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Determination of Antioxidant and Oxidant Potentials of *Pleurotus citrinopileatus* Mushroom Cultivated on Various Substrates

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ABSTRACT
Many mushroom species have been used by people for different purposes, from past to present. Cultivated mushrooms may show different biological effects depending on the content of the substrate they grown on. The present study aimed to determine the total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of *Pleurotus citrinopileatus* Singer mushroom cultivated on five different substrates. The cultivated mushrooms were extracted with ethanol in a Soxhlet device. TAS, TOS and OSI of extracts were determined with Rel Assay kits. The highest TAS (3.125±0.038 mmol/L), TOS (10.786±0.313 µmol/L) and OSI (0.345±0.014) values were determined in the mushrooms grown on 90% beech sawdust+10% bran. The lowest TAS (2.316±0.042), TOS (1.246±0.044) and OSI (0.054±0.001) values were obtained from the mushrooms grown on 100% poplar sawdust.

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Farklı Yetiştirme Ortamlarında Üretilen *Pleurotus citrinopileatus* Mantarının Antioksidan ve Oksidan Potansiyelinin Belirlenmesi

ÖZET
Geçmişten günümüze birçok mantar türü insanlar tarafından farklı amaçlar için kullanılmaktadır. Kültür mantarları, kullandıkları substratın içeriğine bağlı olarak farklı biyolojik etkiler gösterebilir. Bu çalışmada 5 farklı yetiştirme ortamından elde edilen *Pleurotus citrinopileatus* Singer mantarının toplam antioksidan seviyeleri (TAS), toplam oksidan seviyeleri (TOS) ve oksidatif stres indekslerinin (OSI) belirlenmesi amaçlanmıştır. Elde elde edilen mantarlar Soxhlet cihazında etanol ile ekstrakte edilmiştir. Ekstraktların TAS, TOS ve OSI değerleri Rel Assay kitleri kullanılarak belirlenmiştir. En yüksek TAS (3.125±0.038), TOS (10.786±0.313) ve OSI (0.345±0.014) değerleri %90 kayın talaşı +%10 kepek ortamında yetiştirilen mantarlarda belirlenmiştir. En düşük TAS (2.316±0.042), TOS (1.246±0.044) ve OSI (0.054±0.001) değerleri ise %100 kavak talaşı ortamında yetiştirilen mantarlardan elde edilmiştir.

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INTRODUCTION

Reactive oxygen species (ROS) increase in living organisms as a result of environmental and metabolic activities. In response to this increase, the endogenous antioxidants produced in the organisms play an active role and suppress the oxidant ROS. In cases where endogenous antioxidants are inadequate against ROS, the molecular structure of the organism may degrade. The degradations, called oxidative damage, might lead to serious health problems such as Parkinson's, Alzheimer's, cancer and cardiovascular disorders (Bolisetty and Jaimes, 2013; Li et al., 2013; Akyol et al., 2015; Selamoglu et al., 2016; Bozdoğan et al., 2018; Akata et al., 2019; Sevindik, 2019).

Exogenous antioxidants, which are supplemented when endogenous antioxidants produced in humans are inadequate against oxidant compounds, are very important in preventing oxidative damage. Being one of the several natural sources of exogenous antioxidants, mushrooms play an important role in human diet. Edible mushrooms are collected from their natural environment and consumed by humans throughout the history. However, especially after the second half of the 20th century, mushroom cultivation became popular and turned into an industry of billions of dollars turnover annually (Pilz et al., 2001; Yılmaz et al., 2017). In addition to the strong nutritional properties, mushrooms are also important medicinal natural resources because of containing the secondary metabolites. Research demonstrated that mushrooms are also important natural sources used in the treatment of AIDS (Acquired Immune Deficiency Syndrome) patients in Africa, as well as wound healing, immune system strengthening and tumor-inhibiting properties (Dai et al., 2009; Baba et al., 2012; Cheung, 2013; Zhang et al., 2014).

It is important to analyze mushroom species in order to identify and offer as new natural medical sources. Previous studies have reported pharmacological effects of *P. citrinopileatus* such as antioxidant, antibacterial, anticancer and antihyperlipidemic (Hu et al., 2006; Lee et al., 2007; Chomcheon et al., 2013; Yıldız et al., 2017). There are no studies in the literature determining the oxidative stress status of the *P. citrinopileatus*. In the present study, TAS, TOS and OSI values of *Pleurotus citrinopileatus* cultivated on various substrates were determined. The study also aimed to examine which compost medium is more suitable for the medical usages of *P. citrinopileatus* mushroom.

