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Heavy metals content and the role of *Lepiota cristata* as antioxidant in oxidative stress

Abstract

The present study aimed to determine antioxidant/oxidant potential and heavy metal content of *Lepiota cristata* (Bolton) P. kumm. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the mushroom were determined using Rel Assay kits. Fe, Zn, Cu, Pb and Ni content were measured by a wet decomposition method using a Perkin Elmer AAnalyst 400 AA spectrometer. The study findings demonstrated that TAS value of *L. cristata* was 2.210±0.068, TOS value was 24.357±0.129 and OSI was 1.103±0.030. It was also determined that Fe content was 381.07±4.847, Zn content was 73.93±2.078, Cu content was 14.16±0.876, Pb content was 8.517±0.623, and Ni content was 3.09±0.30. Thus, it was determined that the mushroom had antioxidant potential. However, due to the high oxidant content, it was considered that the mushroom could only be used as an antioxidant source by collecting samples that grow in adequate regions where the mushroom exhibits low oxidative stress index after determination of the compounds with antioxidant effect in these samples. Furthermore, it was determined that *L. cristata* Fe, Zn and Ni content were within the ranges reported previously in the literature, however Cu content was lower and Pb content was higher when compared to the ranges reported in the literature.

Keywords: *lepiota cristata*, antioxidant, oxidant, oxidative stress, heavy metals.

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Introduction

Mushrooms have been significant natural sources over the years due to their taste, economic and ecological significances, as well as their medical properties.[1] Mushrooms contain several essential amino acids as well as vitamins (thiamin, riboflavin, ascorbic acid, ergosterol and niacin).[2,3] Mushrooms can be considered functional nutrients that provide health benefits due to their medical properties in addition to their nutritional value. It was reported that several mushrooms possess antioxidant properties as well. Mushroom organism also contains antioxidant compounds such as phenolics, polysaccharides, tocopherols, flavonoids, carotenoids, glycosides, ergothioneine and ascorbic acid.[3–5] Due to these compounds, mushrooms are natural antioxidant sources that alleviate the effects of oxidative stress induced by oxidant compounds in living organisms. Thus, the determination of antioxidant potential of mushrooms is very important for the identification of new natural resources that could be used against diseases induced by oxidative stress. Mushrooms are significant saprotrophic organisms in addition to their use as medical and nutritional sources. Mushrooms store several elements based on the substrate content in their habitat. Due to these properties, mushrooms could also be used as pollution indicators. Thus, the pollution in their habitats could be detected by identifying the elements accumulated in this organisms.[6] In their natural habitat, the fruiting bodies of *L. cristata* are scattered or clustered over the land and they are usually observed in late summer or early fall. *L. cristata* is a saprophyte that commonly grows in paths, ditches, lawns, roads, gardens and roadsides.[7] In the present study, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and Fe, Cu, Zn, Pb and Ni content were determined in *L. cristata* (Bolton) P. Kumm ethanol extracts.

Material and method

Laboratory studies

Lepiota cristata samples used in the study were collected in Gaziantep province. Morphological (shape, color, size) and ecological characteristics of the samples were recorded in the field conditions. The microscopic characteristics of the specimens transported to the laboratory under appropriate conditions were determined by light microscopy using a%3 KOH solution (Leica DM750). The specimen was identified morphologically using the references of Breitenbach et al.[8–11] After the identification of the collected mushroom samples, they were dried at 40°C. Then, they were pulverized in a mechanical grinder. Pulverized mushroom samples were extracted with ethanol (EtOH) in a Soxhlet apparatus (Gerhardt EV 14). The mushroom extracts were then concentrated under pressure at 40°C in a rotary evaporator (Heidolph Laborator 4000 Rotary Evaporator) and stored at +4°C.

