

Some Physical and Chemical Properties of Fruitbodies of *Cordyceps militaris* Collected in Turkey

Özge SÜFER^{1*}, Fuat BOZOK², Hatıra TAŞKIN³, Saadet BÜYÜKALACA³

¹Osmaniye Korkut Ata University, Faculty of Engineering, Department of Food Engineering, Osmaniye, TURKEY

²Osmaniye Korkut Ata University, Faculty of Arts and Science, Department of Biology, Osmaniye, TURKEY

³Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, TURKEY

*Corresponding author: ozgesufer@osmaniye.edu.tr

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Abstract

Aim of study: Turkish *Cordyceps militaris* was collected in order to determine its certain quality characteristics such as color, texture, total phenolics level and antioxidant activity.

Material and method: Samples (not cultivated, wild) which were harvested from a field of Istanbul in 2018 were analyzed by using Eurx genematrix universal isolation kit for determining total genomic DNA. Color and textural properties of *C. militaris* body were measured by a colorimeter (according to Munsell system) and texture analyzer (applying texture profile analysis, TPA) respectively. The methanolic extract of *C. militaris* was subjected to total phenolic determination (by Folin Ciocalteu method) and antioxidant activity (by DPPH method) tests.

Main results: L*, a*, b*, C*, H° (color) values were recorded as 34.96, 18.84, 21.22, 28.41 and 48.57 respectively. Hardness of samples was 26.25 N. Total phenolic amounts and DPPH radical scavenging activity of specimens were found as 2316.22 mg gallic acid equivalent (GAE)/L extract and 3.98 mg trolox equivalent (TE)/L extract.

Highlights: The recommendation of this preliminary study is that *C. militaris* may be useful in various food formulations as an ingredient because of its high bioactive molecule and antioxidant potential.

Keywords: Biological Activity, *Cordycipitaceae*, Medicinal Macrofungi

Türkiye'den Toplanan *Cordyceps militaris*'in Bazı Fiziksel ve Kimyasal Özellikleri

Öz

Çalışmanın amacı: Türk *Cordyceps militaris*'i, renk, doku, toplam fenolik düzeyi ve antioksidan aktivite gibi bazı kalite özelliklerinin belirlenmesi amacıyla toplanmıştır.

Materyal ve metod: 2018 yılında İstanbul'daki bir araziden toplanan mantar örnekleri (kültür ortamında yetişmemiş, yabani) Eurx genematrix universal isolation kit kullanılarak toplam genomik DNA'nın belirlenmesi için analiz edilmiştir. *C. militaris* gövdesinin renk ve dokusal özellikleri sırasıyla bir renk ölçer (Munsell sistemine göre) ve doku analizörü (doku profili analizi, TPA uygulanarak) vasıtasıyla tayin edilmiştir. *C. militaris*'in metanol içerisinde hazırlanmış özütü, toplam fenolik tayinine (Folin Ciocalteu yöntemi) ve antioksidan aktivite testine (DPPH yöntemi) tabi tutulmuştur.

Sonuçlar: L*, a*, b*, C*, H° (renk) değerleri sırasıyla 34.96, 18.84, 21.22, 28.41 ve 48.57 olarak kaydedilmiştir. Örneklerin sertliği 26.25 N'dir. Numunelerin toplam fenolik miktarı ve DPPH radikali süpürme aktivitesi 2316.22 mg gallik asit eşdeğeri (GAE)/L özüt ve 3.98 mg troloks eşdeğeri (TE)/L özüt olarak bulunmuştur.

Önemli vurgular: Bu ön çalışmanın önerisi, *C. militaris*'in yüksek biyoaktif molekül ve antioksidan potansiyelinden dolayı, çeşitli gıda formülasyonlarında bir besin ögesi olmasının yararlı olabileceğidir.