MATERIALS and METHODS

Substrates

No trees were cut down throughout the study. The sawdusts were obtained from sawmill located in the Karadeniz Technical University Campus (KTU) (Trabzon/Turkey). The substrates used in this study are presented in Table 1. Mycelium was supplied from a commercial firm.

Mushroom cultivation

The sawdusts were soaked to 70-80% humidity and stored for one day. Next day, to ensure homogeneity, moisture and autoclavable bags filled with sawdust were mixed and sterilized in the autoclave at 121 ° C for 1.5 hour. After sterilization, they were moved to the fume cabinet for cooling. Substrates were inoculated with spawn of 3% of the sawdust weight (Küçükomuzlu and Pekşen, 2005). The bags were counted in the Mushroom Culture Laboratory (KTU) and allowed to incubate. The mycelium colonizations were completely wrapped within 10 days, and the harvest was initiated on the 17th day. Harvested mushrooms (Figure 1) were prepared for the extraction process.

Table 1. Substrates used in the study
Çizelge1. Çalışmada kullanılan substratlar

Materials	Name in Latin
90% beech sawdust +10% wheat bran	*Fagus orientalis* Lipsky.
100% beech sawdust	*F. orientalis* Lipsky.
100% walnut sawdust	*Juglans regia* L.
100% poplar sawdust	*Populus nigra* L.
100% alder sawdust	*Alnus glutinosa* (L.) Gaertner

Figure 1. *Pleurotus citrinopileatus* Singer
Şekil 1. *Pleurotus citrinopileatus* Singer

Extraction of mushroom samples

P. citrinopileatus samples obtained from different compost combinations were dried at ±40°C about 8 hours (Profilo, PFD1350W, Turkey). After the drying process, 30 g mushroom samples were pulverized and extracted with 200 mL ethanol at 50 °C about 6 hours in a Soxhlet device (Gerhardt EV 14). The extracts were the concentrated in a rotary evaporator (Heidolph Laborator 4000 Rotary Evaporator).

Determination of TAS, TOS and OSI

Rel Assay kits were used to calculate TAS, TOS and OSI values of mushroom samples. Analyzes were conducted with 5 replicates. TAS values calibrator: Trolox. TOS values calibrator: Hydrogen peroxide. TAS results were shown mmol Trolox equiv./L. TOS results were shown µmol H_2O_2 equiv./L (Erel, 2004; Erel, 2005). The following Equation 1 was used to calculate the OSI (AU: Arbitrary Unit) values obtained by dividing the TOS value to TAS value (Erel, 2004).

$$OSI\ (AU) = \frac{\text{TOS,µmol/L}}{\text{TAS,mmol/L X 10}} \quad (1)$$

RESULT and DISCUSSION

TAS, TOS and OSI values were determined using ethanol extracts of *P. citrinopileatus* cultivated on various substrates. The results of this study are presented in Figure 2-4. All values are presented as mean±standart deviation (SD). Also, number of mushroom samples n=6 and experiments were made as 5 parallels.

