Journal of Integrative and Anatolian Medicine
Bütünleyici ve Anadolu Tıbbı Dergisi

Cilt/ Volume: 1 **Sayı / Issue:** 1
Yıl/Year: 2019

Yayıncı / Publisher
Sağlık Bilimleri Üniversitesi
University of Health Sciences

Araştırma Makalesi / Rersearch Article Bütünleyici ve Anadolu Tıbbı Dergisi, 1(1): 24-32, 2019

Journal of Integrative and Anatolian Medicine, 1(1): 24-32, 2019

Determination of Antioxidant Activities of Some Wild Mushroom Species in Tokat Region

Duygu MISIRLI[1]*, Mahfuz ELMASTAŞ[1], İbrahim TÜRKEKUL[2]

[1] Hamidiye Faculty of Pharmacy, University of Health Sciences, Istanbul, Turkey

[2]Department of Biology, Faculty of Arts and Science, University of Gaziosmanpaşa, Tokat, Turkey

*Sorumlu Yazar / Corresponding Author: Duygu MISIRLI, e-mail: duygumisirli@gmail.com

ABSTRACT

Wild edible mushrooms can be eaten for health and play an important role in maintaining a healthy life by creating synergy due to the various bioactive components it contains. However, many species that may contain bioactive compounds have not been investigated.

The purpose of this study was to investigate the antioxidant capacity of wild mushroom species which are grown in Tokat region with names *Leatiporus sulpherus, Ramaria sp., Cantharellus aerruginascens, Verpa conica, Verpa bohamica, Tricholoma terreum, Agaricus sp., Helvella elastica* in vitro. In addition, total phenolic compound and vitamin E levels were analyzed in mushroom samples. In order to determine the antioxidant capacity of mushroom samples, free radical scavenging activity (DPPH), reducing power activity and ABTS•$^{+}$ [2,2´-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] cation radical scavenging activity analyzes were performed.

The results were compared with concentrations of Butylated hydroxy toluene (BHT), Butylated hydroxy anisole (BHA) and α-tocopherol. Consequently, the mushroom species with the highest radical removal activity are *Ramaria sp*., *V. bohamica* and *H. elastica*, respectively. The highest value of vitamin E, was found in *Agaricus sp. (1444.1 mg/kg), C. aerruginascens (1370.8 mg/kg)* and *Ramaria sp. (1204.2 mg/kg)* respectively. The highest amount of total phenolic contains was found to be in the *Ramaria sp. (6.57 g/kg)*. These results may encourage further studies to assess nutritional and medicinal properties of selected mushroom species.

Keywords: Antioxidant activity, wild mushrooms, vitamin E

Tokat Bölgesinde Yetişen Bazı Yabani Mantar Türlerinin Antioksidan Aktivitelerinin Belirlenmesi

ÖZET

Yabani yenilebilir mantarlar sağlık için yenebilir ve içerdiği çeşitli biyoaktif bileşenler nedeniyle sinerji yaratarak sağlıklı bir yaşam sürdürmede önemli bir rol oynayabilir. Bununla birlikte, biyoaktif bileşikler içerebilen birçok tür araştırılmamıştır.

Bu çalışmanın amacı Tokat bölgesinde yetişen *Leatiporus sulpherus, Ramaria sp., Cantharellus aerruginascens, Verpa conica, Verpa bohamica, Tricholoma terreum, Agaricus sp., Helvella elastica* isimli doğal mantar türlerinin antioksidan kapasitesini araştırmaktır. Ayrıca, mantar örneklerinde total fenolik bileşik ve E vitamini seviyeleri analiz edilmiştir. Mantar örneklerinin antioksidan kapasitesini belirlemek için serbest radikal giderme aktivitesi (DPPH), indirme gücü tayini, ABTS • $^{+}$ [2,2´-Azino-bis (3-etilbenzotiyazolin-6-sülfonik asit)] katyon radikali giderme aktivite testleri yapılmıştır.

Sonuçlar standart olarak kullanılan Bütillenmiş hidroksi toluen (BHT), Bütillenmiş hidroksi anisol (BHA) ve α-tokoferol konsantrasyonları ile karşılaştırıldı. Radikal giderme aktivitesi en yüksek olan mantar türlerinin sırasıyla *Ramaria sp., V. bohamica ve H. elastica* olduğu, E vitamini yüksek olan mantar türlerinin ise *Agaricus sp*. (1444.1 mg/kg), *C. aerruginascens* (1370.8 mg/kg) ve *Ramaria sp.* (1204.2 mg/kg) olduğu belirlendi. Total fenolik bileşik miktarının en yüksek olduğu türün *Ramaria sp.* (6.57 g/kg) olduğu tespit edildi. Sonuç olarak bu çalışma, seçilen mantar türlerinin beslenme ve tıbbi özelliklerini değerlendirmek için daha fazla çalışmaya teşvik edebilir.

Anahtar Kelimeler: Antioksidan aktivite, yabani mantarlar, E vitamini

1. INTRODUCTION

A balance between free radical production and antioxidant defense (both enzymatic and non-enzymatic) is essential for normal organism functions. Products that have antioxidant activity in nature can help the endogenous defense system of the human body. In this regard, antioxidants in the diet are protective compounds that reduce oxidative stress (Elmastaş et al., 2006; I. Gülçin, Sat, Beydemir, & Küfrevioglu, 2004; Oktay, Gülçin, & Küfrevioğlu, 2003)Family: Lauraceae.

