry straw and tea leaves fiber. After the soaking process, 1% gypsum was added to adjust the pH of the substrate. Homogeneous substrate mixtures were prepared by mixing component materials based on their dry weight (w/w). The mixtures were wetted for 48 h and the moisture content of substrates was adjusted to approximately 70%. The substrate mixtures were filled (1 kg wet weight) into the heat-resistant polypropylene bags. The substrates were sterilized in an autoclave at 121±1 °C for 1.5 hours and inoculated with 4% (w/w) mushroom spawn. Then, the bags were incubated in the dark at 25±2 ºC. When the substrates were completely colonized by mushroom mycelium, the bags were exposed to fluorescent light for a 12 h at 10-15 °C room temperature and 80-90% relative humidity for the formation of fruiting bodies in a controlled room according to Pekşen and Yakupoglu (2009).

Preparation of P. ostreatus extraction samples: The mushroom samples were dried at 40 °C for 3 days in an oven. Samples were grinded and sieved. Then, 25 mL of methanol was added on to 2.5 mg of powdered mushroom sample, and the mixture was shaken at 300 rpm on a shaker (Heidolph Promax 2020) at room temperature for 24 hours.

Methanol has a low boiling point (65 °C) and a polarity index of 6.6. We used methanol as an extraction material due to its high capacity to dissolve polar compounds. The extraction solutions was filtered three times before the antioxidant and antimicrobial activity (Sulistiany et al., 2016; Bakir et al., 2018; Bozdogan et al., 2018). The obtained filtrates were used to determine antioxidant and antimicrobial activity.

Antimicrobial activity of Pleurotus ostreatus methanol extracts: An agar well diffusion method was used to test antimicrobial activities of methanol extracts from *P. ostreatus* (Perez et al., 1990). Tested microorganisms were *Escherichia coli* (ATCC 25922), *Yersinia pseudotuberculosis* (ATCC911), *Pseudomonas aeruginosa* (ATCC 43288), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (Roma 702), *Mycobacterium smegmatis (*ATCC607) and yeasts; *Candida albicans* (ATCC 60193) and *Saccharomyces cerevisiae* (RSKK 251). Mueller Hinton agar (MHA) was used for bacteria, Potato Dextrose agar (PDA) for yeasts and Brain Heart Infusion agar (BHIA) for *M. smegmatis.* Each microorganism was suspended in Mueller-Hinton broth (Difco, Detroit, MI) and diluted approximately 10^6 colony forming unit (cfu) per ml. The microorganism suspensions were coated on the surface of MHA, PDA and BHI agar (Difco, Detriot, MI) plates and then dried. Each well was put in 50 µL of *P. ostreatus* methanol extract samples. The bacteria were incubated at 37±0.1 °C for 24 hours and yeast at 25±0.1 °C for 72 hours after the extract samples were placed in the agar well. Each test was performed in triplicate. Ampicillin (10 µg), fluconazole (5 µg) were used as the standard drug. Methanol (70%) was used as the control solvent.

Analyses of total phenolic content, FRAP and DPPH Activity;

Analyses of Total Phenolic Content: The total phenolic content (TPC) of the extracts was determined by the Folin–Ciocalteu method (Singleton et al., 1999; Doğan & Cemhan, 2021). TPC was calculated as gallic acid equivalents from the calibration curve of gallic acid standard solutions (1 – 0.03125 mg/mL) and expressed as mg of gallic acid equivalents (GAE) g of the samples. The analyses were carried out in triplicate, and the average value was calculated in each case.

Determination of Antioxidant Activity of Mushroom Extracts;

Determination Ferric reducing / antioxidant power (FRAP):The FRAP method is used to test the total antioxidant capacity of the extract 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 **(**Benzie & Strain, 1999**;** Earnshaw et al., 2012). The preparation of working FRAP reagent was carried out by mixing 25 mL of 300 mM 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 1 mL of the sample and 3 mL of freshly prepared FRAP reagent was incubated at 37 °C for 4 min. After incubation, the absorbance values of the samples were read in spectrophotometer at 593 nm wavelength. A calibration curve prepared with an aqueous solution of ferrous sulfate $FeSO_4.7H_2O$ in the range of 100-1000 µM was used. $FeSO_4.7H_2O$ was also tested under the same conditions as a standard antioxidant compound. FRAP values were expressed in weight of the samples as µmol of ferrous equivalent Fe (II) per g sample.