Anahtar Kelimeler: Biyolojik Aktivite, *Cordycipitaceae*, Tıbbi Makromantar



Introduction

Cordyceps militaris which belongs to the family *Cordycipitaceae* (Ascomycota) is an entomopathogenic fungal species that lives on pupa and larva of lepidopteran insects as parasitic (Kirk, Cannon, David & Stalpers, 2008; Masuda, Urabe, Sakurai & Sakakibara, 2006). *Cordyceps* species are known as medicinal macrofungi in Far East Countries such as China and Japan. Also, in East Asia, cultivated *C. militaris* is accepted as a folk tonic food (Wu et al. 2014).

An increment of reactive oxygen species in healthy cells may cause several pathological effects like DNA damages, diabetes, cancer and so on. In order to eliminate these health risks, discovering and developing new natural antioxidants will be useful for living organisms (Liu, Jia, Kan & Jin, 2013). The species belonging to *Cordyceps* genus includes various bioactive compounds (cordycepin, ophiocordin and some polysaccharides etc.) that have antioxidant, antimicrobial, anti-inflammatory, antitumor activities (Yoo et al 2004; Won & Park, 2005; Zhan, Dong & Yao, 2006; Reis et al., 2013). Especially cordycepin was extensively investigated because of its inhibiting action on tumors. The mechanism of cordycepin on tumors based on the inhibition of migration and invasion of cancer cells, blockage of metastasis and disruption between cancer and mesenchymal stromal cells (Jin et al., 2018).

On the other hand, the amino acid contents of cultivated fruit bodies of *C. militaris* were also described by Zhan et al. (2006) and the major ones were isoleucine, glutamic acid, arginine and aspartic acid. Hypolipidemic, antitumor, antioxidant, antibacterial and anti-inflammatory effects of some *Cordyceps* species were also previously assessed by many researchers (Yang et al., 2000; Yoo et al., 2004; Won and Park, 2005; Leung, Zhao, Ho & Wu, 2009; Huang, Siu, Wang, Cheung & Wu, 2013; Reis et al., 2013; Chen et al., 2014; Jing et al., 2014; Wang et al., 2015).

Cordyceps militaris was first reported from Turkey very recently, from Istanbul province (Akata, Kabaktepe & Akgül, 2016). This drawing scientific attention species however has not been studied in Turkey in respect of its biological activity and nutritional properties. In this paper we report

certain physical and chemical characteristics of collected from nature Turkish *C. militaris*.

Materials and Methods

Macrofungi sample, DNA isolation, PCR and sequencing

Macrofungi samples (voucher no FBozok00139) were collected from Istanbul province of Turkey on 15 April 2018 (Figure 1) and deposited at Fungarium of Osmaniye Korkut Ata University. Total genomic DNA was isolated from fresh samples by using Eurx genematrix universal isolation kit with slight modifications (concentrations and amounts of RNase (10 µl, 10 mg/ml) and Proteinase K (10 µl, 20 mg/ml) were increased). ITS gene region was amplified with ITS1F and ITS4 primers (White, Bruns, Lee & Taylor, 1990). PCR conditions were as follows: 94°C for 5 min, followed by 30 cycles for 30 s at 94°C, for 45 s at 53°C, for 60 s 72°C and final extension for 5 min at 72°C. DNA sequence analysis was made by BigDye Terminator (version 3.1) Sequencing Kit using ITS1F-ITS4 primers again and raw sequence was edited by Sequencer version 5.4.5 (Gene codes, Ann Arbor, MI, USA) and aligned by Bioedit programme. This sequence was deposited in GenBank as accession MK131341. When compared to the sequences in the public GenBank database, the sequence obtained in the present study shows similarity 100% with JX488477 (China), HQ591382 (Taiwan), AB070375 (Japan), JX488481 (South Korea), JN121122 (Germany), JX488479 (China) and JN488478 (USA).

Moisture Determination

Moisture content of fresh samples was measured according to the air oven method of AACC (1995) numbered as 44-15A.

