

# Phenolic Contents, Oxidant/Antioxidant Potential and Heavy Metal Levels in *Cyclocybe cylindracea*

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## ABSTRACT

**Introduction:** Mushroom species have been used for medical purposes and as nutrients since the old times. The present study aimed to determine phenolic content, antioxidant activity and heavy metal content of *Cyclocybe cylindracea* (DC.) Vizzini & Angelini mushroom. **Materials and Methods:** Phenolic contents were screened with HPLC devices. TAS, TOS and OSI values were determined using Rel Assay kits. Heavy metal content were determined with atomic absorption method using an atomic absorption spectrophotometer. **Result and Discussion:** Gallic acid, hesperidin, catechin, syringic acid and hydroxybenzoic acid were detected in the mushroom. It was determined that the heavy metal content in *C. cylindracea* were generally at optimal levels and the TOS value of mushroom was high. **Conclusion:** *C. cylindracea* could be consumed as a good antioxidant source as long as it is obtained from safe areas.

**Key words:** *Cyclocybe cylindracea*, Antioxidant, Oxidant, Oxidative stress, Heavy metal, Phenolic contents.

## INTRODUCTION

Most of mushroom species that could conduct a cosmopolitan life in all habitats could be consumed as food and for medical purposes because of their biological properties.<sup>1,2</sup> Mushrooms are not preferred only for their texture and taste, but they are also rich in protein and amino acids and poor as a calorie source.<sup>3</sup> In addition to their nutritional value, mushrooms were reported to have medical effects such as antibacterial, antiviral, anti-tumor, antioxidant, anti-inflammatory and immune system-booster.<sup>4-8</sup>

Mushrooms include many secondary metabolites such as phenolic compounds, polyketides, terpenes and steroids.<sup>9</sup> It is known that these secondary metabolites show important medicinal properties in spite of lacking nutritive values.<sup>10</sup> These secondary metabolites play a vital role in the antioxidant defense mechanisms of biological systems.<sup>11</sup> Mushrooms can also help to reduce the effects of oxidative stress due to their

antioxidant potential. Because of oxidative stress induced by free radicals, serious health problems such as neurological, cardiologic diseases, cirrhosis, diabetes, leukemia and necrosis may occur.<sup>12,13,14</sup> Investigation of antioxidant activity and oxidative stress index of mushrooms as potent natural antioxidant used in inhibition of oxidative stress is very important to determination of new natural antioxidant materials.

Heavy metal contents in mushrooms are higher than agricultural plants, vegetables and fruits.<sup>15</sup> Due to their saprotrophic properties, mushrooms can also be used as natural pollution indicators through element levels accumulated in their bodies due to substrate content during organic matter catabolism.<sup>16,17</sup> Elements such as Fe, Co, Cu, Mn, Mo, Sr, V and Zn that are required for living organisms in trace amounts can accumulate in living organisms over time and lead to undesirable

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consequences.<sup>18</sup> Thus, to determine the element levels of mushrooms is important to avoid the health problems that may arise due to their consumption via the food chain.

The present study aimed to determine total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and heavy metal contents (Fe, Cu, Zn, Pb, Ni, Mn, Co, Cd and Cr) in *Cyclocybe cylindracea* (DC.) Vizzini and Angelini mushroom.

## Experimental

*Cyclocybe cylindracea* (DC.) Vizzini and Angelini mushroom samples were used in this study collected at Oğuzeli (Gaziantep Province / Turkey) region.

### Laboratory Studies

The mushroom samples were dried at the incubator at 40°C, then pulverized in a mechanical grinder, and 30 g samples were extracted with ethanol at 50°C for about 6 h in the soxhlet device (BUCHI Extraction System Model B-811). The extracts were concentrated under pressure with a rotary evaporator and were stored at 4°C until analyzed (BUCHI Rotavapor Model R-144).

### Determination of Phenolic Contents

Extracted mushroom samples were processed with some modifications of the method developed by Caponio *et al.*<sup>19</sup> using SHIMADZU system HPLC and DAD detector: Injection volume was adjusted to 20 µL. Acetic acid (3%) was used as mobile phase A and methanol was used as mobile phase B (Table 1) and the flow rate was regulated to 0.8 mL per min. Chromatographic separation was conducted with Agilent Eclipse XDB-C18 column (250x4.6 mm id 5 µM) at 30°C.