Free Radical-Scavenging Activity of DPPH: The DPPH assay was performed using the method described by Molyneux (Molyneux, 2004) to determine the radical scavenging capacity of the metholic extracts. This method is based on the sweeping of the DPPH radical by antioxidants due to a redox reaction. Briefly, 0.1 mM DPPH (750 µL) dissolved in methanol and mushroom extract sample (750 µL) were mixed equal volume in eppendorf. The resulting mixture values were then measured at 517 nm by spectrophotometer. The values were expressed as SC_{50} (mg/mL), and the concentration of the samples were found to cause 50% scavenging DPPH radicals. There is a reverse correlation between SC_{50} values and free radical scavenging activity.

Statistical analysis: The experiment was carried out in Completely Randomized Design with eight replicates for production and with three replicates for antimicrobial. The data obtained from the experiment were analyzed using the ver. SPSS 12.0 statistical program. The significant differences among the mean values were compared by DUNCAN multiple range test.

RESULTS AND DISCUSSION

In this study, the antioxidant and antimicrobial activities of mushroom extracts grown on different substrates were determined. The antibacterial and antioxidant activity results of methanol extracts of these mushroom grown on different substrates are presented in Tables 1-2. All the mushroom extracts (except for those linden and oak logs) revealed antimicrobial activity, showing different inhibition zones for each microorganism (Fig 1). Since *Pleurotus ostreatus* could not be grown on linden and oak logs, its antimicrobial and antioxidant activity data could not be obtained. However, *P. ostreatus* was grown on substrates containing linden and oak sawdust, and its data are in Tables 1–2. It was determined that the extract of mushrooms obtained from the 80OS+20TW substrate was the most effective antimicrobial agent against to all of bacteria and yeasts investigated. *M. smegmatis* and Gram-

positive bacteria are sensitive to mushroom extracts whereas Gram-negative bacteria are less sensitive. The wild mushroom extract is more effective against yeast while it is determined that it has lower effectiveness against other microorganisms (Table 1).

Table 1. Antimicrobial activities of methanol extracts of *P. ostreatus* grown on different substrates.

Extract samples	**Microrganisms and inhibition zone (mm)**								
	Gram Negative		**Gram Positive**				**No Gram**	**Yeast**	
	Ec	**Yp**	**Pa**	**Sa**	**Ef**	**Bc**	**Ms**	**Ca**	**Sc**
100PS (poplar)	-	6	9	6	8	6	16	-	8
80PS+20WB	6	6	8	6	8	8	10	-	6
80PS+20TW	-	7	9	7	7	6	14	-	-
100OS (oak)	-	10	10	6	6	6	14	-	-
80OS+20WB	-	7	8	6	8	6	14	-	8
80OS+20TW	10	7	12	10	10	10	10	-	10
100BS (beech)	6	6	6	6	6	10	15	-	7
80BS+20WB	-	7	8	-	6	6	12	-	6
80BS+20TW	6	6	8	6	7	8	12	-	7
100AS (alder)	-	8	9	6	7	6	14	-	-
80AS+20WB	-	7	9	6	-	6	14	-	8
80AS+20TW	-	7	6	6	6	7	16	-	6
100LS (linden)	-	-	8	8	7	6	16	-	-
80LS+20WB	-	7	9	6	10	7	16	-	8
80LS+20TW	6	6	6	6	6	6	14	-	8
Poplar Log	-	6	9	6	-	7	14	-	-
Beech Log	-	6	9	6	-	8	12	-	8
Alder Log	7	8	8	6	-	8	18	6	10
Wild mushroom	7	9	7	6	-	6	12	6	12
Met. cont.	-	-	-	-	-	-	-	12	10
Amp (10 µg/ml)	10	18	18	35	10	15	-	-	-
Flu (5 µg/ml)	-	-	-	-	-	-	-	25	>25

Ec*: Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bc: *Bacillus cereus* 702 Roma, Ms; *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251, Amp.: Ampisilin, Flu.: Fluconazole, (-): No activity, Met. cont.; Methanol control. (PS: Poplar sawdust, OS; Oak sawdust, BS; Beech sawdust, AS; Alder sawdust, LS; Linden sawdust, WB; wheat bran, TW; Tea waste).

