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Change of Total Phenolic Substance, Total Flavonoid Substance, Ergothioneine Concentration, Antioxidant and Antibacterial Properties in Edible Mushrooms Under The Influence of UV-B Radiation

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Abstract

Aim of study: This study investigated the effects of UV-B radiation on antioxidant, phenolic and flavonoid, ergothioneine, amino acids, and antimicrobial properties in four edible mushroom species.

Area of study: The mushrooms were verified according to their microscopic and macroscopic features at Kastamonu University, Faculty of Forestry.

Material and method: DPPH measurements, total phenolic, total flavonoid, ergothioneine, and amino acid analyses, antibacterial measurements were also performed for mushroom extracts.

Main results: Results showed that low UV-B enhanced antioxidant and antibacterial activities, with *L. edodes* and *P. ostreatus* exhibiting the highest antioxidant levels. *P. eryngii* showed a steady increase in phenolic and flavonoid contents. Ergothioneine and glutamic acid concentrations peaked under low UV-B.

Research highlights: The findings indicate that UV-B exposure significantly influences the biochemical properties of mushrooms.

Keywords: Total Flavonoid Substance, Edible Mushrooms, Antioxidant Properties, Antimicrobial Properties, UV-B Radiation, Ergothioneine

UV-B Işınlarının Etkisi Altında Yenilebilir Mantarlarda Toplam Fenolik Madde, Toplam Flavonoid Madde, Ergotionin Konsantrasyonu, Antioksidan ve Antibakteriyel Özelliklerin Değişimi

Öz

Çalışmanın amacı: Bu çalışma, UV-B radyasyonunun yenilebilir mantarlarda antioksidan, fenolik ve flavonoid, ergotionin, amino asitler ve antimikrobiyal özellikler üzerindeki etkilerini araştırmıştır.

Çalışma alanı: Mantarların mikroskobik ve makroskobik özellikleri, Kastamonu Üniversitesi Orman Fakültesinde doğrulanmıştır.

Materyal ve yöntem: Mantar ekstreleri için, DPPH ölçümleri, toplam fenolik, toplam flavonoid, ergotionin ve amino asit analizlerinin yanı sıra antibakteriyel ölçümleri de yapılmıştır.

Temel sonuçlar: Sonuçlar, düşük UV-B'nin antioksidan ve antibakteriyel aktiviteleri artırdığını göstermiştir. *L. edodes* ve *P. ostreatus* en yüksek antioksidan seviyelerini gösterirken, *P. eryngii* fenolik ve flavonoid içeriğinde sürekli bir artış göstermiştir. Ergotionin ve glutamik asit konsantrasyonları düşük UV-B altında en yüksek seviyeye ulaşmıştır.

Araştırma vurguları: Bulgular, UV-B maruziyetinin mantarların biyokimyasal özellikleri üzerinde önemli bir etkisi olduğunu göstermektedir.

Anahtar Kelimeler: Toplam Flavonoid Madde, Yenilebilir Mantarlar, Antioksidan Özellikler, Antimikrobiyal Özellikler, UV B Işınları, Ergotionin

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Introduction

Mushrooms have been used worldwide for thousands of years in daily life and as traditional medicine (Debnath et al., 2017). Today, mushroom powders produced by drying can be used as flavoring and preservatives in functional foods on an industrial scale (Chang, 2008; Onbaşılı et al., 2015). Mushrooms are also evaluated for medicinal properties, such as lowering blood cholesterol and sugar and preventing heart diseases (Chang & Miles, 2004). Mushrooms are low in fat and calories and contain high dietary fiber. They are rich in carbohydrates and vitamins (Kalač, 2009). The most important indicator of nutritional values is amino acids, which are abundant in their content (Sudheep & Sridhar, 2014; Teklit, 2015). Aspartic acid and glutamic acid are proof of the flavour-enhancing properties of mushrooms (Kalač, 2016). Furthermore, mushrooms contain sweet amino acids like glycine, threonine, and alanine; bitter ones such as methionine histidine, isoleucine, arginine, and; as well as unpleasant amino acids including tyrosine and lysine (Pomeranz, 2012; Ferreira et al., 2016).

Mushrooms are an effective antioxidant candidate thanks to polyphenolic compounds and flavonoids (Mau et al., 2002; Jean-Philippe, 2005; Alispahić et al., 2015; Smolskaitė et al., 2015; Reis et al., 2017; Sun *et al.*, 2017; Bakır et al., 2018a). In particular, ergothioneine, which is found in the content of many mushrooms between 0.21 and 2.6 mg/g dry weight (dw), is an amino acid that exhibits unique antioxidant properties as a potent hydroxyl radical scavenger (Akanmu et al., 1991; Dubost et al., 2007; Ey et al., 2007; Cheah & Halliwell, 2012).

Considering the recent development of resistance of pathogenic bacteria to various antimicrobial drugs, fungi with their antimicrobial properties are being studied as an alternative treatment (Dülger et al., 2004; Hearst et al., 2009; İnci et al., 2019). Bawadekji et al. (2017) conducted a study investigating the edible, nutritious, and antibacterial properties of *Pleurotus ostreatus* extracts. It has been reported that *P. ostreatus* stem extracts show significant inhibition against *C. albicans*, *P aerugino*, and *S. aureus*. In a study examining the in vitro antibacterial activities of *Pleurotus eryngii* samples, it was reported that the *P. eryngii* extract exhibited antimicrobial effects against *K. pneumonia*, *S. aureus*, and *B. megaterium*, although not to the same extent as the control antibiotic (Uzun et al., 2004).

