Bartın Orman Fakültesi Dergisi | Journal of Bartin Faculty of Forestry
2024, 26(1): 7–12 | DOI: 10.24011/barofd.1386438

Investigation of the Phenolic Contents and Antioxidant Activities of Some Natural Edible Mushroom Species

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Article History

Received: 05.11.2023
Accepted: 24.01.2024
Published: 29.01.2024

Research Article

Abstract − In recent years, edible mushroom species have become a part of the daily human diet due to their high protein content. These mushrooms have also gained popularity in alternative medicine practices due to their chemical composition and antioxidant properties. This study aimed to determine the biologically important antioxidant activities and total phenolic content of four different mushroom species, each with its unique appearance and habitat: *Amanita caesarea* (*Aca*), *Clitocybe geotropa* (*Cge*), *Cordyceps militaris* (*Cmi*) and *Lentinula edodes* (*Led*). The antioxidant activities of the mushroom species were determined using the DPPH radical scavenging method, and the percentage inhibition and IC_{50} values were reported. The analysis of inhibition values at various concentrations revealed that both *Cmi* and *Aca* mushrooms demonstrated higher antioxidant activity when compared to *Led* and *Cge* mushrooms across all tested concentrations. Moreover, the phenolic content of the methanolic extracts, quantified in gallic acid equivalents (GAE), were determined to be 37.04±0.35, 52.04±0.41, 19.33±0.11, and 21.63±0.15 mg GAE/g for *Cmi*, *Aca*, *Led*, and *Cge*, respectively. In conclusion, a direct correlation was noted between the overall phenolic content and the antioxidant activity of the various mushroom species.

Keywords − DPPH radical scavenging method, Amanita caesarea, Clitocybe geotropa, Cordyceps militaris, Lentinula edodes.

Doğal Tüketilebilir Bazı Mantar Türlerinin Fenolik İçeriklerinin ve Antioksidan Aktivitelerinin Araştırılması

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Makale Tarihçesi

Gönderim: 05.11.2023
Kabul: 24.01.2024
Yayım: 29.01.2024

Araştırma Makalesi

Öz − Son yıllarda yenilebilir mantar türleri, yüksek protein içeriğinden dolayı günlük insan beslenmesinin bir parçası haline gelmiştir. Bu mantarlar kimyasal bileşimleri ve antioksidan özellikleri nedeniyle alternatif tıp uygulamalarında da popülerlik kazanmıştır. Bu çalışma, her biri kendine özgü görünüm ve yaşam ortamına sahip dört farklı mantar türünün biyolojik olarak önemli antioksidan aktivitelerini ve toplam fenolik içeriğini belirlemeyi amaçlamıştır: *Amanita caesarea* (*Aca*), *Clitocybe geotropa* (*Cge*), *Cordyceps militaris* (*Cmi*) ve *Lentinula edodes* (*Led*). Mantar türlerinin antioksidan aktiviteleri DPPH radikal süpürme yöntemi kullanılarak belirlenmiş ve yüzde inhibisyon ve IC_{50} değerleri rapor edilmiştir. Farklı konsantrasyonlarda elde edilen inhibisyon değerlerine göre *Cmi* ve *Aca* mantarlarının her konsantrasyonda *Led* ve *Cge* mantarlarına göre daha yüksek antioksidan aktivite sergilediği tespit edilmiştir. Ayrıca, metanolik özütlerin fenolik içeriği galik asit eşdeğerleri (GAE) cinsinden belirlenmiş olup, *Cmi* için 37.04±0.35, *Aca* için 52.04±0.41, *Led* için 19.33±0.11 ve *Cge* için 21.63±0.15 mg GAE/g olarak tespit edilmiştir. Sonuç olarak mantar türlerinin toplam fenolik içeriği ile antioksidan aktivitesi arasında doğrusal bir ilişki gözlendi.

Anahtar Kelimeler − DPPH radikal sönümleme metodu, Amanita caesarea, Clitocybe geotropa, Cordyceps militaris, Lentinula edodes.