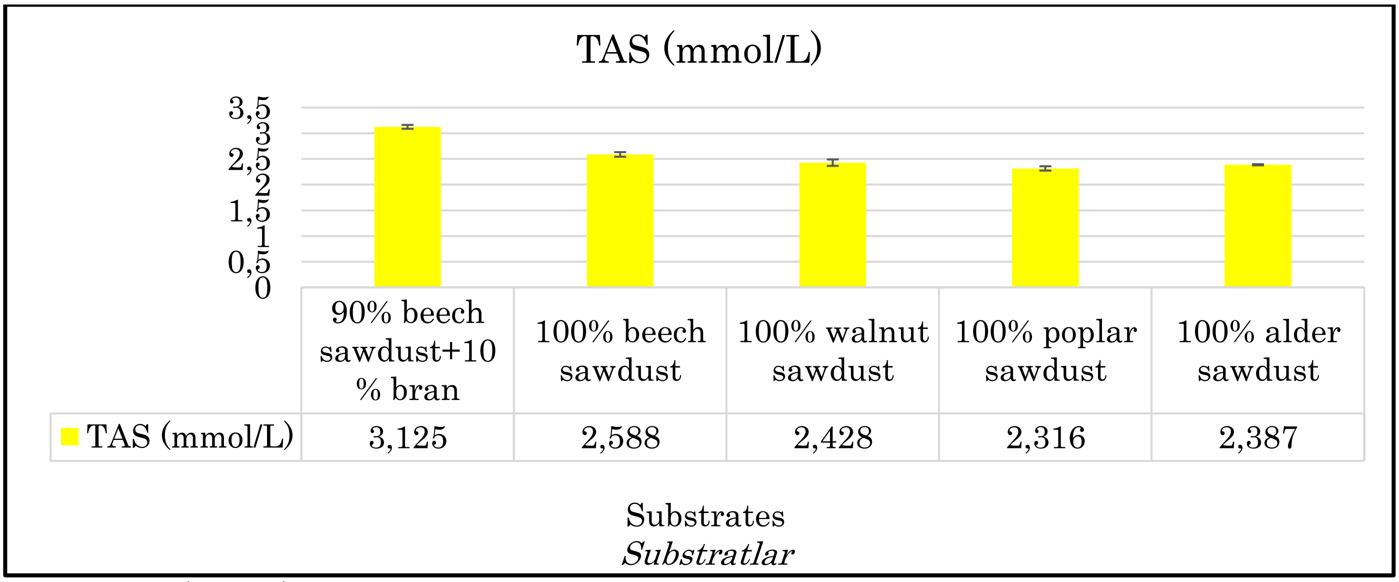

Figure 2. TAS (mmol/L) values of *P. citrinopileatus* ethanol extracts cultivated on various substrates
Şekil 2. Çeşitli substratlar üzerinde yetiştirilen P. citrinopileatus etanol ekstraktlarının TAS (mmol / L) değerleri

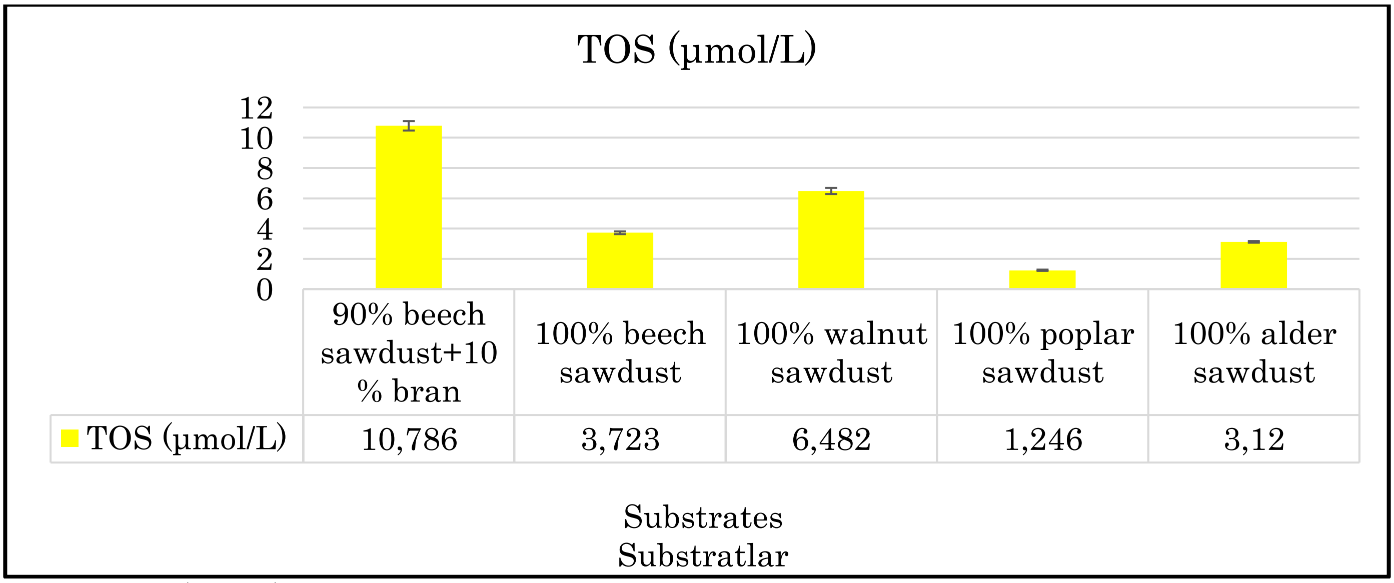

Figure 3. TOS (µmol/L) values of ethanol extracts of *P. citrinopileatus* cultivated on various substrates
Şekil 3. Çeşitli substratlar üzerinde yetiştirilen P. citrinopileatus'un etanol ekstraktlarının TOS (µmol/L) değerleri