The flavors and medicinal properties of edible mushrooms are gaining increasing significance in our diets (Bakır et al., 2018b). Therefore, many studies have explored the inclusion of mushrooms, often referred to as a natural pharmacy, in daily diets. Increasing the amount of Vitamin D in mushrooms exposed to UV-B radiation is one of these studies (Karadeniz et al., 2019). It has been reported that UV-B rays increase the amount of polyphenolic compounds, such as some flavanols and flavonoids in some vegetables and fruits (Castagna et al., 2014). Phytosterols such as ergosterol, abundant in mushrooms, undergo photolysis to produce vitamin D when exposed to UV-B light (Schneider et al., 2011). However, a review of the existing literature reveals no study that simultaneously investigates the changes in total phenolic content, total flavonoids, antioxidant activity, ergothioneine, and amino acid levels under UV-B radiation. In addition, the variation of antimicrobial activities of mushrooms with UV-B radiation dose was not investigated. This study aims to examine the effects of UV-B radiation applied to *L. edodes*, *A. bisporus (Abi)*, *P. ostreatus (Pos)*, and *P. eryngii (Per)* mushrooms on total phenolic and total flavonoid substances, antioxidant and antimicrobial activity, ergothioneine and amino acid amounts.

Material and Methods

Trolox and DPPH were employed for antioxidant activity assays; Aluminum (III) chloride ($AlCl_3$) and Quercetin were used for total flavonoid analysis. Methanol was utilized for flavonoid extraction, while disodium carbonate (Na_2CO_3), Gallic acid, and Folin-Ciocalteu's phenol reagent were applied for total phenolic content determination. All of these chemicals were sourced from Merck KGaA, Darmstadt, Germany. Additionally, methimazole and ergothioneine standards for ergothioneine analysis were also obtained from the same supplier. All chemicals used were of

analytical grade, and deionized water was used at every stage.

Spectroscopic analyses were conducted using the SHIMADZU UVmini-1240 UV-Visible spectrophotometer manufactured by Shimadzu Corp., Kyoto, Japan. Ergothioneine analyses were carried out using an Agilent Eclipse XDB-C18 (5 µm, 4.6 x 150 mm) HPLC column manufactured by Shimadzu, Japan. The SIL-20AC HT automatic sample injection unit was used with a flow rate of 0.7 mL/min, utilizing 1% methanol (adjusted to pH 5 with boric acid) as the mobile phase, and the column oven was set to a temperature of 25°C. Calibration curves of the method with a detection limit of 1 µg/g were linear between 0.2 and 11.0 µg/mL ergothioneine.

LC/MS-MS determinations were made for amino acid analysis. A Zorbax Eclipse AAA (3.5 µm- 4.6 X 150 mm) column was used and samples were taken in an injection volume of 0.2 µL. The mobile phase consisted of methanol with 1% formic acid, flowing at a 1 mL/min rate, and the column oven temperature was set to 40°C. The resultant unit was found to be µM, and the dilution factor was reflected.

UV-B Application on Mushroom Samples

Samples of *Abi*, *Led*, *Per*, and *Pos* were verified by Prof. Dr. Sabri Ünal and Assist. Prof. Dr. Mertcan Karadeniz from the Faculty of Forestry, Kastamonu University, according to their microscopic and macroscopic properties. These mushroom species were sliced at an average thickness of 0.5-1 cm with a slicer. They took horizontal sections from their stems and caps and distributed them into pans with a suitable width on the UV line conveyor (Figure 1). Three different groups were formed for each mushroom: Group 1, the control group that did not receive UV-B; Group 2, the group that received low UVB dose (200-222mj/cm²); The third group consists of fungal groups that were applied high UVB dose (390-430 mj/cm²). Before starting the application, energy measurements on the conveyor were measured in mj/cm2 using a UV-Integrator (Beltron). For low and high UV-B radiation applications, the conveyor speeds varied at different rates, and the total exposure time was treated in the range of 23-25 seconds. Afterward, the mushrooms were dried in a room dryer (NUVE KD 400, Turkey) at 30°C for 48 hours and homogeneously pulverized for analysis.

Figure 1. Application to the mushroom samples: (a) UV-B conveyor system, (b) Sliced and panned mushroom samples, (c) dried and powdered mushroom sample

Preparation of Mushroom Extracts Exposed to UV-B Rays

Mushroom extracts were obtained following a slightly modified version of the protocol by Bakır et al. (2018a). 1 g of the dried mushroom was weighed and dissolved in 10 mL of an 80% methanol solution for extraction. After standing at room temperature for 60 minutes, the mixture was prepared and then filtered through filter paper. The obtained homogenate was centrifuged at 3600 rpm for 5 minutes (at 180 °C). The supernatant (100 mg/mL) obtained from this treatment was

used for DPPH, total phenolic, and flavonoid measurements (Lee et al., 2019).

Preparation of DPPH Calibration Solutions

Sample system solutions for DPPH radical scavenging measurements were prepared by diluting from 3.0x10-4 M DPPH (1,1-diphenyl-2-picryl hydrazyl) solution prepared as a stock. For this, 3 mL stock DPPH solution was completed as a 6 mL reaction mixture using different volumes of trolox (TR) or mushroom extract and adding sufficient methanol. The $1.0x10^{-4}$ M TR solution used as a standard was prepared in 80% methanol. Thus, the DPPH radical scavenging measurements of the mushroom extracts were followed by the color change observed by reducing the maximum absorbance of the DPPH radical solution at 517 nm by antioxidants (Hatano et al., 1988; Frankel & Meyer, 2000; Okan et al., 2013). In this study, the absorbance changes in the solution were measured across five different concentrations ranging from 0.20 to 8.00 mg/mL for each mushroom extract added. The results were expressed as inhibition (%) for mushroom extracts, and the percent radical scavenging activity was calculated by the formula (Equation 1):

$$Inhibition\ (\%) = \left[\frac{A0-A1}{A0}\right] x100 \quad (1)$$

A_0: Control absorbance
A_1: Absorbance in the presence of the samples

Then, IC_{50} (mg/mL) values, which express 50 % antiradical activity required for initial DPPH radical scavenging, were calculated for TR and mushroom extracts (Mukherjee et al., 2011).