Color Analysis

Colorimetric measurements of specimens were identified in triplicate using a portable device (Konika Minolta, Chroma meter, CR-400, Japan). L* (brightness), a* (redness-greenness) and b* (yellowness-blueness) were measured. Chroma (C*) and hue angle (H°) values were also calculated from equation 1 and 2 respectively.



Figure 1. *Cordyceps militaris* (photographed by Mr. Naim Güleç)

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

Equation 1. The quality of color (chroma) equation

$$H^\circ = \arctan\left(\frac{b^*}{a^*}\right)$$

Equation 2. The mathematical expression of hue angle

Texture Profile Analysis (TPA)

Texture analysis of *C. militaris* was performed by a texture analyzer (Brookfield, CT3, load cell:4500 g, USA). The samples were compressed 1 mm and test and post speed were 1 mm/s and 2 mm/s respectively. Trigger load was 3 N and a cylindrical probe which had 2 mm diameter and 20 mm length was used. Five measurements were taken. Tested textural parameters were hardness, springiness, adhesiveness, gumminess and chewiness.

Extraction of Bioactive Compounds

Before total phenolics and antioxidant activity analysis, bioactive molecules were extracted from *C. militaris* by using the modified method of Bennett et al. (2011). 1 gram of chopped sample was diluted with 10 ml 100% methanol. After blending for 15 s (Wisd VM-10 Vortex mixer, Italy), mixture was put in an ultrasonic water bath (Selecta-HD 5 liters) at 25°C for 20 min and then it was centrifuged (Hettich Zentrifugen, Rotina 380, Germany) at 3500 rpm during 15 min. The supernatant was collected and used for experiments immediately.

Total Phenolic Content

Total phenolic determination was conducted according to the method of Li et al. (2015) with some modifications. 0.5 ml extract was reacted with 0.5 ml Folin-Ciocalteu reagent. After that, 3 ml 10% Na₂CO₃ solution was added and the mixture was left for incubation in dark for 30 minutes. The absorbance was read at 760 nm by a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) and result was given as mg gallic acid equivalents (GAE). 100% methanol was accepted as blank and analysis was carried out triplicate.

Antioxidant Activity (DPPH method)

0.1 ml extract was added to 2 ml 0.063 M 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution. The absorbance of mixture was read at 517 nm by a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) after 30 min waiting in dark place (Aghraz et al 2018). The result was expressed as mg 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) equivalents (TE). The test was performed in triplicate.

Statistical Analysis

The data of bioactive compounds was analyzed using Student's t-test ($p < 0.05$) by using SPSS version 18 (IBM, USA) which was a common statistical software. Means and standard deviations were also evaluated by same computer programme.

Results and Discussion

The calculated moisture level of *C. militaris* was 79.49%±1.07 ($p < 0.05$). Li, Yang, Yang, Wang & Chen (2019) claimed that *C. militaris* had high moisture content and easily perishable. Hence, drying may retard

microbial deterioration and help to preserve quality. Furthermore, the drying effect on the antioxidant activity of polysaccharide from *C. militaris* was studied by Chen, Liu, Yang, Zhou & Yang (2016) and freeze drying was reported as suitable method among hot air and spray drying.

Color is one of the most important parameters which have the ability of influencing the consumers' choice in a food product. Table 1 presents the color properties of macrofungi and L^* , a^* and b^* values were recorded as 34.96 ± 0.86 , 18.84 ± 2.69 and 21.22 ± 0.97 respectively ($p < 0.05$). When a^* value increase, the redness of sample also enhances. Characteristic orange-red color (high a^*) of *C. militaris* may be originated from bioactive compounds like lycopene and cyanidin-3-glucoside and significant correlations between red pigments and redness of *C. militaris* have been reported by Li et al (2019). On the other hand, chroma defines the purity of color, however hue angle expresses strict distinction between colors. Chroma was 28.41 ± 2.40 and hue angle was 48.57 ± 3.17 in this study.