TAS, TOS and OSI tests

TAS, TOS and OSI values of the mushroom ethanol extracts were measured with Rel Assay brand commercial kits (Assay Kit Rel Diagnostics, Turkey). TAS and TOS tests were conducted on 5 different mushroom samples in 5 replicates. In TAS tests, Trolox was used as calibrator and hydrogen peroxide was used as calibrator in TOS tests.[12,13] OSI (Arbitrary unit: AU) values were calculated with the following formula and the results were indicated in percentages.[13]

$$OSI\left(AU\right)=\frac{TOS,\mu mol\,H_2O_2\,equiv./L}{TAS,mmol\,Trolox\,equiv./L\,X10}$$

Determination of heavy metal content

The mushroom samples were dried in an incubator at 40°C. Dried mushroom samples were pulverized in a grinder. Three 1gr specimens were taken from the mushroom samples in three replicates and 50mL glass beakers. Then, 10mL concentrated HNO_3 was added to the beakers and stored at room temperature for 24-48 hours. These samples were heated on the heating plate until they turned into sediment. Then, 10mL concentrated HCI was added and the heating process was reconducted. After the reheating, 20mL of diluted HCI was added into the beakers and the samples were filtered. Heavy metal analysis was conducted with a Perkin Elmer (AAnalyst 400) device.[14]

Result and discussion

TAS, TOS and OSI

In the present study, the TAS value of *L. cristata* was determined as 2.210±0.068, the TOS value was determined as 24.357±0.129 and the OSI value was determined as 1.103±0.030. There were no previous studies that determined the total antioxidant and oxidant potential and oxidative stress status of *L. cristata*. Oxidative stress studies on different mushroom species reported that *Cyclocybe cylindracea* TAS value was 4.325, the TOS value was 21.109 and the OSI was 0.488.[15] It was reported that the TAS value of *Pholiota limonella* was 2.378, TOS value was 4.742 and the OSI value was 0.199.[16] In previous studies, it was determined that the TAS values of *Auricularia auricula* and *Trametes versicolor* were 1.010 and 0.820, TOS values were 23.910 and 17.760, and OSI values were 2.367 and 2.166, respectively.[17] In other studies, the TAS value of *Pleurotus eryngii* was reported as 1.93 and the TAS value of *Auricularia polytricha* was reported as 0.93.[18,19] It was found in the present study that the antioxidant potential of *L. cristata* was higher when compared to previous studies conducted on *A. auricula, T. versicolor, P. eryngii* and *A. polytricha*. It was also determined that the antioxidant potential of *L. cristata* was lower when compared to *C. cylindracea* and *P. limonella* mushrooms. It was considered that the above-mentioned differences were due to the differences in the potential of these mushrooms to produce antioxidant compounds. Analysis of the oxidant compounds produced by *L. cristata* and the environmental and physical factors demonstrated that the mushroom had higher TOS value when compared to that of *A. auricula, T. versicolor, C. cylindracea* and *P. limonella* mushrooms. It was suggested that the the differences among the TOS values were due to the differences between the regions they were collected and metabolic differences between the mushroom species and resulting variances in oxidant compound production capacities. The OSI value demonstrates the rate the antioxidant compounds produced by the mushrooms inhibit the oxidant compounds produced by the mushroom. Analysis of the oxidative stress levels demonstrated that *L. cristata* exhibited a lower OSI value when compared to *A. auricula* and *T. versicolor* mushrooms. On the other hand, it was observed that *L. cristata* had a higher OSI value when compared to *C. cylindracea* and *P. limonella* mushrooms. Thus, it was determined that *L. cristata* had antioxidant potential. In this context, it was suggested that *L. cristata* could be consumed as a natural source after the antioxidant compounds in samples collected in regions with suitable oxidative stress index are determined.