Antibacterial properties of the *P. ostreatus* extract samples were examined, and it was found that the mushroom extracts cultivated in a bag medium had a larger range of activity and wider spectrum. It was observed that the antimicrobial activity of the *P. ostreatus* extracts increased, and the spectrum of action expanded with the addition of tea waste (20%) to oak sawdust. Tea (*Camellia sinensis* (L.) Kuntze) is rich in phenolic compounds such as catechins and theaflavins, and caffeine content, gallic acid, theobromine alkaloids (Serpen et al., 2012). Although the content of these compounds in tea wastes is low, it has been determined that they can contribute to antimicrobial activity when tea wastes are used in mushroom substrates. In the antimicrobial activity test, the tuberculosis agent *Mycobacterium smegmatis* was determined to be the most sensitive microorganism against *P. ostreatus* extracts (Table 1). All extracts tested had an inhibition zone of 10-18 mm in diameter against *M. smegmatis*, while it was determined that most effective the antimicrobial effect of the mushroom extract grown on the alder tree. It was determined that the extracts with the best anti-tuberculosis (16 mm) activity among the mushroom extracts grown in the plastic bag substrate were the 100PS, 80AS+20TW, 100LS, and 80LS+20WB groups, respectively. It was determined that mushroom extracts grown on different substrates mixtures were effective against Gram-positive bacteria (except in the 80AS+20WB group), and the most sensitive microorganism was *P. aeruginosa* (Table 1). It has been observed that the content of the growth medium in culturable mushrooms can change the antimicrobial activity. It greatly affects the nutritional value of the mushroom of the grown substrate (Sturion & Oettere, 1995).

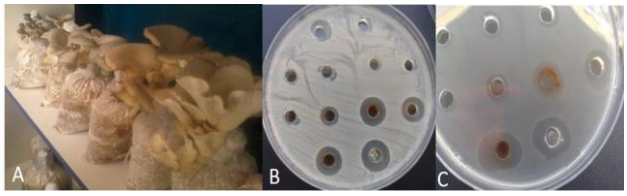

Figure 1. Pre-harvest view of *P. ostreatus* grown in plastic bags(a), and Antimicrobial activities of *P. ostreatus* methanol extracts grown on different substrates (b) *S. aureus* and (c) *E. faecalis.*

The antimicrobial potential of mushroom species depends on the origin of the samples, the extract method and type, the grown substrate, and the bacterial and fungal species investigated (Alves et al., 2012a; Harikrishnan et al., 2011; 2012). Methanol extracts of *Pleurotus* species grown on different substrates (Sorghum grain residue substrate and wheat grain substrate) were examined. According to the Gashaw et al. (2020) study, *P. ostreatus* extract has antimicrobial activity against *Pseudomonas aeruginosa* (16.4 mm zone diameter) and *E. coli* (19.8 mm zone diameter). In the same study, it was stated that *P. florida* extract formed 18.6 mm and 14.8 mm inhibition zones against *E. coli* and *Streptococcus faecalis*, respectively (Gashaw et al., 2020). Petroleum ether and acetone extracts

of *P. ostreatus* showed antimicrobial activity against *Staphylococcus* spp. (7.0–7.6 mm), *Bacillus* spp. (7.1–7.8 mm), *S. thyphi* (7.0–7.5 mm), *E. coli* (7.0–8.2 mm), *K. pneumoniae* (7.0–7.1 mm) ve *Candida* spp. (8.0–8.3 mm) (Akyuz & Kırbağ, 2009). In a study on the antibacterial activity of *P. ostreatus* (Demirhan et al., 2007), acetone extract did not show any effect against microorganisms but ethyl acetate extract showed antibacterial effect against *P. aeruginosa*, *E. coli*, and *S. aureus* (11.3 mm, 8.7 mm, and 10.0 mm in diameter, respectively). In addition, methanol extract of the *P. ostreatus* showed antimicrobial activity against *K. pneumonia* and *A. haemolyticus* (Yılmaz et al., 2016). Similar results were observed in our study. The crude extracts (hexane: chloroform) of three wild mushroom species (*Pleurotus sajor-caju*, *P. tuber-regium*, and *Lentinus squarrosulus*) collected from the forests of Cameroon were reported to have MIC values in the range from 6.25 mg/mL to 12.5 mg/mL against Gram-positive bacteria, and *M. smegmatis.* Four small Ramariolides A–D molecules isolated from *Ramaria cystidiophora* were tested in vitro antimicrobial activity against two *Mycobacterium* species. It was reported that purified Ramariolid A was effective against *M. smegmatis* and *M. tuberculosis* with MIC of 8 μg/mL and 64−128 μg/mL, respectively (Centko et al., 2012). In our study, it was observed that *P. ostreatus* methanol extracts grown on different substrates had high antimicrobial activity against *M. smegmatis* (10-16 mm). It was determined that the methanol extracts of *P. ostreatus* grown on different logs had a zone diameter of 12-18 mm against *M. smegmatis*, and the best antimycobacterial activity was found in those grown on alder logs. In addition, it is the first study to determine the antimycobacterial activities of methanol extracts of *P. ostreatus* grown on different substrates. These values, which emerged as a result of the various studies, are compatible with the fact that the extracts of *P. ostreatus* contain antimicrobial compounds against certain fungal and bacterial pathogens (Demirhan et al., 2007; Hearst et al., 2009; Gashaw et al., 2020).