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1. Introduction

Natural edible mushrooms contain many bioactive compounds, including phenolic compounds that have antioxidant properties. For this reason, in recent years there has been a noticeable increase in research on the phenolic contents and antioxidant activities of mushroom species. (Kim et al., 2008). Antioxidants play important roles in preventing chronic diseases and protecting the body against oxidative stress. Therefore, information on antioxidant activity and the phenolic content of mushrooms is important in determining potential health benefits (Kozarski et al., 2015).

Many studies have been conducted to determine the phenolic compound concentrations and antioxidant activities of various edible mushroom species. In a study conducted in Korea, the phenolic compound content and antioxidant activity of five medicinal and edible mushroom species were examined, and the result of this research showed that these mushrooms have different concentrations of phenolic compounds and antioxidant activity and therefore they can be a good source of natural antioxidants (Kim et al., 2008).

Amanita caesarea (*Aca*), also called Caesar mushroom, which is distributed in Asia and Southern Europe, is at the center of medical studies, especially due to its phenolic content and antioxidant activity. Numerous studies investigating the phenolic content and antioxidant activities of *A. caesarea* have also revealed insights into its potential therapeutic uses. A study revealed that a polysaccharide extracted from *A. caesarea* has great potential in the treatment of Alzheimer's disease (Li et al., 2017; Hu et al., 2021).

Another mushroom species that attracts attention with its medicinal properties is *Clitocybe geotropa* (*Cge*). As a result of their study on the antioxidant and antigenotoxic abilities of this mushroom in 2020, Sevindik and his colleagues. identified a number of phenolic compounds such as protocatechuic acid, p-hydroxybenzoic acid, abscisic acid and cinnamic acid (Sevindik et al., 2020).

Cordyceps mushrooms, which are found in Asian cuisine, are known to contain phenolic compounds known for their antioxidant properties. In particular, *Cordyceps sinensis* was found to contain three times more total phenolics compared to *Ganoderma lucidum* (Ciric et al., 2020). In a later study, it was stated that *C. militaris* (*Cmi*) had antioxidant activity (Barido et al., 2020).

Lentinula edodes (*Led*), also known as Shiitake, is a type of edible mushroom that is also well known for its health benefits. As a result of a study, it was revealed that there is a connection between the bioactive components of *L. edodes* and its antioxidative, immune regulatory and anticancer properties, and it was stated that this was thought to be caused by the polysaccharides, phenolic compounds, ergosterols and terpenoids found in *L. edodes* (Wu et al., 2023). Moreover, another study revealed that *L. edodes* exhibited various pharmacological activities (Bisen et al., 2010).

These results emphasize the importance of phenolic contents and antioxidant activities of natural edible mushrooms. A better understanding of this issue can play an important role in the creation of functional foods and dietary supplements. These data may provide valuable information regarding the potential use of mushrooms as natural substitutes for synthetic antioxidants. Therefore, more studies are needed in this area to fully investigate the potential of mushrooms as a source of antioxidants and phenolic compounds.

The aim of this study is to determine the antioxidant activities and total phenolic content of four different mushroom species (*A. caesarea*, *C. geotropa*, *C. militaris*, and *L. edodes*) that have been the subject of important medical and biological studies.

2. Material and Methods

2.1. Chemicals

The SHIMADZU UVM-1240 UV-VIS spectrophotometer was utilised to measure the absorbances with a pair of identical quartz cuvettes, each with a thickness of one centimetre (Shimadzu Corp., Kyoto, Japan). Deionized water obtained from the Milli-Q system (18.2 MX/cm^3, Human Power I Plus, Korea) was used throughout every stage of this study.

In the study methanol was used for preparation of mushroom extracts. DPPH (1,1-diphenyl-2-picryl hydrazyl) used for determination of antioxidant activity. Folin & Ciocalteu′s phenol reagent, Gallic acid (3,4,5-Trihydroxybenzoic acid) and Carbonic acid disodium salt (Na_2CO_3, anhydrous, powder) were used for determination of total phenolic substance. All cehmicals were supplied by Merck KGaA, Darmstadt, Germany.

