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# Comparison of the effect of UVB application on the antioxidant properties of different mushroom species with vitamins $D_2$ and $D_3$

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**ABSTRACT**: This study aimed to determine the effect of UVB application on different mushrooms and their antioxidant properties and compare their properties with pure vitamins $D_2$ and $D_3$. In the study, as without UVB, with medium UVB and with high UVB, respectively; 3 ethanol extracts each from White button (E1, E2 and E3), Shiitake (E4, E5 and E6) and King oyster mushroom (E7, E8 and E9) were prepared. The antioxidant activities of the samples were compared with pure vitamin D2 and D3 solutions using DPPH, FRAP, and CUPRAC methods. The results show that vitamins $D_2$ (0.277 mMFeSO4/mg extract) and $D_3$ (0.380 mM$FeSO_4$/mg extract) had the highest FRAP values. In mushroom species, it was determined that E3 (0.216 mM$FeSO_4$/mg extract) and E9 (0.273 mM$FeSO_4$/mg extract) mushroom extracts showed higher activity. Similarly, it was detected that vitamins $D_2$ (0.609 mMrolox equivalent/mg extract) and $D_3$ (1.149 mMrolox equivalent/mg extract) had the highest CUPRAC values. In mushroom species, E3 (0.324 mMrolox equivalent/mg extract) and E9 (0.292 mMrolox equivalent/mg extract) extracts had the highest CUPRAC values. According to the DPPH experiment, E6 ($IC_{50}$:0.386 mg/ml) and E9 ($IC_{50}$:0.411mg/ml) extracts showed stronger free radical scavenging activity compared to other extracts, while vitamin D forms showed lower activity than mushroom extracts. Ultimately, it was concluded that the antioxidant properties of mushrooms were positively influenced by the application of UVB.

**KEYWORDS**: Mushrooms; antioxidant activity; ultraviolet rays; ergocalciferol; cholecalciferol.

## 1. INTRODUCTION

Mushrooms are regarded as a rich source of many nutrients, such as riboflavin, copper, selenium, vitamin D, potassium, chitin, dietary fibre, and β-glucan [1]. They also possess a multitude of secondary metabolites, encompassing polypeptides, steroids, terpenes, and phenolic compounds [2]. Particular attention is paid to phenolic compounds because of their potent antioxidant properties [3]. Vitamin D, which has two main forms, ergocalciferol (vitamin $D_2$) and cholecalciferol (vitamin $D_3$), is a fat-soluble vitamin that can be obtained through diet and is also a secosteroid prohormone produced in the skin by ultraviolet B (UVB) from sunlight [4,5]. While vitamin $D_3$ is synthesised from the 7-dehydrocholesterol in the skin via UVB radiation and obtained through animal-based foods, vitamin D2 is produced by exposing mushroom ergosterol to UV rays [6,7].

Research on improving the antioxidant qualities of fresh and edible mushrooms shows that applying UVB radiation yields notable results in this area [8,9]. Similarly, various studies indicate that UV treated mushrooms are a potential plant-based source of dietary vitamin $D_2$, due to their conversion of ergosterol to ergocalciferol [10,11]. Moreover, UVB light is considered safe and suitable for mushrooms because it enhances their nutrient profile and does not cause detrimental changes in their composition [12]. Therefore, enhancing both the nutritional profile and antioxidant capacity of mushrooms through this methodology may be regarded as an innovative strategy.

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Cells experience heightened oxidative stress, primarily due to the increased generation of reactive oxygen species (ROS). This stress happens when the body's antioxidant defence mechanisms exceed their ability to neutralise ROS formation. Excessive ROS levels can potentially damage cellular components, encompassing nucleic acids, lipids, and proteins [13]. Uncontrolled free radical production has been linked to more than 100 diseases, including atherosclerosis, diabetes, various cardiovascular diseases, cirrhosis, neurological disorders, cancer, lung diseases, drug-induced deafness, Parkinson's disease, etc. [14].