Determination of Total Phenolic Substance

The Folin-Ciocalteu reagent method was used to measure the total phenolic content of the mushroom extracts. For this purpose, 4.5 mL of deionized water and 0.1 mL of Folin-Ciocalteu reagent mixture were kept for 3 minutes, and then 0.3 mL of Na_2CO_3 (2%) solution and 0.1 extract solution were added. After 2 hours, the samples' absorption at 760 nm was measured (Slinkard & Singleton, 1977; Chandler & Dodds, 1983). The gallic acid solution was used as a standard for comparison.

Phenolic compound concentrations were calculated using the following Equation (2), based on the standard curve of gallic acid within the 0.025 - 2.025 M range:

$$\text{Abst.} = 0.0051\left(\text{mg}\frac{\text{GA}}{\text{g}}\right) + 0.0031 \quad (2)$$
$$R^2 = 0.992$$

Determination of Total Flavonoid Substance

The total flavonoid content of mushroom extracts was measured using 1 mL of the extract or quercetin solution mixed with 1 mL of a 2% methanol solution of aluminum trichloride ($AlCl_3$) (Arvouet-Grand et al., 1994). After the 15-minute dwell time, sample absorbances were measured at 415 nm against a blank sample.

For total flavonoid compound concentrations, the Equation (3) obtained from quercetin used as standard in the 0.025-2.025 M working range was used;

$$\text{Abs.} = 0.0029\left(\text{mg}\frac{\text{QE}}{\text{g}}\right) + 0.0011 \quad (3)$$
$$R^2 = 0.999$$

Preparation of Mushroom Extract for Ergothioneine Analysis

The sample preparation step for ergothioneine assays was performed similarly to the procedure outlined by Sapozhnikova *et al*. (2014). For this, 100 mg of powdered mushroom sample and 10 µg of ISTD methimazole mixture were mixed by adding 10 mL of 75 % by volume methanol solution. This mixture in a polypropylene centrifuge tube was shaken vigorously in a NUVE ST-30 brand shaker for 90 minutes and then centrifuged at 5000 rpm for 5 minutes. 0.3 mL of supernatant samples were taken from these extracts, and each was transferred separately to 2.7 mL of 30% by volume methanol solution so that the samples were ready for HPLC analysis.

Preparation of Mushroom Extract for Amino Acid Analysis

A 0.5 g mushroom sample was burned with 20 mL of HCl at 110°C for 18–24 hours. Afterward, 20 mL of distilled water was added, and the mixture was evaporated at

70°C. The final volume was adjusted to 25 mL with distilled water in a balloon flask. The prepared 20 mg/mL sample was then analyzed for amino acids using the LC/MS-MS device.

Antibacterial Activity

The antimicrobial activity of the samples was evaluated against ten bacteria, including four Gram-positive strains (ATCC®10876, 25922, 7677, 7829, 27853, 14028, 25923*)*, and B1018). MHB (Merck) or MHA (Merck) was used for culturing the bacterial strains. At the same time, *C. albicans* and *S. cerevisiae* (ATCC®10231 *and* 9763) were grown using SDB (Difco) or SDA (Oxoid) for yeast and fungal cultures.

Disc Diffusion Assay

Antimicrobial activity was evaluated using the method described by Ronald (1990). For bacterial cultures, 20 mL of MHA was used, while 20 mL of SDA was poured into each petri dish for fungal and yeast cultures. All bacterial strains were incubated in MHB at 37°C for 24 hours, whereas yeast and fungal strains were cultured in SDB at 27°C for 48 hours. The overnight cultures were diluted with broth, and the final concentrations were adjusted to 10^8 cells/mL for bacteria and 10^7 cells/mL for yeast and fungi, based on the 0.5 McFarland turbidity standard. Finally, 100 µL of each diluted suspension was evenly spread over the agar surface in petri dishes. 6 mm sterile paper discs were then placed on the agar, and 30 µL of each sample (1 mg/mL) was applied to the discs. Ampicillin was used as positive control for the fungi, yeast, Nystatin, and bacteria. Ethanol was used as a negative control. After 24 hours of incubation at 37°C for antibacterial and 27°C for antifungal activities, the inhibition zones formed on the medium were measured in millimeters. All tests were performed in triplicate.

Statistical Analysis

Descriptive statistical analysis and significant effects between antioxidant activity and total phenolic and flavonoid substance concentrations against UV-B radiation dosages applied to fungi were investigated using SPSS ver.13 (SPSS Inc., USA).

Results and Discussion

DPPH Radical Scavenging Activity of Mushrooms

In this study, the DPPH scavenging activity of the mushroom was determined using a Trolox calibration curve, following the method described by Gonzalez et al. (2021) ($y = 28.264x + 9.7918$; $R^2 = 0.9845$, where y represents percent inhibition and x represents the Trolox concentration).

The inhibition percentages of mushroom samples at various concentrations for the control group exposed to low, high, and no UV-B radiation are presented in Figure 2. In all mushroom samples, it was observed that the inhibition percentages were found to increase directly with the rising concentrations. Figure 2 indicates that the increase in inhibition percentages is more pronounced for *Per* exposed to high UV-B doses and for *Pos* exposed to low UV-B doses compared to the other samples. Akata et al. (2012) reported that methanolic extracts of *Pos* exhibited a strong antioxidant effect of 96.16% at a concentration of 2.72 mg/mL. In our study, the *Pos* strain showed inhibition at 8 mg/mL (69%). We found inhibition of *Per* strain at 4 mg/mL (37.52%). Lo (2005) said that ethanolic extract (68.35%) at 5 mg/ml showed DPPH quenching in his study with the same type of mushroom. Choi et al. (2006), in their study investigating the effect of temperature on the antioxidant activity of *Led*, showed that the raw mushroom sample extracts had DPPH radical scavenging activity (45.1%).41 Similarly, *Led* showed inhibition at 8 mg/mL (45.12%) in this study.