2.2. Preparation of Mushroom Extracts

The taxonomy of fresh *A. caesarea* and *C. geotropa* collected from Kastamonu province was determined by Prof. Dr. Sabri ÜNAL from the Faculty of Forestry at Kastamonu University. The morphological identification of *L. edodes*, which were grown in a culture medium, and *C. militaris* obtained from Istanbul was also performed. The mushrooms were cut into small pieces and dried for 48 hours at 30°C in a room dryer (NUVE KD 400, Turkey). The dried mushrooms were subsequently ground into a homogenous powder for use in the analyses. Mushroom extracts were prepared following a standard protocol provided by Bakır et al., 2018 with slight modifications (Bakır et al., 2018). The produced samples were placed in dark-colored bottles and then dissolved in 10 mL of a 75% methanol solution and kept in a mixer at 25°C for 4 hours. After that, the homogenate was centrifuged for 10 minutes at 7500 rpm (18°C). In the end 100 mg/mL of the supernatant was collected and used for total phenolic and DPPH assays (Lee et al., 2004).

2.3. Determination of Antioxidant Activity

Using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method, the antioxidant activity of the mushroom samples was determined based on the decreasing colour change of the purple solution of the DPPH radical, detected at 517 nm. The solutions prepared for this application were kept in dark at 24 degrees for 30 minutes. (Bozdoğan et al., 2018).

For this purpose, a stock solution of DPPH in methanol was prepared at a concentration of $4.0x10^{-5}$ M. Each mushroom solution was prepared in the range of 2.50-20.00 mg/mL, and measurements were taken for four different concentrations. The percentage inhibitions of the mushrooms were calculated using the following formula (2.3):

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100 \quad (2.3)$$

Where A_0 is the absorbance in the control, and A_1 is the absorbance in the presence of the samples. Antiradical activity, also known as IC_{50} (mg/mL), defines the amount of antioxidant required to reduce the initial DPPH concentration by 50% (Frankel and Meyer, 2000).

2.4. Determination of Total Phenolic Compound

The determination of the total phenolic component in the methanol extracts is based on the spectrophotometric determination of the maximum absorbance observed at 760 nm using the Folin-Ciocalteu reagent as a standard method. In this study, a solution of gallic acid was used as the standard. 0.1 mL of Folin-Ciocalteu reagent and 4.5 mL of deionized water were added in order to apply this procedure. Three minutes later, 0.1 mL of the extract solution and 0.3 mL of Na_2CO_3 (2%) solution were added, and the mixture was vigorously shaken. Then the solutions were kept in dark at 24°C for 45 minutes (Slinkard and Singleton, 1977; Chandler and Dodds, 1983).

Each mushroom's phenolic compound concentrations were determined using the following formula (2.4), which was derived from the typical gallic acid graph:

$$\text{Absorption} = 4.8\times10^{-3}.\text{Gallic Acid (mg)} + 0.042 \text{ , } R2 = 0.992 \quad (2.4)$$

2.5. Statistical Analysis

Descriptive statistical analysis was used to determine the significant link between the antioxidant concentration of the mushrooms using SPSS software (SPSS Inc., Chicago, IL, USA) for Windows version 13.

3. Results and Discussion

The concentration equations and total phenolic values used in the calculation of IC_{50} values with negative correlation for the comparison of antioxidant activities of mushroom extracts are presented in Table 1. Accordingly, the %50 DPPH free radical scavenging activities (IC_{50}) found for *Cmi*, *Aca*, *Led*, and *Cge* were 0.97±0.08, 0.15±0.02, 2.05±0.12, and 1.87±0.11 mg/mL, respectively. Additionally, the gallic acid equivalents (GAE) of total phenolics for *Cmi*, *Aca*, *Led*, and *Cge* were found to be between 37.04±0.35, 52.04±0.41, 19.33±0.11, and 21.63±0.15 mg/g, respectively.