Faced with oxidative stress, the organism triggers diverse defence mechanisms to alleviate or counterbalance the detrimental effects of ROS. These responses are based on a rise in the activity of antioxidants and related enzymes [15]. By interacting with free radicals, antioxidants are compounds that can terminate chain reactions [16]. Hence, it is thought that the consumption of antioxidant-rich foods might contribute to alleviating the adverse consequences of oxidative damage [17].

In recent years, mushrooms have attracted attention in terms of the antioxidant compounds they contain. Mushrooms are also thought to provide a wider range of health benefits than pure vitamin D, as they are a natural food source, and both their antioxidant content and vitamin $D_2$ levels increase with exposure to UVB [8-11,18,19].

In this regard, the objective of this study is to evaluate how UVB application affects the antioxidant properties of different mushrooms and to compare their antioxidant properties with vitamins $D_2$ and $D_3$.

## 2. RESULTS

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) experiment revealed that E6 ($IC_{50}$:0.386 mg/ml) and E9 ($IC_{50}$:0.411 mg/ml) mushroom extracts exhibited stronger activity to scavenge free radicals in comparison to the other extracts. It was also shown that vitamin D forms showed lower scavenging activity than other extracts except E7. All of the extracts, however, were found to have less capacity to scavenge free radicals than the standard ascorbic acid (Table 1).

Based on an analysis of the extracts' ferric ion reduction capabilities, vitamins $D_2$ (0.277 mM$FeSO_4$/mg extract) and $D_3$ (0.380 mM$FeSO_4$/mg extract) were shown to have the highest ferric reducing antioxidant power (FRAP) values. The ferric ion reduction capacity of E9 (0.273 mM$FeSO_4$/mg extract) mushroom extract was found to be higher than that of other extracts in the category of mushrooms. Furthermore, as with the DPPH analysis, it was discovered that all extracts had lower levels of ferric ion-reducing activity than the reference compound, BHA (Table 1).

Vitamins $D_2$ (0.609 mMTE/mg extract) and D3 (1.149 mMTE/mg extract) showed the greatest cupric ion reducing antioxidant capacity **(**CUPRAC) values when the Cu (II) reduction potentials of the extracts were assessed. It was discovered that the extracts E9 (0.292 mMTE/mg extract) and E3 (0.324 mMTE/mg extract) had a greater capacity to reduce Cu (II) in mushroom species than the other extracts. However, all samples had lower CUPRAC values compared to the standard compound (Table 1).

**Table 1.** Antioxidant activities of the samples

| Extracts/standards | DPPH ($IC_{50}$: mg/mL) | FRAP (mM$FeSO_4$/mg extract) | CUPRAC (mMTE/mg extract) |
|---|---|---|---|
| | mean ± SD | mean ± SD | mean ± SD |
| E1 | 0.861 ± 0.013$^{d,e}$ | 0.107 ± 0.024$^{f}$ | 0.146 ± 0.009$^{e,f}$ |
| E2 | 0.722 ± 0.016$^{c,d}$ | 0.199 ± 0.017$^{e}$ | 0.284 ± 0.002$^{d}$ |
| E3 | 0.598 ± 0.05$^{b,c}$ | 0.216 ± 0.002$^{d,e}$ | 0.324 ± 0.102$^{d}$ |
| E4 | 0.584 ± 0.121$^{b,c}$ | 0.100 ± 0.007$^{f}$ | 0.108 ± 0.037$^{f}$ |
| E5 | 0.519 ± 0.027$^{b,c}$ | 0.105 ± 0.005$^{f}$ | 0.176 ± 0.007$^{e,f}$ |
| E6 | 0.386 ± 0.043$^{b}$ | 0.110 ± 0.005$^{f}$ | 0.247 ± 0.075$^{d,e}$ |
| E7 | 1.226 ± 0.090$^{f}$ | 0.109 ± 0.027$^{f}$ | 0.071 ± 0.009$^{f}$ |
| E8 | 1.091 ± 0.228$^{e,f}$ | 0.260 ± 0.003$^{c,d}$ | 0.240 ± 0.003$^{d,e}$ |
| E9 | 0.411 ± 0.109$^{b}$ | 0.273 ± 0.004$^{c}$ | 0.292 ± 0.017$^{d}$ |
| $D_2$ | 1.094 ± 0.068$^{e,f}$ | 0.277 ± 0.041$^{c}$ | 0.609 ± 0.025$^{c}$ |
| $D_3$ | 1.151 ± 0.048$^{f}$ | 0.380 ± 0.046$^{b}$ | 1.149 ± 0.063$^{b}$ |
| BHA | | 5.81 ± 0.004$^{a}$ | |
| Ascorbic acid | 0.004±0.001$^{a}$ | | 5.683±0.337$^{a}$ |