Antioxidant Properties and Total Phenolic Contents: It was determined that presence of antioxidant activity in mushroom extracts showed statistically significant changes depending on the used cultivation substrates. The highest amount of total phenolic material (4.772 mgGAE/g) was detected in the mushroom extract grown on alder logs while the lowest amount (1.016 mg GAE/g) was determined for the mushroom extract grown on beech logs (Fig 2a). It was observed that the total amount of phenolic material was lowest in 100% sawdust substrat (except for OS group and 80LS + 20TW) when different substrate media (PS, OS, BS and AS groups) were evaluated among themselves. The total phenolic compounds in the BS group were found to increase the phenolic content by 50% in the addition of wheat bran (80BS+20WB; 4.309 mg GAE/g) and tea waste (80BS+20TW; 4.749 mg GAE/g). The content of total phenolic contents in the mushroom extract collected from the natural environment was measured to be 1.630 mg GAE / g, and it is thought that the amount of phenolic compounds of the mushroom extract may be related to the content of the region or the growth medium. *P. ostreatus* was grown on three different tropical trees (*Pycnanthus ongoleubis, Ceiba pentandra* and *Cananium* sp.) in a study by Oyetayo and Ariyo (2013). The total phenolic contents of *P. ostreatus* were determined to be 0.89-2.63 μg/g and the highest phenolic content was obtained when grown on *Pycnanthus ongoleubis*.

Table 2. Total phenolics, FRAP, and DPPH-SC_{50} values in methanol extracts of *P. ostreatus* grown on different substrates.

Mushroom substrate	Total polyphenol (mgGAE/g sample)	FRAP (μmol$FeSO_4.7H_2O$/g)	DPPH –SC_{50} (mg/mL)
PS (Poplar)	1.683±0.0031l**	3.995±0.005m**	19.007±0.002c**
80PS+20WB	2.246±0.0020h	6.200±0.026f	11.199±1.069f
80PS+20TW	1.833±0.0031k	4.371±0.005l	15.824±0.001d
OS (Oak)	2.050±0.0010i	2.674±0.004p	21.015±1.000b
80OS+20WB	1.845±0.0006k	2.653±0.001p	18.688±0.009c
80OS+20TW	0.839±0.0010n	3.375±0.005o	14.900±0.012e
BS	2.627±0.0006f	5.773±0.011g	7.7953±0.291h
80BS+20WB	4.309±0.0006b	8.902±0.009a	8.5960±0.001h
80BS+20TW	4.749±0.0010a	8.387±0.015c	8.0237±0.002h
AS (Alder)	2.178±0.0602ı	3.391±0.001o	22.922±0.002a
80AS+20WB	3.832±0.0020d	7.535±0.005e	8.369±0.001h
80AS+20TW	2.805±0.0025e	3.855±0.009n	10.370±0.010g
LS (Linden)	2.465±0.0010g	5.173±0.010h	5.509±0.003ı
80LS+20WB	3.922±0.1141c	7.654±0.004d	5.904±0.002ı
80LS+20TW	1.818±0.0010k	4.541±0.010k	9.997±0.001g
Poplar log	2.847±0.0015e	5.025±0.001ı	19.104±0.002c
Beech log	1.017±0.0015m	2.245±0.005r	14.627±0.562e
Alder log	4.770±0.0015a	8.770±0.010b	4.650±0.044i
Wild mushroom	1.630±0.0025l	4.733±0.011i	5.387±0.214ıi
			Troloks mg/mL 0.004±0.001

(PS: Poplar sawdust, WB: Wheat bran, TW: Tea waste, OS: Oak sawdust, BS: Beech AS: Alder sawdust, LS: Linden sawdust) There were no significant differences among means indicated with the same letters at P<0.01 level.