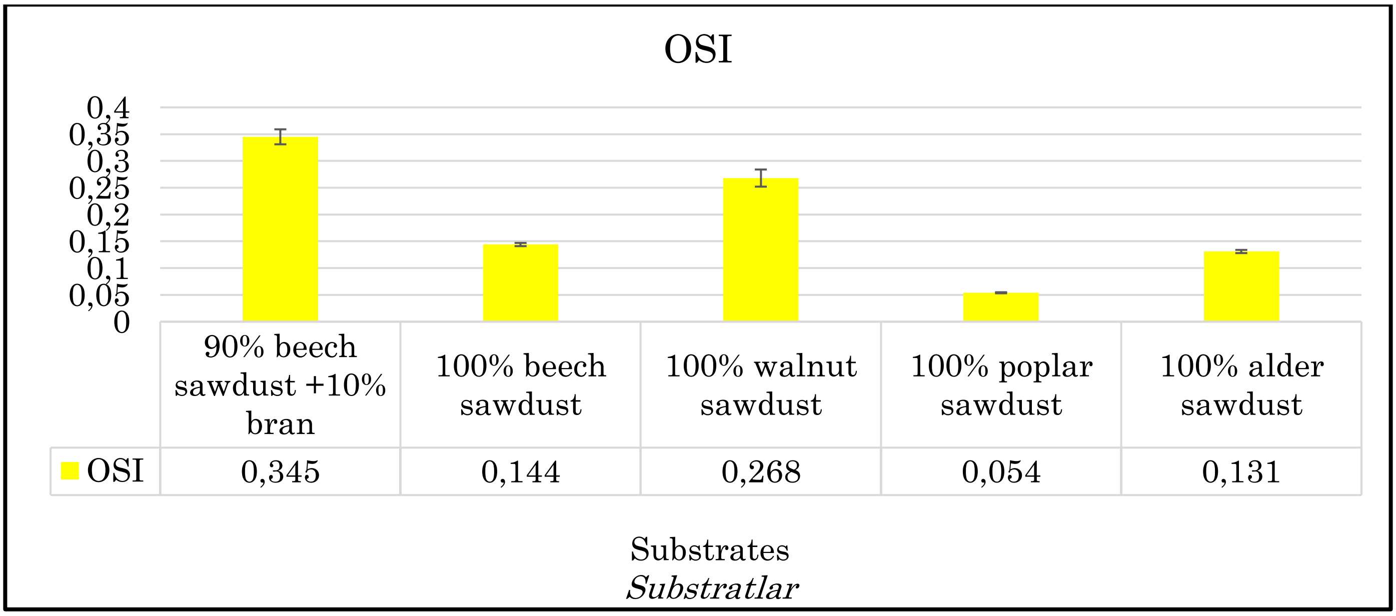

Figure 4. OSI values of *P. citrinopileatus* ethanol extracts cultivated on various substrates
Şekil 4. Çeşitli substratlar üzerinde yetiştirilen P. citrinopileatus etanol ekstraktlarının OSI değerleri