[a] Values are means ± standard deviation of triplicates.

[b] Values in a column with different superscripts are significantly different ($p<0.05$, Tukey).

[c] E1: White button mushroom with non-UVB, E2: White button mushroom with medium UVB, E3: White button mushroom with high UVB, E4: Shiitake mushroom with non-UVB, E5: Shiitake mushroom with medium UVB, E6: Shiitake mushroom with high UVB, E7: King oyster mushroom with non-UVB, E8: King oyster mushroom with medium UVB, E9: King oyster mushroom with high UVB, BHA: Butylated Hydroxyanisole

## 3. DISCUSSION

According to the main study outcomes, applying UVB to mushrooms significantly improved their antioxidant capacity.

Nowadays, mushrooms, recognised for their noteworthy biological properties and nutritional value, are a focal point of scientific inquiry, owing to their substantial influence on human health. The antioxidant capacities of mushrooms, particularly their ability to prevent free radical-induced cellular damage, are a major contributor to their health benefits [20].

### 3.1. DPPH radical scavenging activity test

The assay on DPPH radical is useful in predicting antioxidant activities by inhibiting lipid oxidation. Therefore, the degree of free radical scavenging is determined by DPPH radical scavenging technique. The DPPH radical scavenging test is widely utilised because it produces rapid analytical results in a relatively brief period [21].

The $IC_{50}$ value of Agaricus bisporus ranges from 1.4 to 77.5 mg/ml, according to DPPH experiments on this mushroom offered in the scientific literature [22-24]. The $IC_{50}$ values of Agaricus bisporus with no UVB, medium UVB, and high UVB were reported to be 0.861, 0.722, and 0.598 mg/ml, respectively, in this investigation. Furthermore, these mushrooms demonstrated a superior capacity for scavenging DPPH radicals compared to results documented in the current literature.

Nam et al. reported that the $IC_{50}$ value of Lentinula edodes varied between 0.88-0.94 mg/ml, according to its growing environment [25]. In research examining the antioxidant properties of the water extract of Lentinula edodes, the $IC_{50}$ value of this mushroom was determined as 48.3 µg/ml [26]. The antioxidant properties of various Lentinula edodes sections (peel, fruiting body, gills, inner cap, and stipe) were investigated in a different study, and it was discovered that the samples' $IC_{50}$ values ranged from 0.129 to 0.213 mg/ml [27]. An investigation into how UVB exposure affects Lentinula edodes' antioxidant activity revealed that UVB treatment increased the mushroom's capacity to scavenge free radicals (DPPH) [9]. The $IC_{50}$ values of Lentinula edodes without UVB, with medium UVB, and with high UVB were determined to be 0.584, 0.519, and 0.386 mg/ml, respectively, in this search. Upon reviewing the literature, it was noted that there was inconsistency between the results obtained from the studies analyzing the $IC_{50}$ value of Lentinula edodes.

An investigation by Gąsecka and colleagues on the antioxidant characteristics of Pleurotus eryngii revealed an $IC_{50}$ value of 7.34 mg/ml [28]. $IC_{50}$ values of samples taken from different parts of Pleurotus eryngii (peel, fruiting body, gills, inner cap, and stipe) were observed to vary between 0.171 and 6.331 mg/ml in another study [27]. According to a study that assessed the antioxidant properties of excreted parts of Pleurotus eryngii, the $IC_{50}$ value of this mushroom ranged from 0.41 to 0.52 mg/ml [29]. $IC_{50}$ values of Pleurotus eryngii without UVB, with medium and high UVB, were 1.226, 1.091, and 0.411 mg/ml, respectively, in this investigation. As with Lentinula edodes, inconsistent results were obtained when the $IC_{50}$ levels of Pleurotus eryngii were examined by reviewing the literature.