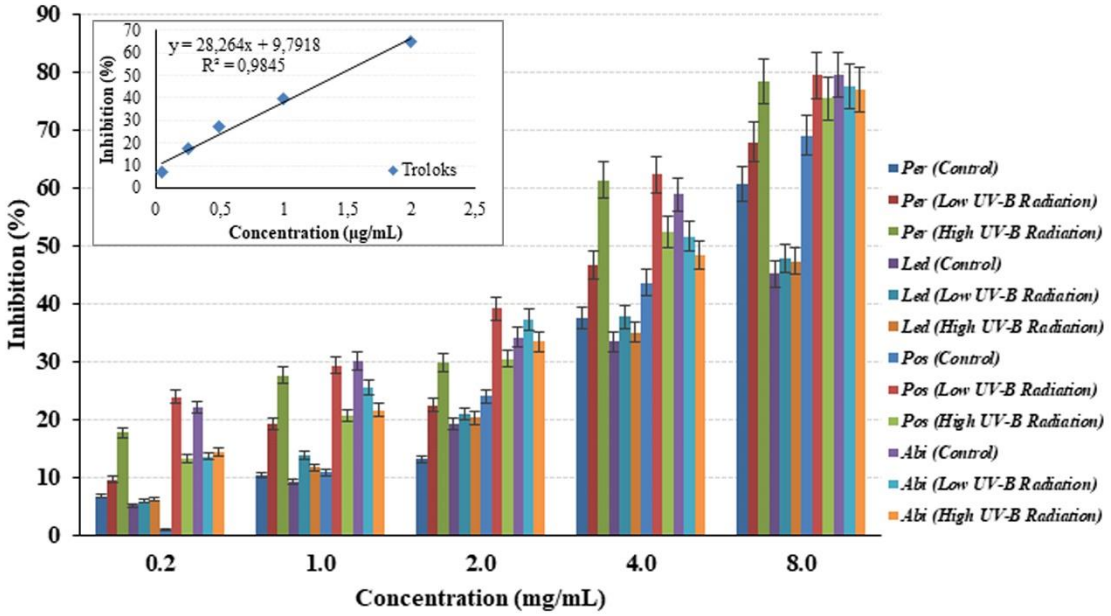

Figure 2. Change in DPPH radical scavenging inhibition (%) of mushroom species treated with low and high UV-B dose

Examination of Total Phenolics, Total Flavonoids, and Antioxidant Activities

We comparatively examined the effects of UV-B radiation on antioxidant activity, total flavonoid, and total phenolic content among different types of mushroom powder samples (Table 1). The IC_{50} values we obtained using the DPPH method did not follow a definite order according to the change in the UV-B dose of the mushroom. For example, antioxidant activity increased in the transition from low to high dose for *Per*, compared to the control group, but decreased for *Led*, *Pos*, and *Abi*. The 95% confidence interval for the difference test between concentration inhibition pairs showed a significance level of $p<0.05$ for both high and low UV-B treatments across all fungal species. The standard deviation (SD) for the comparisons of concentration inhibition was found to be 3.115. Additionally, a relative correlation between the control and UV-B dosage was observed for each mushroom. Accordingly, it showed good significance for low UV-B dosage ($p<0.05$; $r=0.925$) and high UV-B dosage ($p<0.05$; $r=0.919$), calculated with IC_{50} data. UV-B application caused a decrease in antioxidant activity for *Abi*, while it increased for *Per*, *Led*, and *Pos*. This study used trolox for comparison, and the IC_{50} value was found to be 1.42 µg/mL. It was observed that the UV-B dose affected antioxidant activity and caused similar changes in terms of total phenolic and total flavonoid substances. It was found that total flavonoid substances increased significantly with the application of UV-B dose, especially for *Per*. For *Per*, it is clear that this increase is the cause of the change in antioxidant activity. Again, for *Abi*, it was observed that the total amount of phenolic substance was not affected by low and high UV-B application. However, the antioxidant activity changed the same way as the total amount of flavonoids. Although the change in total phenolic values for all mushroom samples with UV-B application was in parallel with antioxidant activities, the changes in total flavonoid substances were higher. This result indicates that total flavonoid substances are more effective in the change of antioxidant activity. A significant negative correlation was found between IC_{50} and total phenolic compounds ($p<0.05$; $r = -0.624$), as well as between IC_{50} and total flavonoid compounds ($p<0.01$; $r = -0.768$). The difference test between IC_{50} and total flavonoid substance pairs was found at $p<0.01$ significance level and showed their dependent relationship. Jeena et al. (2016) reported the total polyphenolic content value as 1.32 mg/g for *Pos* in their study using Oyster mushroom varieties. In our study, this

value was found to be 7.73 mg/g for the *Pos* (Control) group.

Gasecka et al. (2016) in their mushroom enrichment study with selenium and zinc, total phenolic content 9.64±0.33 and 7.91±1.02 mg/g extract, total flavonoid content 2.11±0.19 and 1.26±0.17 mg/g were found as the extract for untreated raw *Pos* and *Per*. Likewise, when we examined the control groups, we found that the total phenolic and flavonoid content of *Per* was lower than that of *Pos*. However, interestingly, when we applied different doses of UV-B, the total flavonoid content increased quite significantly for *Per*. Such that, in the application of high UV-B dose, the IC_{50} value was 3.92 mg/mL for Per, and 4.47 mg/mL for *Pos*; that is, it showed higher antioxidant activity than *Pos*. Lee et al. (2019) investigated the effects of different cooking methods on vitamins, minerals and bioactive components in Shiitake Mushrooms and found the polyphenolic and flavonoid contents for raw *Led* as 28.56 mg/g and 4.13 mg/g, respectively.

We detected lower phenolic and flavonoid contents for the dried and powdered raw *Led* than the other mushroom species studied in this study. However, after applying different UV-B doses, we observed a significant increase in total phenolic and flavonoid content. The control group's total phenolic and flavonoid values for *Abi* decreased with UV-B application.

In a previous study using different solvent extraction, total phenolic and flavonoid contents were found to be 22.96 and 14.13 mg/mL for *Abi* (Brown) and 16.67 and 30.13 mg/mL for *Abi* (White), respectively.