Heavy metal content

Mushrooms have the capacity to biologically store most heavy metals in their fruiting body. Mushrooms could absorb the metals from the substrate via their mycelia. The metal concentrations in the fruiting bodies are affected by the age of mycelium and the interval between fructification (formation of the fruiting body).[20] These properties of the mushrooms vary across the species. Thus, different species of mushrooms might indicate the presence of various elements in the environment. In the present study, Fe, Cu, Zn, Pb and Ni content of *L. cristata* were investigated. The present study findings are presented in Table 1 as mg/kg±Std. In previous studies, it was reported that the Cu content of *Lepiota cristata* collected in Balıkesir province (Turkey) was 41.67 and the Zn content was 45.58.[21] In a different study, it was found that the Fe content in *L. cristata* collected at Larger Menderes River Basin (Turkey) was 444.8, Cu content was 26.41, Ni content was 3.866, Zn content was 50.04, and Pb content was 2.413.[22] Furthermore, in previous studies, the lowest and highest element content of different mushroom species were determined as 14.6-835 for Fe, 29.8-306 for Zn, 64.8-290 for Cu, 0.04-6.88 for Pb and 1.18-5.14 mg.kg^{-1} for Ni.[23–25] *L. cristata* used in the present study was collected in Gaziantep province (Turkey) and it was determined that its Fe, Cu and Ni content were lower when compared to samples collected at Larger Menderes River Basin. It was also determined that Zn and Pb content were higher when compared to those collected at Larger Menderes River Basin. Furthermore, it was observed that *L. cristata* mushroom used in our study had higher Zn content and lower Cu content when compared to the samples collected in Balikesir. On the other hand, it was found that Fe, Zn and Ni content of *L. cristata* collected in Gaziantep province were within the ranges reported in the literature. It was also determined that the Cu content was lower and the Pb content was higher when compared to the values reported in the literature. It was suggested that the differences among the findings could be due to the differences between regional pollution, even though the investigated mushroom species was the same. It was observed that the mushroom stored more Pb when compared to the findings in the literature and samples collected in different regions. Thus, it was suggested that *L. cristata* mushroom could be utilized as a Pb pollution indicator (Figure 1).

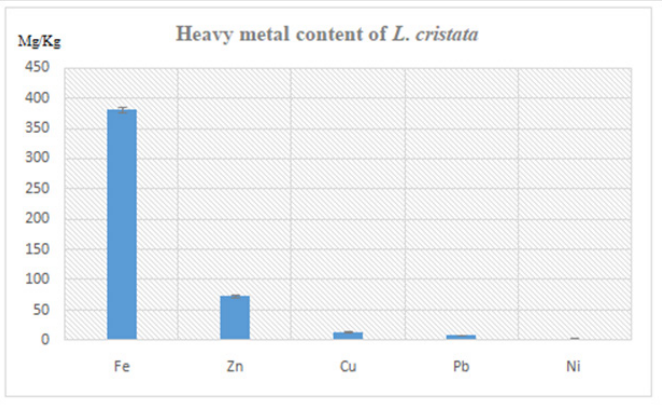

Figure 1 Heavy metal content of *L. Cristata.*

Table 1 Heavy metal content of *L. cristata*

	Fe	Zn	Cu	Pb	Ni
L. cristata	381.07±4.847	73.93±2.078	14.16±0.876	8.517±0.623	3.09±0.30

Values are presented as mean±SD; number of mushroom samples n=6; experiments were made as 3 parallels.

Conclusion

In the present study, total antioxidant status, total oxidant status, oxidative stress index of *L. cristata* mushroom was determined for the first time in the literature. It was observed that the mushroom demonstrated antioxidant potential in the study. In addition, it was identified that the oxidative stress index was high due to high levels of oxidant compounds. It was considered that the mushroom could only be used as an antioxidant source by collecting samples that grow in adequate regions where the mushroom exhibits low oxidative stress

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index after determination of the compounds with antioxidant effect in these samples. Furthermore, it was determined that the heavy metal levels in the mushroom were in the value ranges reported in the literature in general, however Pb levels were higher in the mushroom when compared to the other elements. Thus, it was considered that the mushroom could be used as a Pb pollution indicator.

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Conflict of interest

Author declares that there is no conflict of interest.

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