### 3.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay, as an electron transfer-based method, measures the ferric ion ligand complex reduction by antioxidants in an acidic environment to the ferrous complex [30]. This assay assesses the reducing potential of a sample as a means to gauge its antioxidant capacity. Increased antioxidant activity is correlated with increased reduction potential [31].

Agaricus bisporus had FRAP values ranging from 1.35 to 11.62 µMTE/g in a study where Smolskaitė and colleagues tested the antioxidant properties of different mushroom species [32]. Gan et al., in their antioxidant analysis study on aqueous and 60% ethanol extracts of different mushrooms, determined the FRAP values of Agaricus bisporus as 186.72 and 84.69 µM $Fe^{2+}$ equivalent/g extract, respectively, according to these extraction types [23]. The FRAP values of Agaricus bisporus with no UVB, medium UVB, and high UVB were found to be 0.107, 0.199, and 0.216 mM$FeSO_4$/mg extract, respectively, in this research. Reviewing the data, it became clear that, similar to other analyses, the FRAP values of Agaricus bisporus demonstrated variations in the literature.

Investigating the impact of heat and extraction duration on the antioxidant capacities of Lentinula edodes, Baptista et al. observed variations in the FRAP values of the mushroom samples, ranging from 0.434 to 1.197 mMTE/g of extract [33]. The FRAP value of Lentinula edodes was 64.79 mg/ml, according to research conducted by Carneiro et al. [34]. A study on how UVB rays affect the antioxidant properties of

Lentinula edodes revealed that UVB application increased the FRAP value, parallel to this investigation [9]. The findings of the current study demonstrated that the FRAP values of Lentinula edodes without UVB, with medium UVB, and with high UVB were 0.100, 0.105, and 0.110 mM$FeSO_4$/mg, respectively. It is imperative to underscore that FRAP values reported for Lentinula edodes show discrepancies across various studies in the literature.

During an examination of the antioxidant properties of Pleurotus species, the reported FRAP values for Pleurotus eryngii exhibited a range of 13.30 to 19.63 $Fe^{2+}$/g extract levels [35]. In an alternative investigation, the FRAP value for Pleurotus eryngii was determined to be 18.25 mg TE/g extract [36]. The FRAP values of Pleurotus eryngii without UVB, with medium UVB, and with high UVB were evaluated as 0.109, 0.260, and 0.273 mM$FeSO_4$/mg, respectively, in this experiment. Consistent with other analyses, the results identified in this study differ from those documented in the existing scientific literature.

### 3.3. The cupric ion reducing antioxidant capacity (CUPRAC) method

A copper reduction test called the CUPRAC assay was developed as an alternative to the FRAP test. In the CUPRAC test, copper acts as the oxidizing agent instead of iron. This procedure quantifies the potential of the antioxidants to convert cupric ($Cu^{2+}$) ions to cuprous ($Cu^{+}$) ions [30].

The CUPRAC values of Agaricus bisporus were from 0.07 to 1.19, according to a study searching the antioxidant activity of Agaricus species [37]. A study examining the bioactive characteristics of various mushroom species discovered that, depending on the extraction method, the CUPRAC value of Agaricus bisporus measured between 49.69 to 132.40 µMTE/g extract [38]. According to this study, Agaricus bisporus with no UVB, medium UVB, and high UVB had CUPRAC values of 0.146, 0.284, and 0.324 mM/mg extract, respectively. In comparison, it could be seen that, like other analyses, the results in the literature were in contradiction.

Lentinula edodes was reported to have CUPRAC values that differ from 8.04 to 19.51 mgTE/g extract in a study assessing its antioxidant activity [39]. In another investigation, the reported CUPRAC values for Lentinula edodes spanned from 0.155 to 2.578 mg/ml [40]. According to findings from a parallel investigation, the CUPRAC values of Lentinula edodes measured between 42.38 and 171.60 µMTE/g extract [32]. The current investigation yielded CUPRAC values of 0.108, 0.176, and 0.247 mMTE/mg extract for Lentinula edodes with no UVB, medium UVB, and high UVB, respectively. The outcomes were different when compared with relevant research in the literature.