The highest TAS value was observed as 3.125±0.038 mmol/L, in the ethanol extracts of mushrooms cultivated on 90% beech sawdust+10% bran. The other TAS values were found close to each other. The highest TOS was observed in the extracts of mushrooms cultivated on 90% beech sawdust+10% bran as 10.786±0.313 µmol/L. The lowest TOS was found on 100% poplar sawdust (1.246±0.044 µmol/L). It was determined that the highest OSI value, that indicate the rate of the extent to which the oxidant compounds produced due to the environmental and inherent effects in the mushroom, was tolerated by the endogenous antioxidants (0.345 ± 0.014) obtained from the 90% beech sawdust+10% wheat bran substrate. Mushrooms have several antioxidant enzymes. By this means they have reduced coenzymes in addition to reduce some molecules such as phenolic compounds with various electron sources (Kalač, 2016; Sevindik, 2018). The identification of TAS values containing all enzymatic and non-enzymatic molecules that mushrooms potentially produce is very important for the identification and discovery of new antioxidant natural resources. In the present study, the highest antioxidant potential was seen mushroom cultivated on 90% beech sawdust+10% wheat bran substrate. This situation can be attributed to the diversity of substrate used by the mushroom. There are no previous studies on the oxidative stress status of *P. citrinopileatus* mushroom. However, *Trametes versicolor, Auricularia auricula, Ompholatus olearius, Helvella leucomelaena* and *Sarcosphaera coronaria* TAS values were determined as 0.820, 1.010, 2.827, 2.367 and 1.066, respectively, and their TOS values were determined as 17.760, 23.910, 14.210, 55.346 and 41.672, and OSI values were reported as 2.166, 2.367, 0.503, 2.338 and 3.909, respectively in previous oxidative stress studies on wild mushrooms (Akgul et al., 2017; Sevindik et al., 2017; Sevindik et al., 2018). In other studies, TAS values of *Pleurotus eryngii* and *Auricularia polytricha* mushrooms were determined as 1.93 and 0.93, respectively (Yildirim et al., 2012; Avcı et al., 2016). It was observed that the TAS value of *P. citrinopileatus*, cultivated on 90% beech sawdust+10% wheat bran substrate. was found higher when compared to the mushrooms reported in those studies. There were some differences between the literature results. These differences may be due to the different antioxidant production capacity of different mushroom species growing in different substrates. Mushrooms produce endogenous antioxidant compounds as a defense mechanism against oxidative damage (Ramírez-Anguiano et al., 2007). Thus, the high antioxidant capacity of *P. citrinopileatus* exposed that the mushroom had high tolerance to oxidative damage. It was also considered that the mushroom could be used as a supplementary antioxidant source to decrease the oxidative damage in human body.

Analysis of the TOS values demonstrated that *P. citrinopileatus* had lower TOS values when compared to *T. versicolor, A. auricula, O. olearius, H. leucomelaena* and *S. coronaria* mushrooms reported in the literature (Akgul et al., 2017; Sevindik et al., 2017; Sevindik et al., 2018). These mushrooms were wild and collected from the nature unlike our study. The differences in TOS values can be due to the differences in growth conditions and metabolic processes. It was reported that natural products which have antioxidant activity such as mushrooms may help the endogenous defense system (Ferreira et al., 2009). However, when compared to the wild mushrooms reported in the literature (Akgul et al., 2017; Sevindik et al., 2017; Sevindik et al., 2018), it was observed that the cultivated *P. citrinopileatus* mushroom was more adequate for the growth of the mushroom. It can be noted that cultivation mushrooms can be more suitable for consumption since they are less affected by the environmental factors and thus, produce lower levels of endogenous oxidant compounds.

OSI value demonstrates the extent to which the mushrooms inhibit oxidant compounds that they endogenously produce as a result of environmental and metabolic mechanisms with endogenous antioxidants. In the present study, it was identified that the OSI values for *P. citrinopileatus*, cultivated on different substrates, were low. It was seen that *P. citrinopileatus* had a lower OSI value when compared to *T. versicolor, A. auricula, O. olearius, H. leucomelaena* and *S. coronaria* mushrooms investigated in previous studies (Akgul et al., 2017; Sevindik et al., 2017; Sevindik et al., 2018). These findings indicated that oxidative stress induced by endogenous oxidant molecules produced by *P. citrinopileatus* was better inhibited by TAS that includes all enzymatic and none-enzymatic systems, and consequently, OSI values were lower.

CONCLUSION

Many natural antioxidants such as mushrooms are being widely investigated for their qualified capacity to defend cells and organisms from degradation brought on by oxidative stress. In the study, antioxidant/oxidant potentials and oxidative stress status of *P. citrinopileatus* mushroom cultivated in different composts were determined. It was observed that the mixture of 90% beech+10% bran, exhibited the highest antioxidant potential. The lowest antioxidant potential was seen in the 100% poplar. The all test mushrooms cultivated on different synthetic composts exhibited a low oxidative potential. Therefore, it can be stated that cultivated *P. citrinopileatus* mushroom had a lower oxidative stress status. In conclusion, it was determined that *P. citrinopileatus* had antioxidant potential and this potential varied based on the substrate used. It was also found that *P. citrinopileatus* cultivated in culture medium was healthier due to the lower oxidant compound levels.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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