Table 1
Concentration equations calculated by the DPPH method of *Cmi, Aca, Led and Cge* at different concentrations, IC_{50} values and total phenolic values.

	Total Phenolic (mg GAE/g)	**IC_{50} (mg/mL)**	**Concentration Equations**	**R^2**
Cmi	37.04±0.35	0.97±0.08	y=15.27x+35.20	0.930
Aca	52.04±0.41	0.15±0.02	y=12.05x+48.14	0.958
Led	19.33±0.11	2.05±0.12	y=13.77x+21.77	0.991
Cge	21.63±0.15	1.87±0.11	y=13.86x+24.12	0.948

*Data are expressed as mean value ± standart deviation (SD) of three parallel measurements.

It has been observed that the inhibition values of the methanol extracts of all mushroom samples increase with concentration. Inhibition values in the concentration range of 2.50-20.00 mg/mL varied as follows for *Cmi*, *Aca*, *Led*, and *Cge* respectively: % 45.79-91.58; % 57.83-93.30; % 33.94-75.93; and % 34.03-76.56 (Figure 1).

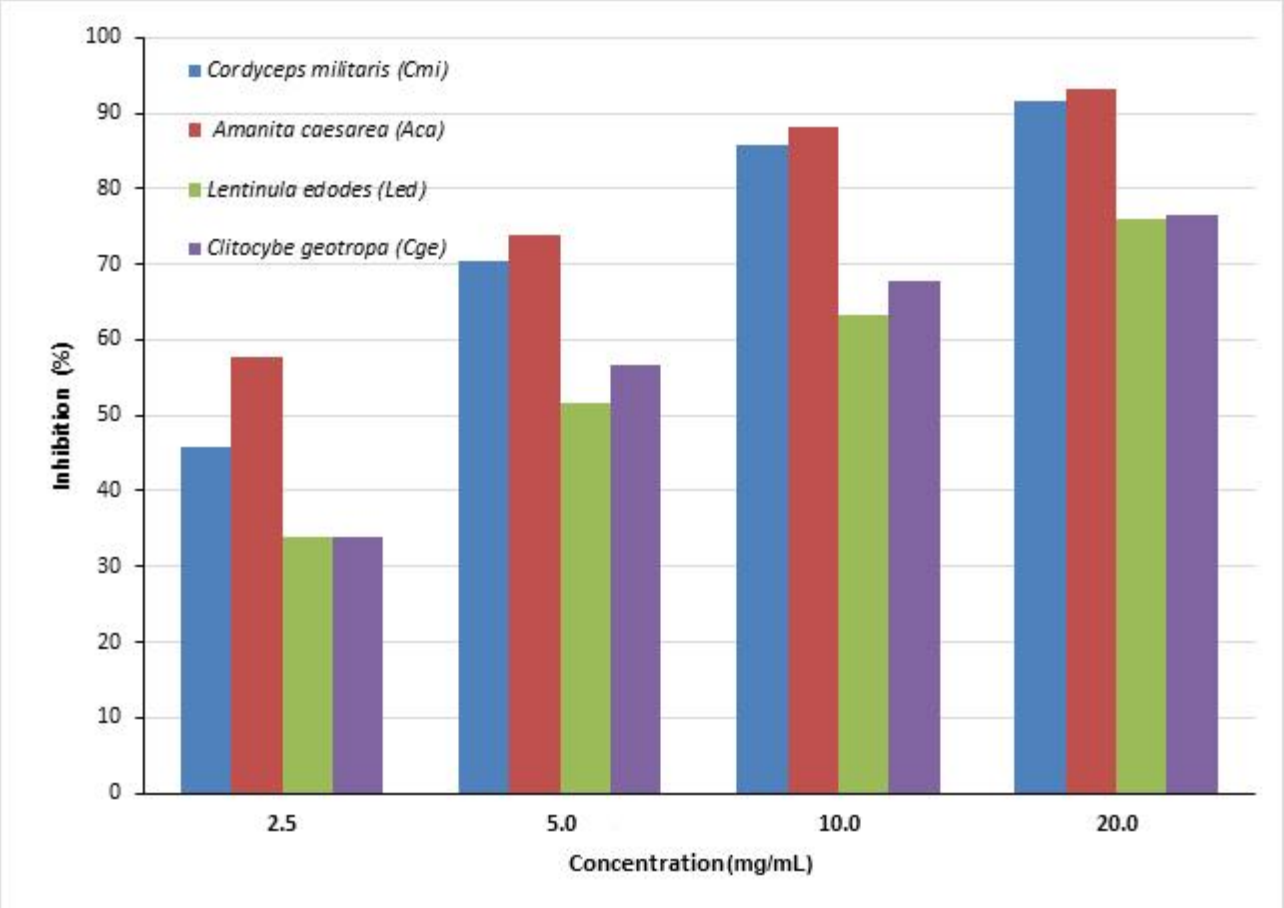

Figure 1. Free radical-scavenging capacities of the extract measured in different concentrations (measured by DPPH assay). The calculated results are given as mean ± SEM (standard error of the mean).

Accordingly, *Cmi* and *Aca* mushrooms showed higher % inhibition values (% 45.79-91.58 and % 57.83-93.30) at every concentration compared to *Led* and *Cge* (%33.94-75.93 and % 34.03-76.56). In the study conducted by Sarıkürkçü et al. (2010), it was observed that the inhibition value of *Aca* (%70.1) was higher than *Cge* (%61.3) as in our study at 2.5 mg/mL concentration, while *Cge* (%83.2-%86.1) was higher than *Aca* (%73.9-%79.6) at 5 mg/mL and 10 mg/mL concentrations. In addition, Doğan and Akbaş (2013) determined the inhibition value of *Aca* as 40.9% at a concentration of 0.5 mg/mL in their study. For *Cmi*, it showed 70% inhibition at a concentration of 0.5 mg/mL, while this value was reported by Joshi et al. (2017), it was found to be around 40%. Also previous studies on plants and mushrooms have shown a strong correlation between rich total phenolic content and antioxidant activity values (Gülçin et al., 2003).