Pleurotus eryngii was identified with CUPRAC values ranging from 0.125 to 2.657 mg/ml in a study exploring the antioxidant properties of different mushroom species [40]. The CUPRAC values of the three Pleurotus eryngii forms tested in this work were 0.071, 0.240, and 0.292 mMTE/mg extract for the forms with no UVB, medium UVB, and high UVB, respectively. Consistent with prior research, the findings of this study exhibited variances compared to the documented literature.

This observed situation is predicted to arise from a multitude of factors, such as variety among species, variations in cultivation methods, environmental conditions, extraction times and techniques, developmental phases during harvest, and different parts of the mushrooms having different components.

This study is envisioned to provide support to the current literature, considering the limited research available on the effects of UVB application on mushrooms and their antioxidant properties. Moreover, the absence of any literature documenting a concurrent examination of these mushroom species, coupled with a comparative analysis of their antioxidant properties against vitamins $D_2$ and $D_3$, emphasizes the originality of this study and highlights its strength. Another notable aspect of this research stems from the lack of studies assessing vitamins $D_2$ and $D_3$ using DPPH, FRAP, and CUPRAC methods, which contributes to the distinctive strengths of the current study. However, a thorough review of the literature reveals a clear impact of extraction methods on antioxidant activity. Thus, the utilization of a single extraction method in this study imposes a limitation on the research. Furthermore, scientific literature illustrates the variability in antioxidant properties among different parts of mushrooms, so the fact that different parts of the mushroom were not examined in this study constitutes another limitation of the study.

## 4. CONCLUSION

Consequently, it was determined that UVB application positively affected the antioxidant properties of mushrooms in this study. However, to fully determine the effect of UVB on the antioxidant properties of mushrooms, further studies should be conducted on more mushroom species, using different extraction methods and applying UVB to different parts of the mushrooms. Additionally, future research could focus

on the bioavailability of the enhanced nutrient profiles in mushrooms resulting from UVB application to better understand their potential health benefits.

## 5. MATERIALS AND METHODS

### 5.1. Supply of samples

White button (Agaricus bisporus), Shiitake (Lentinula edodes), and King oyster mushrooms (Pleurotus eryngii) with and without UVB application were obtained from the Elit Engineering Food Industry. Vitamins $D_2$ (SIGMA PHR1238) and $D_3$ (SIGMA BP787) were purchased from Sigma Aldrich.

### 5.2. Drying mushrooms and UVB application

The drying process was applied to non-UVB-treated mushrooms. The mushrooms were dried at 55 °C for approximately 15-17 hours by turning the trays 90° in the same direction every 2-3 hours and were pulverized with a homogenizer.

UVB-treated mushrooms were dried using the same method and sliced into 5-15 mm pieces. Then, UVB was applied to some of the three mushroom species at a medium level (200-222 mj/cm²) and to some at a high level (390-430 mj/cm²) for 90 seconds. After the process, the mushrooms were pulverized with a homogenizer.

### 5.3. Classification of mushrooms

Classification of mushrooms according to UVB application is shown in Table 2.

**Table 2.** Classification of mushrooms according to UVB application

| UVB Application | Mushroom Species | | |
|---|---|---|---|
| | **Agaricus bisporus** | **Lentinula edodes** | **Pleurotus Eryngii** |
| Non-UVB | E1 | E4 | E7 |
| Medium UVB | E2 | E5 | E8 |
| High UVB | E3 | E6 | E9 |

[a] E: Extract

[b] E1: White button mushroom with non-UVB, E2: White button mushroom with medium UVB, E3: White button mushroom with high UVB, E4: Shiitake mushroom with non-UVB, E5: Shiitake mushroom with medium UVB, E6: Shiitake mushroom with high UVB, E7: King oyster mushroom with non-UVB, E8: King oyster mushroom with medium UVB, E9: King oyster mushroom with high UVB

### 5.4. Extract preparation

Each sample weighed 10 grams and was mixed with 100 ml of ethanol. After waiting for 48 hours, it was filtered with coarse filter paper. After repeating this procedure three times, the resulting filtrate was evaporated in a steam bath to obtain a solid extract. Before usage, the extracts were kept under storage conditions at +4 ºC.