Table 1. Total phenolic and total flavonoid contents of mushroom species treated with low and high UV-B dose

Mushrooms	IC_{50} (mg/mL)	Total Phenolic (mg GA/g)	Total Flavanoid (mg QE/g)
Per (Control)	6.38±0.57	3.42±0.24	0.14±0.04
Per (Low UV-B Radiation)	5.28±0.52	3.71±0.26	2.23±0.14
Per (High UV-B Radiation)	3.92±0.33	4.79±0.35	2.71±0.15
Led (Control)	8.33±0.71	2.93±0.18	0.21±0.05
Led (Low UV-B Radiation)	7.70±0.65	3.32±0.22	0.53±0.07
Led (High UV-B Radiation)	7.92±0.66	3.13±0.21	0.44±0.06
Pos (Control)	5.40±0.54	7.73±0.54	1.10±0.09
Pos (Low UV-B Radiation)	3.48±0.29	8.22±0.57	1.57±0.12
Pos (High UV-B Radiation)	4.47±0.38	7.83±0.54	1.49±0.12
Abi (Control)	3.71±0.30	4.79±0.35	1.93±0.13
Abi (Low UV-B Radiation)	4.17±0.35	4.50±0.32	1.01±0.08
Abi (High UV-B Radiation)	4.43±0.35	4.50±0.32	1.44±0.12

Examination of Ergothioneine Measurements

Mushrooms can serve as a source of naturally occurring ergothioneine in daily food intake. In this study, the ergothioneine concentrations were found for *Abi, Pos, Per*, and *Led* in mushroom powder samples with and without UV-B radiation, 0.280-0.304 mg/g dw (*Abi*), 2.185-3.169 mg/g dw (*Pos*), 1.314-1.578 mg/g dw (*Per*), and 0.600-0.741 mg/g dw (*Led*), respectively (Figure 3). The ergothioneine amounts of the control group mushroom samples were found in the order of *Pos*> *Per*> *Led*> *Abi*. In other words, the oyster mushroom strains (*Pos* and *Per*) exhibited significantly higher average concentrations of ergothioneine compared to shiitake and button mushrooms (*Led* and *Abi*).

Similar to our study, Kalaras et al. (2017) found ergothioneine values of 1.21±0.25 mg/g for *Pos* (Gray Oyster), 0.41±0.18 mg/g for *Abi* (White), and 0.92±0.29 mg/g for *Led* (Shiitake). In the study of Nguyen et al. (2012), ergothioneine values were found to be 24.17±4.01 and 78.98±3.72 mg/kg for *Abi* (Brown) and *Abi* (White) on a wet weight basis, respectively. In their study, Bao et al. (2010) showed that ergothioneine amounts to be 3.17±0.11 for Per and 2.84±0.04 mg/mL for Led. As with phenolics and flavonoids, the amount of ergothioneine may vary due to differences in the growing medium and

substrate materials that vary from crop to crop. Sapozhnikova et al. (2014) reported that ergothioneine concentrations in white, brown *Abi*, oyster (*Pos*), and shiitake (*Led*) mushroom samples ranged from 0.4-10.4 mg/g dry weight. They also showed that UV-B radiation did not significantly affect these values. In this study, it was observed that *Abi* was not significantly affected by low and high UV-B radiation, and again, *Led* changed slightly inversely with increasing UV-B dose. However, it was observed that the ergothioneine concentrations measured in our study changed significantly from control to low and high UV-B applications for *Pos* and *Per* mushrooms. For these species, ergothioneine concentrations were increased in the *Per* species, except for high UV-B application.

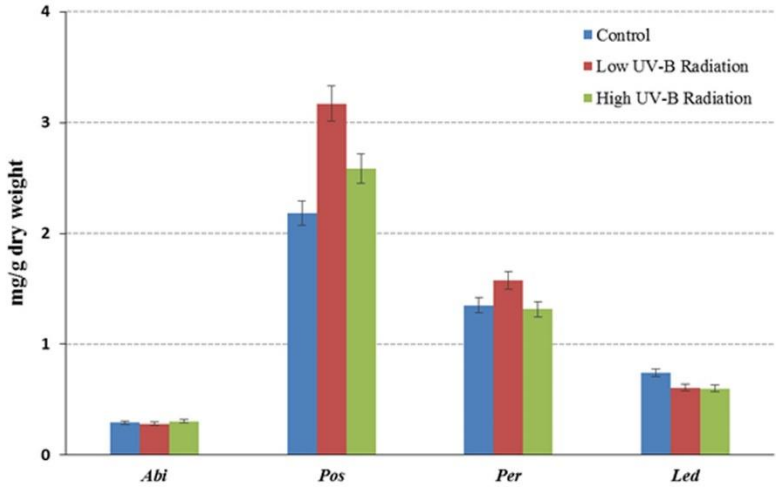

Figure 3. Mean concentrations of ergothioneine (mg/g dry weight) in low and high UV-B dosed fungal species, error bars represent standard deviations for n = 3

Examination of Free Amino Acid Measurements

This study identified eight essential, five conditionally essential, and four non-essential amino acid types in four fungal species exposed to different UV-B doses (Table 2). Especially known to contribute to the primary flavor of mushrooms, glutamic acid was the primary non-essential amino acid type in all mushroom species (Yamaguchi et al., 1971; Sato et al., 1985; Dutta et al., 2013). Numerous studies have reported that glutamic acid, known for its anti-cancer properties, is the most abundant total free amino acid (TAA) in mushrooms (Sato et al., 1985). This study found that the highest concentration was found in *Pos* and the lowest concentration in *Per* among the control groups. The correlation between the amino acid content of each mushroom species was significant at the $p<0.01$ level in applying different UV-B dosages, and the (r) values ranged between 0.790 and 0.996.

When the amounts of glutamic acid were examined, it was determined that all mushroom species exposed to low UV-B dose increased compared to the control group. Compared to the control group, this increase was the highest in *Led*, with 87.83%. However, in the application of a high UV-B dose, this increase was detected in *Pos* with a rate of 20.79 % compared to the control group.