Great textural properties in edible mushrooms can directly affect the preferences of individuals as well as color attributes. The chewing motion is simulated by TPA (Ma, Zhang & Zhao, 2019) and the results of TPA of *C. militaris* are shown in Table 1. Hardness (37.10 N) of *Boletus edulis* stems was reported higher than *C. militaris* (Jaworska & Bernaś, 2010). Also, *Agaricus bisporus* had a harder texture (55.68 N) than both *B. edulis* and *C. militaris* (Jaworska, Bernaś, Biernacka & Maciejaszek, 2010).

Folin-Ciocalteu technique enables to detect total polyphenol levels and total phenolics determination can be accepted as a measure of antioxidant activity. DPPH procedure is a well-known method for rapid testing the natural antioxidant capacities (Xu et al., 2012). Total phenolics and DPPH radical scavenging activity of *C. militaris* were found as 2316.22 ± 45.23 mg GAE/L extract (or 23.16 mg GAE/g sample) and 3.98 ± 0.24 mg TE/L extract (or 0.03 mg TE/g sample) respectively (on wet basis) ($p < 0.05$). These results indicate that wild *C. militaris* procured from Istanbul has a good potential of antioxidant activity. The stronger capability of

C. militaris in reducing oxidant molecules is coming from higher concentration of polysaccharides (Nie, Cui, Xie, Phillips & Phillips, 2013).

While regarding previous studies, Yu, Wang, Shiow & Duh (2006) assessed the total phenolic amount and antioxidant capacity of water extracts of *C. militaris* and *C. sinensis* fruitbodies. They found the polyphenolic contents of both species was $60.2 \mu\text{g GAE/mL}$ and $31.8 \mu\text{g GAE/mL}$ respectively and the antioxidant activity increased depending on the dose increased. These findings may demonstrate that methanol which has a high polarity might be more efficient than water for extraction of polyphenols from *Corydceps* species taking into account the results reported in this paper. Similarities were declared by Wang et al. (2015) in natural and cultured *C. sinensis* and their polyphenol contents in water extracts were given as 17.07 mg GAE/g for cultured and (averagely) 12.08 mg GAE/g for naturals. Also, *C. militaris* had higher polyphenol content than some wild and commercial mushrooms such as *A. bisporus*, *A. silvaticus*, *A. silvicola*, *Boletus edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides* and *Marasmius oreades* (Barros, Cruz, Baptista, Estevinho & Ferreira, 2008).

Won and Park (2005) investigated the pharmacological activities of 70% ethanolic extract of *C. militaris* (cultured mycelia) and found that the IC₅₀ value (which meant that the required concentration of scavenging the 50% of DPPH solution) of the extract was 0.026 g/mL and this result implied the weak antioxidant activity of related macrofungi when compared with vitamin C as a synthetic antioxidant. Furthermore, Yu et al. (2007) studied in vitro antioxidant activity by hydroxyl radical system of the water-soluble and three different fractions polysaccharide (P50-1, P70-1 and P70-2) from fruiting bodies of cultivated *C. militaris* and found that P70-1 possessed hydroxyl radical-scavenging activity with an IC₅₀ value of 0.548 mg/mL. The comparisons of these researches with our data are difficult because of differences in testing methods.

Conclusion

In this study, color, textural and antioxidant properties and polyphenolic compounds of *C. militaris* from Turkey were investigated. Results of this preliminary study showed that Turkish *C. militaris* has light orange color and its tissue was softer than other mushroom species. High bioactive and antioxidant potentials were discovered in methanolic extract. Hence, *C. militaris* may take part in food formulations as a useful nutrient. Also, its physical properties make it suitable for direct consumption. Future works which will focus on polysaccharides and specific phenolic constituents of *C. militaris* naturally grow from Turkey must be conducted.

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