Certain wild mushrooms, which serve as an important source of antioxidant compounds and are used both as food and medicine, can have medical value due to their antioxidant activities that show a good correlation with their phenolic content (Petrović et al., 2014; Turfan et al., 2018).

The polyphenolic and flavonoid content of mushrooms, which are high in protein and carbs but low in fat calories, is directly linked to their antioxidant properties (Smolskaite et al., 2015; Reis et al., 2017).

The results of the difference test between concentration-inhibition pairs for *Cmi*, *Aca*, *Led*, and *Cge* were found to be significant at the 95% confidence interval with $p<0.01$ level of significance. Total phenolic values, which are directly responsible for the variance in antioxidant properties, were found to have a statistically significant ($p<0.01$, $n=4$) negative correlation value ($r = -0.999$) with antioxidant activity values. The inverse association between IC_{50} and total phenolic content was confirmed by the results.

4. Conclusion

Cordyceps militaris, *Amanita caesarea*, *Lentinula edodes*, and *Clitocybe geotropa* were examined by comparing their DPPH radical scavenging activity and total phenolic content. *C. militaris* and *A. caesarea* exhibited much higher antioxidant activity compared to *L. edodes* and *C. geotropa*. These two mushroom species were also found to have higher total phenolic content compared to *L. edodes* and *C. geotropa*. This study will serve as a resource for the potential use of these mushroom species in the alternative medicine industry.

Acknowledgement

This study was presented at the 5th International Non-Wood Forest Products Symposium.

Author Contributions

Mertcan KARADENİZ: drafted the article and revisions

Temel Kan BAKIR: carried out experiments, data collection, and reporting.

Sabri ÜNAL: designed and planned the study

Conflict of Interests

No potential conflict of interest was reported by the authors.

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