Table 2. Amino acid contents of *A. bisporus* (*Abi*), *P. ostreatus* (*Pos*), *P. eryngii* (*Per*) and *L. edodes* (*Led*) mushroom species that were not exposed to UV-B Radiation (control group) and treated to Low and high UV-B Radiation

	Content (mg/g dry weight)											
Amino Acids	*Led* (Control)	*Led* (Low UVB Radiation)	*Led* (High UVB Radiation)	*Per* (Control)	*Per* (Low UVB Radiation)	*Per* (High UVB Radiation)	*Abi* (Control)	*Abi* (Low UVB Radiation)	*Abi* (High UVB Radiation)	*Pos* (Control)	*Pos* (Low UVB Radiation)	*Pos* (High UVB Radiation)
Leucine (Leu)	0.94±0.00	5.88±0.04	0.69±0.00	4.46±0.03	4.39±0.03	4.36±0.03	6.37±0.04	6.79±0.05	6.48±0.04	6.34±0.04	7.95±0.05	7.19±0.05
Alanine (Ala)	19.50±0.15	37.86±0.23	11.52±0.10	26.89±0.19	31.09±0.20	31.03±0.20	54.49±0.33	41.43±0.25	28.18±0.20	30.18±0.21	44.25±0.26	32.95±0.21
Arginine (Arg)	3.09±0.01	3.10±0.01	2.53±0.00	3.35±0.01	3.53±0.02	3.73±0.01	3.13±0.01	3.04±0.01	2.65±0.01	3.44±0.01	4.10±0.02	3.50±0.02
Aspartic acid (Asp)	35.13±0.22	53.96±0.32	17.82±0.14	35.85±0.22	40.25±0.26	47.49±0.28	46.48±0.28	41.68±0.25	26.42±0.19	30.49±0.20	47.11±0.28	41.72±0.25
Cysteine (Cys)	4.42±0.02	3.42±0.01	2.94±0.00	0.92±0.00	0.82±0.00	1.00±0.00	0.79±0.00	1.08±0.00	0.57±0.00	0.68±0.00	1.10±0.00	0.85±0.00
Glutamine (Gln)	3.15±0.01	2.86±0.01	1.48±0.00	3.41±0.01	3.04±0.01	3.11±0.01	3.95±0.01	4.20±0.02	3.45±0.01	3.97±0.01	3.21±0.00	4.30±0.02
Glutamic acid (Glu)	64.75±0.35	121.62±0.57	73.34±0.38	43.63±0.27	53.07±0.32	52.06±0.32	62.36±0.34	73.13±0.38	72.42±0.38	76.80±0.39	102.96±0.52	92.77±0.44
Histidine (His)	1.16±0.00	1.99±0.00	1.42±0.00	1.41±0.00	1.44±0.00	1.21±0.00	1.31±0.00	1.53±0.00	1.54±0.00	1.77±0.00	2.23±0.00	1.80±0.00
Isoleucine (Ile)	3.18±0.01	5.34±0.02	2.72±0.00	3.95±0.01	4.11±0.01	3.82±0.01	5.67±0.02	6.00±0.03	5.55±0.02	5.71±0.02	6.85±0.03	6.51±0.03
Lysine (Lys)	2.18±0.00	2.93±0.00	1.85±0.00	3.45±0.01	3.39±0.01	2.21±0.00	2.96±0.01	2.73±0.00	4.41±0.02	5.68±0.02	4.35±0.01	5.01±0.02
Methionine (Met)	0.63±0.00	1.26±0.00	0.59±0.00	1.07±0.00	1.24±0.00	0.86±0.00	1.28±0.00	1.18±0.00	1.17±0.00	1.50±0.00	2.15±0.00	1.73±0.00
Phenylalanine (Phe)	1.89±0.00	Nd*	1.52±0.00	Nd*	1.95±0.00	1.91±0.00	2.99±0.01	Nd*	Nd*	Nd*	Nd*	Nd*
Proline (Pro)	8.00±0.08	14.99±0.12	9.39±0.08	9.64±0.08	11.49±0.10	8.92±0.08	16.42±0.14	18.02±0.15	20.91±0.16	17.11±0.14	16.93±0.14	19.19±0.15
Serine (Ser)	18.02±0.15	17.06±0.13	14.39±0.12	19.21±0.15	15.55±0.12	18.68±0.15	22.11±0.17	22.57±0.17	18.52±0.15	21.03±0.17	18.58±0.15	22.34±0.17
Threonine (Thr)	13.07±0.11	16.91±0.13	11.35±0.10	12.08±0.04	15.35±0.12	15.94±0.12	18.93±0.15	18.91±0.15	14.42±0.12	16.99±0.13	19.18±0.15	18.05±0.14
Tyrosine (Tyr)	1.94±0.00	0.83±0.00	1.66±0.00	Nd*	0.82±0.00	2.43±0.00	3.29±0.01	3.40±0.02	Nd*	3.45±0.02	Nd*	Nd*
Valine (Val)	3.35±0.01	7.17±0.06	2.75±0.01	5.97±0.02	7.13±0.06	4.92±0.04	9.09±0.08	8.70±0.08	8.53±0.08	9.10±0.08	9.14±0.08	9.01±0.08