Ruiz-Rodriguez et al. (2014) reported that different growth media did not affect the amount of phenolic substances due to the absence of absorption of phenols by *P. ostreatus* species. The total phenolic content of *P. ostreatus* was reported to be the highest (2.672 ± 0.003 mg GAE/g) when grown in walnut tree sawdust and the lowest (1.073 ± 0.028 mg GAE/g) when grown in peanut waste (Yılmaz et al., 2017). In the study of Yılmaz et al. (2016), total phenolic compounds and antioxidant activities of *P. ostreatus* cultivated on lime leaves were studied. The total amount of phenolic compounds of 1.514 mgGAE/g and the amount of antioxidant compounds of 2.508 μmol FeSO4.7H2O/g were determined in the fungi. The FRAP values of the methanol extracts of the mushrooms were determined to be in the range of 2.245-8.902 μmol $FeSO_4.7H_2O$/g. When the methanol extract values of mushrooms grown on logs were compared, the highest FRAP value was found in alder (8,770 μmol$FeSO_4.7H_2O$/g), and the lowest value was found in beech mushroom (2.245 μmol $FeSO_4.7H_2O$/g). In a comparison of the methanol extract values of mushrooms grown on different substrates, the highest FRAP values were found at 80BS+20WB (8.902 μmol $FeSO_4.7H_2O$/g) and 80BS+20TW (8.387 μmol $FeSO_4.7H_2O$/g), while the

lowest values were found at 80OS+20WB (2.653 µmol $FeSO_4.7H_2O$/g) and OS (2.674 µmol $FeSO_4.7H_2O$/g) (Fig 2b, Table 2). It was observed that methanol extracts of mushrooms grown on different substrates had better DPPH-SC_{50} radical scavenging activity than methanol extracts of mushrooms grown on logs. The methanol extracts of mushrooms grown on LS and BS substrates were determined to have the best DPPH-SC_{50} radical scavenging activity (Fig 2c). In the substrate groups (only in PS, OS, and BS), it was observed that the DPPH-SC_{50} values were increased by adding wheat bran (WB) and tea waste (TW). DPPH-SC_{50} radical scavenging activity of values of the mushroom methanol extracts grown on alder logs (4.650 mg/mL), linden sawdust (5.509 mg/mL), and the wild-type *P. ostreatus* methanol extracts (5.387 mg/mL) was measured similarly (Table 2).