### 5.5. In vitro antioxidant assays

The antioxidant properties of the mushrooms were compared with pure vitamin D2 and D3 solutions by FRAP assay, CUPRAC method, and DPPH radical scavenging test.

In the DPPH experiment, 10 µL each of the samples prepared at different concentrations (0.5-3 mg/mL) and the standard solution (ascorbic acid) prepared at the same concentration were taken, and 240 µL of 0.1 mM DPPH (in methanol) solution was added. The standard solution and the samples to which DPPH solution was added were vortexed for a minute and then kept at room conditions and in the dark for 30 minutes. Absorbances were measured against the reference at 517 nm in a microplate reader. Under the same circumstances, a control was prepared using 10 µL of methanol in place of the samples and standard substance. The average values were determined after the experiment was conducted three times [41].

FRAP assay was performed by modifying the Benzie and Strain method [42]. FRAP reagent was prepared and for 30 minutes kept at 37 °C. After mixing 190 µL of the FRAP reagent with 10 µL of either the

standard [butylated hydroxy anisole (BHA)] or the sample (3 mg/mL), the increase in absorbance against the blank that was prepared by replacing the extract with methanol was measured at 593 nm in the 4th minute. FRAP values for the samples were given as mMFeSO4/mg extract.

The method developed by Apak et al. was used to compute the samples' CUPRAC values [43]. 60 µL each of neocuproin, copper (II) and ammonium acetate buffer solutions were added into the microplate. Then, 60 µL of samples at 0.5 mg/ml concentration and 10 µL of methanol were added. For 60 minutes, the microplate was kept closed at room temperature. At 450 nm, the wavelength of absorption of the samples was measured against the reference. CUPRAC values for the samples were expressed as mMTE/mg extract.

The design of the study is shown in Figure.

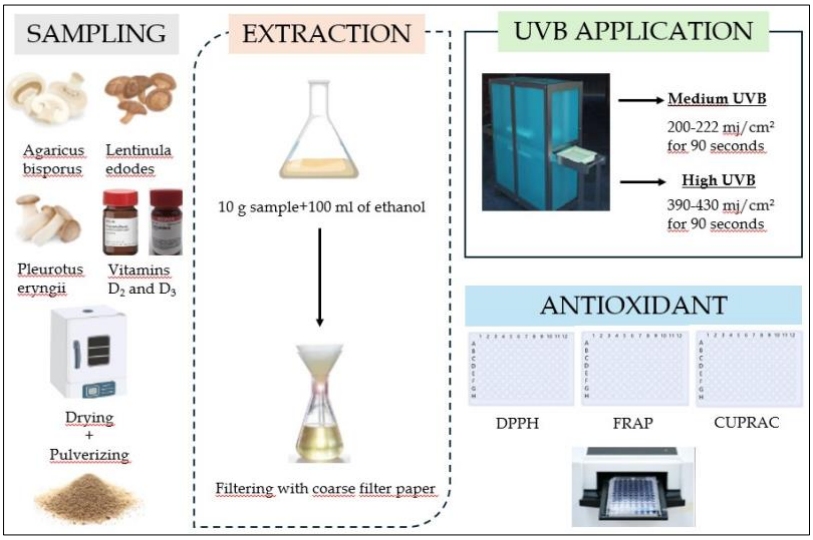


**Figure.** The design of the study

### 5.6. Statistical analysis

Triplicate assays were performed to test the antioxidant capacity, and the findings were expressed as mean ± standard deviations for all data. Using Minitab-18 software, the one-way ANOVA and post-hoc Tukey test were used to evaluate the statistical significance. $P<0.05$ was considered as statistically significant.

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