($p< 0.05$); Nd*: not detected

Antimicrobial Activity

The antimicrobial effect of ethanol extracts of *Abi*, *Led*, *Per*, and *Pos* were tested in vitro against four Gram-positive and four Gram-negative human pathogenic bacteria, as well as a strain of yeast and fungi. As summarized in Table 3, the inhibition zones of Low UV-B Radiation, High UV-B Radiation, and control extracts of the four fungi strains against all test microorganisms were in the range of 8.75±0.005^{b} –31±0.005l mm. The most influential group of alcoholic extracts of mushrooms exposed to rays, respectively, *P. ostreatus* (*Pos*) 18.65±0.005 mm> *A. bisporus* (*Abi*) 16.93±0.005 mm> *P. eryngii* (*Per*) 16.66±0.005 mm> and *L. edodes* (*Led*) 14.66±0.005 mm. As seen in Table 3, the antimicrobial effects of the fungal extracts exposed to both Low UV-B and High UV-B Radiation were most effective against the Gram-negative bacteria among the tested microorganism groups. In contrast, Gram-positive bacteria were inhibited at a slightly lower value. However, it showed a lower activity on fungi and yeast. In twelve different alcohol mushroom extracts, *P. ostreatus* (*Pos*) (Control) fungus species was effective on microorganisms. While it creates a zone of 26.25±0.005^{k}, *P. vulgaris* 24±0.005^{k} and *S. typhimurium* 31±0.005l mm against Gram-negative bacteria *E. coli*, it is most effective against *C. albicans* with a zone diameter of 23±0.005^{h}. While it was observed that the extract was *Abi* (High UV-B Radiation), it was determined that there was *Pos* (Low UV-B Radiation) extract on *S. cerevisiae* with a con diameter of 27*±0.005l. The Gram-positive bacteria *S. aureus* were affected by fungal extracts with a zone diameter of 19.208±0.005 mm, while *P. vulgaris*, a Gram-negative bacterium with a zone diameter of 19.375±0.005 mm, was affected similarly. *B. cereus* was the bacteria that was least affected by all fungal extracts with an average zone diameter of 9.458±0.005 mm. *Led* (High UV-B Radiation), and *Pos* (Control) fungal extracts did not show antibacterial activity on *B. aereus*, while these extracts inhibited other bacteria, yeast and fungus. The ethanolic mushroom extract with the most effective average was *Abi* (Low UV-B Radiation) with a zone diameter of 20,05±0,005 mm, while *Per* (High UV-B Radiation) mushroom extract with a zone diameter of 18.9±0.005 mm was the second. However, the mushroom extract with the lowest microbial activity was the *Led* (Low UV-B Radiation) extract with a zone diameter of 12.3±0.005 mm. As a result, the average activity of the fungal extracts grown by exposure to four different fungi (Low UV-B Radiation) on bacteria was 17,5±0.005 mm with a zone diameter of 16.25±0.005 mm, similar to a 16.75±0.005 mm zone diameter. The antimicrobial activity of the Control group, which was grown without exposure to any UV light, created a zone of 17.75 ± 0.005 mm against the organisms.

Antimicrobial activities of ethanol extracts of *Led*, *Abi*, *Pos*, and *Per*, grown by exposure to low and high UV light (Table 1) are as summarized. Comparable results were found in a previous study (Waithaka et al., 2017). Multiple studies have documented the antibacterial activities of crude extracts from *Abi* against Gram-positive and Gram-negative bacteria (Barros et al., 2008). The methanol extract of a wild *Abi* strain collected from Türkiye exhibited a stronger inhibitory effect on various Gram-positive bacteria compared to Gram-negative ones (Öztürk et al., 2011). Recent findings are consistent with those on a Chinese *Abi* strain, where the button mushroom extract showed no effectiveness against Gram-negative bacteria (Shang et al., 2013). Furthermore, our recent findings showed close antimicrobial activity of extracts obtained due to growing four different species of fungi in both low UV and high UV environments. As a result of growing *Pos* in any medium, it showed a significant antimicrobial effect. Adebayo et al. (2018) found a similar trend for antimicrobial activity. The results of a previous study demonstrated the antibacterial activity of *Per* derived from a wild sample (Akyüz et al., 2010). Results like these suggest a promising potential for developing a potentially active antimicrobial additive from various mushroom origins.

Table 3. The antimicrobial activities of ethanol extracts of *Agaricus bisporus* (*Abi*). *Pleurotus ostreatus* (*Pos*). *Pleurotus eryngii* (*Per*). *Lentinula edodes* (*Led*) species