### Determination of TAS, TOS and OSI Values

The TAS and TOS values of the extracted mushroom samples were determined with Rel Assay brand kits (Rel Assay Kit Diagnostics, Turkey). Trolox was used as calibrator in identification of the TAS values and the results were given as mmol Trolox equiv./L. To determine the TOS values, hydrogen peroxide was used as calibrator

and the results were obtained as µmol H<sub>2</sub>O<sub>2</sub> equiv. /L.<sup>20,21</sup> The formula used to calculate OSI values is in the below:<sup>21</sup>

$$OSI = \frac{TOS, \mu\text{mol H}_2\text{O}_2 \text{ equiv. / L}}{TAS, \text{mmol Trolox equiv. / L}} \times 100$$

### Determination of Heavy Metal Levels

The mushroom samples were dried in the incubator at 40°C and pulverized with a mechanical grinder, weighed and 1 g of samples were placed in 50 mL Erlenmeyer flasks in 3 replicates and 10 mL HNO<sub>3</sub> was added to the flasks and stored at room temperature for 24 to 48 h. The stored erlenmeyer flasks were heated with a hot plate until the solution was cleared. Concentrated HCl (10 mL) was added to the heated erlenmeyer flasks and the firing process was repeated. After the firing process, 20 mL diluted HCl solution was added to the prepared solution, and the filtered solution was prepared for further analyses.<sup>22</sup> The element contents of prepared solutions were determined with a Perkin Elmer (AAAnalyst 400) device.

## RESULTS AND DISCUSSIONS

### Phenolic Contents

The phenolic compounds of *C. cylindracea* were determined using HPLC and the findings are shown in Table 2.

In context of investigations, 5 phenolic compounds were determined such as gallic acid, hesperidin, catechin, syringic acid and hydroxybenzoic acid. Phenolic compounds are known to have many activities, especially antioxidant activity.<sup>23</sup> In some studies, among the various polyphenols, it has been suggested that gallic acid (3,4,5-trihydroxybenzoic acid), a naturally occurring low-molecular-weight triphenolic compound, has strong antioxidant activities.<sup>24</sup> Hesperidin, a member of the flavonoid group, is found in mushrooms such as *Cordyceps sinensis* and *Ganoderma lucidum* in addition to citrus species.<sup>25</sup> It has been reported that hesperidin

Table 1: HPLC gradient program.

Min	0.1	20	28	35	50	60	62	70	73	75	80	81
%A	93	72	75	70	70	67	58	50	30	20	0	93
%B	7	28	25	30	30	33	42	50	70	80	100	7

%A: % 3 Acetic acid, % B: Methanol

Table 2: Phenolic contents of *C. cylindracea*.

Gallic acid	Catechin	Syringic acid	Hydroxy benzoic acid	Hesperidin
2.17 ppm	12.71 ppm	1.72 ppm	6.48 ppm	75.58 ppm

**Table 3: TAS, TOS and OSI Values.**

	TAS	TOS	OSI
<i>C. cylindracea</i>	4.325± 0.133	21.109±1.207	0.488±0.013

Values are presented as mean±S.D.; n=6 (Experiments were made as 5 parallel)

**Table 4: Heavy Metal Contents.**

Elements	Contents mg.kg <sup>-1</sup> ±Std	Literature ranges mg.kg <sup>-1</sup>
Fe	509,18±5,30	14.60-835.00
Cu	60,33±5,60	71.00-95.00
Zn	33,93±3,55	29.80-158.00
Pb	16,54±0,93	2.86-6.88
Ni	0,67±0,05	1.18-5.14
Mn	47,82±1,05	18.10-103.00
Co	5,47±0,20	0.01-8.27
Cd	4,67±0,16	2.71-7.50
Cr	9,63±1,44	10.70-42.70

Values are presented as mean±S.D.; n=3 (Experiments were made as 3 parallel)

has antioxidant, antiallergic, anti-carcinogenic, antihypertensive, antimicrobial and vasodilator properties.<sup>26</sup> Catechins exhibited significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, antimutagenic, probiotic and antimicrobial properties in the result of *in vivo* and *in vitro* studies.<sup>27</sup> Syringic acid is known to exhibit multiple bioactive properties such as strong antioxidant, antiproliferative, antiendotoxic, antimicrobial, anti-inflammatory and anti-cancer effects.<sup>28</sup> In a study, authors stated that hydroxybenzoic acid has antioxidant effect.<sup>29</sup> In our work, high antioxidant activity is might be due to the presence of phenolic compounds as extracted from *C. cylindracea* samples.

In addition, it is suggested that this compound has a higher contribution to antioxidant activity because the highest value of the compounds detected is 75.58 ppm of hesperidin. It should be evaluated separately antioxidant activities of the phenolic compounds identified in the same system to investigate the effective levels of the obtained compounds and to make certainly judgments.

#### TAS, TOS and OSI Values

TAS (mmol/L), TOS (μmol/L) and OSI values of *C. cylindracea* collected in Oguzeli (Gaziantep / Turkey) were determined with Rel Assay commercial kits and are presented in Table 3.