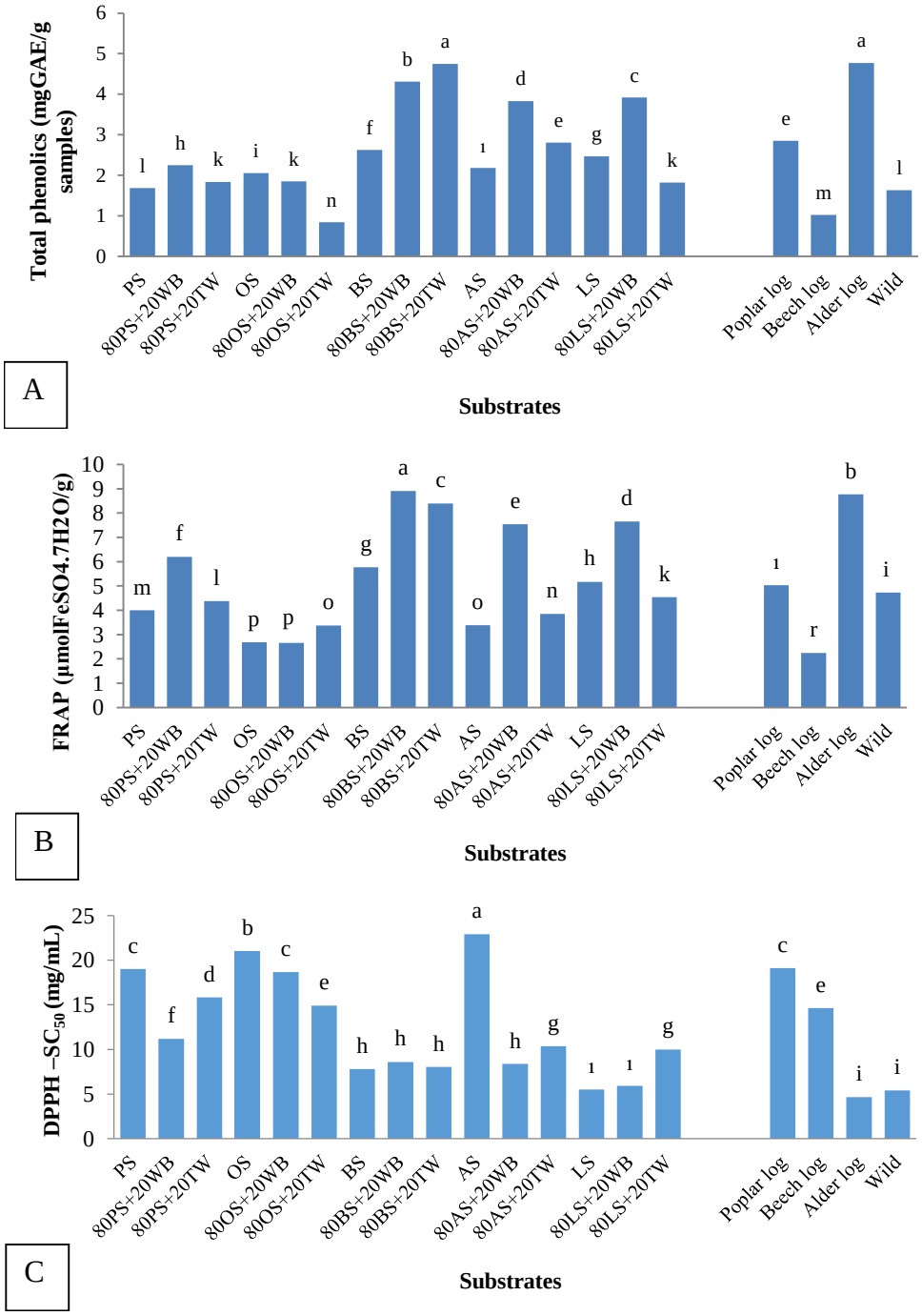

Figure 2. Total phenolics (A), FRAP (B) and DPPH-SC50 (C) in methanol extracts of the *P. ostreatus* obtained from different substrates. There were no significant differences among means indicated with the same letters at P<0.01 level (PS: Poplar sawdust, WB: Wheat bran, TW: Tea waste, OS: Oak sawdust, BS: Beech sawdust, AS: Alder sawdust, LS: Linden sawdust).

When *P. pulmonarius* and *P. ostreatus* were grown in a medium containing sawdust, rice flour, and calcium carbonate, the DPPH radical scavenging IC_{50} values of the mushroom extracts were determined to be 5.61 mg/mL and

8.88 mg/mL, respectively (Arbaayah & UmiKalsom, 2013). Singh et al. (2015) reported that the DPPH-SC_{50} value of *P. ostreatus* mushroom extract was 15.6 µg/mL when mixtures of wheat bran and chickpea straw were used as the growing medium. In our study, the DPPH-SC50 values of *P. ostreatus* grown on different substrates were found to be in the range of 4.650±0.044 - 22.922±0.002, showing similarity to the literature data.

CONCLUSION

The antimicrobial effects of mushrooms are derivatives of antagonistic substances, which include some phenolic compounds, purines, quinones, terpenoids, and phenylpropanoids derivatives synthesized in the fungal structure and are mostly organism-specific. According to the results of our study, it was observed that different growing substrates created differences in terms of the antimicrobial and antioxidant activities of *P. ostreatus* extracts. It was observed that when tea waste was added to the growing medium, it increased the antimicrobial activity or was better than the wheat bran mixture values. In our study, the antimycobacterial activities of methanol extracts of *P. ostreatus* grown on different substrates were revealed for the first time. These results suggest that the addition of tea waste during the preparation of mushroom compost will stimulate mushroom growth and contribute to properties important for health. In future studies, the biological activity of extracts obtained with different solvents from *P. ostreatus* grown on different sawdust substrates with various mixing ratios can be studied in a broader context.

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