Numuneler	**1** ***Per*** **(Control)**	**2** ***Per*** **(Low UV-B Radiation)**	**3** ***Per*** **(High UV-B Radiation)**	**4** ***Led*** **(Control)**	**5** ***Led*** **(Low UV-B Radiation)**	**6** ***Led*** **(High UV-B Radiation)**	**7** ***Pos*** **(Control)**	**8** ***Pos*** **(Low UV-B Radiation)**	**9** ***Pos*** **(High UV-B Radiation)**	**10** ***Abi*** **(Control)**	**11** ***Abi*** **(Low UV-B Radiation)**	**12** ***Abi*** **(High UV-B Radiation)**	**Ampicillin / Nystatin**	**Average activity of fungal extracts on microorganisms**
Bacteria	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	**Mean ±S**	
B. cereus	22.5±0.05^{h}	**9.73±0.003^{d}**	9±0.005^{c}	9±0.005^{c}	13±0.005^{f}	-	-	8.75±0.005^{b}	13±0.005^{f}	20±0.005^{g}	12±0.005^{e}	8±0.005^{a}	43±0.005^{i}	9.458±0.005
M. luteus	-	22.5±0.05^{i}	**20±0.005^{g}**	16±0.005^{e}	15±0.005^{d}	15±0.005^{d}	14±0.005^{c}	13±0.005^{b}	16±0.005^{e}	21±0.005^{h}	10±0.005^{a}	17±0.005^{f}	49.6±0.005^{j}	16.25 ±0.005
L. monocytogenes	14.25±0.005^{e}	13±0.005^{c}	13±0.005^{c}	19±0.005^{h}	17.75±0.005^{g}	22.5±0.005^{k}	21±0.005^{j}	9.5±0.005^{a}	13.75±0.005^{d}	16.5±0.005^{f}	19.25±0.005^{i}	12±0.005^{b}	42±0.005^{l}	17.125±0.005
S. aureus	22±0.005^{j}	20.5±0.005^{h}	12±0.005^{a}	17±0.005^{d}	25.5±0.005^{k}	13±0.005^{b}	16.25±0.005^{c}	19±0.005^{e}	20±0.005^{g}	19.75±0.005^{f}	27±0.005^{l}	21.5±0.005^{i}	49±0.005^{m}	19.208±0.005
E. coli	14±0.005^{c}	24.5±0.005^{j}	16±0.005^{d}	18±0.005^{e}	10±0.005^{a}	11.75±0.005^{b}	26.25±0.005^{k}	22±0.005^{h}	16±0.005^{d}	21.25±0.005^{g}	22±0.005^{h}	23.25±0.005^{i}	42±0.005^{l}	19.458±0.005
P. vulgaris	23.5±0.005^{j}	18±0.005^{c}	14±0.005^{a}	16±0.005^{b}	21.5±0.05^{i}	18±0.005^{c}	24±0.005^{k}	21±0.005^{h}	**16*±0.005^{b}**	20.5±0.005^{g}	**18.5*±0.005^{d}**	**19*±0.005^{e}**	43±0.005^{l}	19.375±0.005
P. aeruginosa	19±0.005^{e}	23.25±0.005^{i}	14±0.005^{a}	-	18±0.005^{d}	17±0.005^{b}	21.75±0.005^{h}	17±0.005^{b}	**18*±0.005^{d}**	20±0.005^{f}	17.5±0.005^{c}	**24*±0.005^{j}**	31±0.005^{k}	17.875±0.005
S. typhimurium	19.25±0.005^{f}	23.5±0.005^{i}	19±0.005^{e}	10.5±0.005^{b}	24.5±0.005^{j}	11±0.005^{c}	**31±0.005^{l}**	16±0.005^{d}	21.41±0.088^{h}	20±0.005^{g}	27±0.005^{k}	24.5±0.005^{j}	42.25±0.005^{m}	18.708±0.005d
C. albicans	23±0.005^{h}	20±0.005^{f}	18.75±0.005^{e}	17.5±0.005^{c}	21±0.005^{g}	16±0.005^{b}	9.5±0.005^{a}	23.75±0.005^{i}	18±0.005^{d}	-	23±0.005^{h}	23±0.005^{h}	16±0.005^{d}	17.291±0.005
S. cerevisiae	19±0.005^{f}	11±0.005^{b}	10±0.005^{a}	-	13±0.005^{c}	18±0.005^{e}	19.5±0.005^{g}	27*±0.005^{l}	21±0.005^{i}	15±0.005^{d}	**22*±0.005^{j}**	20±0.005^{h}	16±0.005^{d}	12.916±0.005
Most effective apology on bacteria	17.5±0.005	18.9±0.005	14.5±0.005	12.3±0.005	16.4±0.005	14.2±0.005	18.5±0.005	17.7±0.005	16.4±0.005	16.3±0.005	20.05±0.005	19.1±0.005		
Mean values of different mushroom groups	*Pleurotus eryngii* (*Per*) 16.66±0.005			*Lentinula edodes* (*Led*) 14.66±0.005			*Pleurotus ostreatus* (*Pos*) 18.65±0.005			*Agaricus bisporus* (*Abi*) 16.93±0.005				

They have a strong demand as antimicrobial agents against microorganisms and can be used to treat infectious diseases caused by these pathogens. The antimicrobial activity of *Per* (ranging from 9 ± 0.005^{c} to 24.5 ± 0.005^{j} mm) appears to vary, as noted by other researchers who reported a range of 4.0 to 29.0 mm. This variability may stem from factors such as the genetic structure of the mushroom species, as well as differences in the physical, bioactive, and biochemical components of the extracts, solvents, and test microorganisms, which are clearly highlighted in comparisons with other mushroom species (Benedict & Brady, 1972; Cochran 1978; Jonathan & Fasidi, 2003; Rosa et al., 2003; Gbolagade et al., 2007). Extracts of diverse fungi obstructed the development of some microorganisms at dissimilar ratios. In conclusion, we found that the broad spectrum of antimicrobial activity of fungal compounds can be attributed to bioactive metabolites of various chemical types. It has been observed that *Per* extracts prepared with ethyl alcohol and exposed to low and high UV light exhibit antimicrobial activity against certain bacteria, yeasts, and fungi. *Led* extracts are recognized for their antibacterial properties, though most studies reported have focused on human pathogens or have been confined to in vitro antimicrobial activity (Pacumbaba et al., 1999; Dülger et al., 2004; Mekawey, 2010). In this study, ethanolic extracts obtained as a result of cultivation of *Led* in low and high UV light showed antimicrobial activity. Similar results according to Kaur et al. (2016) reported.

Conclusions

This study was designed to assess the effects of UV-B radiation on vital natural bioactive compounds in popular and commercially available mushroom species, with the intention of contributing to the food industry. One of the important conclusions drawn from this study is that the change in UV-B dose and antioxidant activity largely depends on the change in total flavonoid substances. Although the total phenolic and amino acid contents are responsible for the natural values of the mushrooms, which affect antioxidant activity, the change effect is lower in the UV-B dose application to the mushrooms. Another result was to observe that the amount of glutamic acid, which is a determining parameter for the taste and flavor of mushrooms, is directly affected by UV-B radiation. This result is an important implication for industrial commercial applications.

One of the results in this study was the evaluation of four different fungal species exposed to low and high UV-B light in terms of their antimicrobial activities. Accordingly, antimicrobial activities were found to be higher in the application of a low UV-B dose. These results suggest that growing mushrooms in low UV light would be more beneficial for obtaining valuable extracts from fungi and increasing secondary compounds that inhibit microorganisms.

Ethics Committee Approval

N/A

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Author Contributions

Conceptualization: R.S.S., S.Ü., T.K.B., M.K.; Investigation: T.K.B, M.K., R.S.S., Ö.E.; Material and Methodology: T.K.B, M.K., R.S.S., Ö.E.; Supervision: S.Ü., T.K.B. R.S.S.; Visualization: T.K.B, M.K.; Writing-Original Draft: T.K.B, M.K.; Writing-review & Editing: T.K.B, M.K.

The entire authors have seen and accepted to publish the version of manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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