As seen in Table 3 TAS value of *C. cylindracea* was 4.325 mmol/L and the TOS value was 21.109 μmol/L. Also OSI value was 0.488. In the previous study, it was found that TAS value of *Tricholoma terreum* (Schaeff.) mushroom samples collected from Mugla province was determined as 0.38, TOS value of the same mushroom

specie was 16.76 and OSI value was 4.41.<sup>22</sup> In other studies, it was determined that the TAS value of *Pleurotus eryngii* (DC.) Quél was 1.93 and the TAS value of *Auricularia polytricha* (Mont.) Sacc. was 0.93.<sup>30,31</sup> In the present study higher TAS and TOS data of *C. cylindracea* were observed compared to previous studies. The high TAS value of *C. cylindracea* indicated that had its rich antioxidant potential. However, similarly higher TOS value indicated that higher amounts of oxidative agents presented in the mushroom due to environmental and metabolic factors. The OSI value, on the other hand, is the ratio of how much of the oxidant compounds produced by environmental and physiological processes in the structure of the living organism could be tolerated by antioxidant molecules that the organisms produced. The *C. cylindracea* OSI level was lower than that of *T. terreum*. These findings demonstrated that *C. cylindracea* was rich in antioxidant compounds that could neutralize oxidant molecules and could produce more antioxidant molecules compared to *T. terreum*. It was reported that the TAS levels of *Omphalotus olearius* (DC.) Singer fungi collected from different regions varied due to regional differences, although TAS values were close to each other.<sup>16</sup> High TAS value of *C. cylindracea* obtained in the present study indicated that this mushroom could be used as a rich natural antioxidant source. However, when the TOS and OSI values are considered, it may be advisable to collect this mushroom from more adequate and pollutant-free regions.

#### Heavy Metal Content

Fe, Cu, Zn, Pb, Ni, Mn, Co, Cd and Cr contents of *C. cylindracea* were determined with heavy metal analyses and presented in mg.kg<sup>-1</sup>. The heavy metal contents are demonstrated in Table 4 as Average ± Std.

Mushrooms are also known to accumulate elements.<sup>16</sup> The results of our studies conducted with previous study reported the lowest and highest element levels as 14.6-835.0 for Fe, 71.0-95.0 for Cu, 29.8-158.0 for Zn, 2.86-6.88 for Pb, 1.18-5.14 for Ni, 18.1-103.0 for Mn and 2.71-7.50 mg.kg<sup>-1</sup> for Cd levels of mushrooms.<sup>32</sup> Furthermore, Co content was reported as 0.01-8.27 mg.kg<sup>-1</sup> and Cr content was indicated as 10.7-42.7 mg.kg<sup>-1</sup>.<sup>15,33</sup> Fe, Zn, Mn, Cd and Co values obtained in our study were within the ranges mentioned above. It was observed that Cu, Ni and Cr values were at the lower and Pb amount was at the higher. The higher Pb

content could be due to the fact that the mushroom samples were collected at a roadside area.

## CONCLUSION

Conducted analyses demonstrated that *C. cylindracea* samples collected at Oguzeli (Gaziantep / Turkey) region contained high levels of Pb, while other heavy metal elements were at normal levels. In addition, bioactive phenolic compounds with antioxidant properties such as gallic acid, hesperidin, catechin, syringic acid and hydroxybenzoic acid have been identified in ethanolic extracts of the mushroom samples. It is suggested that this mushroom could be used as an effective and rich natural antioxidant supplement due to its high antioxidant potential. However, when Pb levels and TOS values are taken into account, it is recommended that these mushrooms should be obtained from cleaner and safer regions.

## CONFLICT OF INTEREST

The Authors declare no conflict of interest.

## ABBREVIATIONS

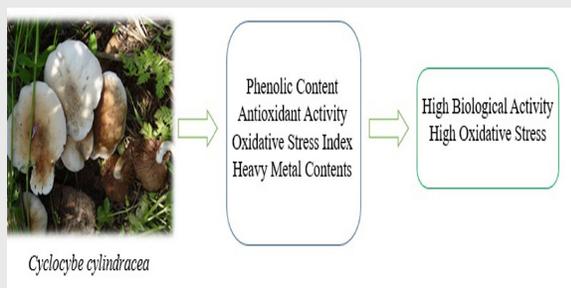
**TAS:** Total Antioxidant Status; **TOS:** Total Oxidant Status; **OSI:** Oxidative Stress Index; **HPLC:** High Performance Liquid Chromatography.

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## PICTORIAL ABSTRACT



## SUMMARY

Ethanol extracts of *Cyclocybe cylindracea* were prepared. Then phenolic contents, total antioxidant level, total oxidant level, oxidative stress index and heavy metal contents were determined. It has been determined that *C. cylindracea* may be a natural antioxidant source at the end of the studies. Heavy metal levels were found to be at appropriate levels. Gallic acid, hesperidin, catechin, syringic acid and hydroxybenzoic acid were also determined in the mushroom.

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