In addition to their nutritional value, many edible mushroom species have long been used for medical purposes in many countries of the world. In addition, many non-edible and poisonous mushroom species have been found to have important medicinal properties (Sharma et al., 2018). It is observed that the use of mushrooms against various diseases has become widespread as a result of the development of mushroom production possibilities in culture conditions. Mushrooms have antioxidant, antiinflammation, antidiabetic, antiviral and antimicrobial effects as well as immunological and anticancer properties (Elsayed, El Enshasy, Wadaan, & Aziz, 2014; Friedman, 2016; Gallego et al., 2019; Singh, Walia, & Kennedy, 2019; Su et al., 2019; Wu et al., 2007)sustained antiviral responses vary in different cohorts, and high costs limit the broad use of direct-acting antivirals (DAAs.

The purpose of this study was to search the antioxidant activities of *L. sulpherus, Ramaria sp., C. aerruginascens, V. conica, V. bohamica, T. terreum, Agaricus sp., H. elastica,* which are wild mushrooms grown in Tokat region, and to determine the amount of vitamin E.

2. EXPERIMENTAL

2.1. Collection of Mushroom Species

The mushroom species used in this study were obtained from Tokat-Merkez, divided into small pieces and dried in the shade. The dried samples were stored in the dark until antioxidant studies were performed.

2.2. Extraction

The dried 8 different mushroom samples were milled in the blender and pulverized. Approximately 5 g of each mushroom species were sampled and extracted with methanol-dichloromethane solvent system at a rate of 1:1. Solvents of the crude extracts were removed and quantified (Table 1). Stock solutions were prepared with a concentration of 1mg/mL from each extract.

Table 1. Amount of mg of crude extracts obtained from mushroom samples

Mushroom species	Quantifications (mg)
Leatiporus sulpherus	622.8
Ramaria sp.	843.3
Cantharellus aerruginascens	973.3
Verpa conica	1099.9
Verpa bohamica	576.8
Tricholoma terreum	557.1
Agaricus sp.	883.4
Helvella elastica	647.5

2.3. Antioxidant Activity

2.3.1. Scavenging DPPH· Radicals

The free radical scavenging activity of the mushroom extracts was measured using 1 mM 1,1-diphenyl-picryl-hydrazyl/(DPPH·). Samples were prepared from stock solution in concentrations of 10, 20 and 30 µg/µL. Then 1 mL of stock DPPH solution was added to each sample tube. After that the absorbance was measured at 517 nm in spectrophotometer. Mushrooms extracts were compared with BHT, BHA and α-tocopherol. % Free radical scavenging activity of the samples was calculated by the following formula.

$$\% \text{ Result} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

2.3.2. Scavenging ABTS•+ Radicals

$ABTS^{\cdot+}$ removal activity was determined according to the study in literature (Re et al., 1999). First, a 2 mM $ABTS^{\cdot+}$ solution was prepared. To this solution, twice the volume of 2.45 M potassium persulfate solution was added to give $ABTS^{\cdot+}$ solution and incubated in the dark for 6 hours. The test tubes were taken from stock solutions at concentrations of 40, 80 and 120 µg/mL respectively, 1 mL of $ABTS^{\cdot+}$ solution was added and the total volume was completed to 4 mL with phosphate buffer. After that the absorbance was measured at 734 nm in spectrophotometer.

2.3.3. Reducing Power

The reducing power of mushroom extracts was determined by the method of Oyaizu (Oyaizu, 1986). 40, 80 and 120 µg/mL of the stock solutions were taken into test tubes, respectively. 0.2 M phosphate buffer (pH:6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] were added to each test tube to a total volume of 2.5 mL and mixed. The mixture was incubated

at 50 ºC for 20 minutes. 2.5 mL of 10% trichloroacetic acid (TCA) was added to the reaction mixture and centrifuged at 3000 rpm for 10 minutes. 2.5 mL of the supernatant was taken and 0.5 mL of 0.1% $FeCl_3$ solution was added. After that the absorbance/was measured at 700 nm in spectrophotometer.

2.3.4. Determination of Total Phenolic Compounds

Total phenolic compounds were determined by Slinkard and Singleton methods (Slinkard & Singleton, 1977). 1 mL (1 mg/mL) was taken from each sample and placed in 50 mL flasks. 40 mL of distilled water and 1 mL of Folin Ciacalteuse reagent were added. After 3 minutes, 3 mL of 2 % Na_2CO_3 solution was added and incubated for 2 hours. After that the absorbance was measured at 760 nm in spectrophotometer. Gallic acid was used as the standard for the calibration curve.

2.3.5. Determination of Vitamin E

Mushroom samples in methanol were extracted with petroleum ether. The supernatant (petroleum ether phase) was used. The solvent was removed in the evaporator. Stock solutions were prepared from the solvent-extracted extracts to 1 mg/mL. Standard solutions of α-tocopherol (vitamin E) in different concentrations were prepared from this stock solution. Measurements were performed at 295 nm wavelength on HPLC device.

3. RESULTS and DISCUSSION

Mushroom extracts at different concentrations (10-30 ug/uL) were compared with BHT, BHA and α-tocopherol at the same concentrations. As can be seen in Figure 1, *Ramaria sp*. has been found to have the best radical scavenging effect among other mushroom species.

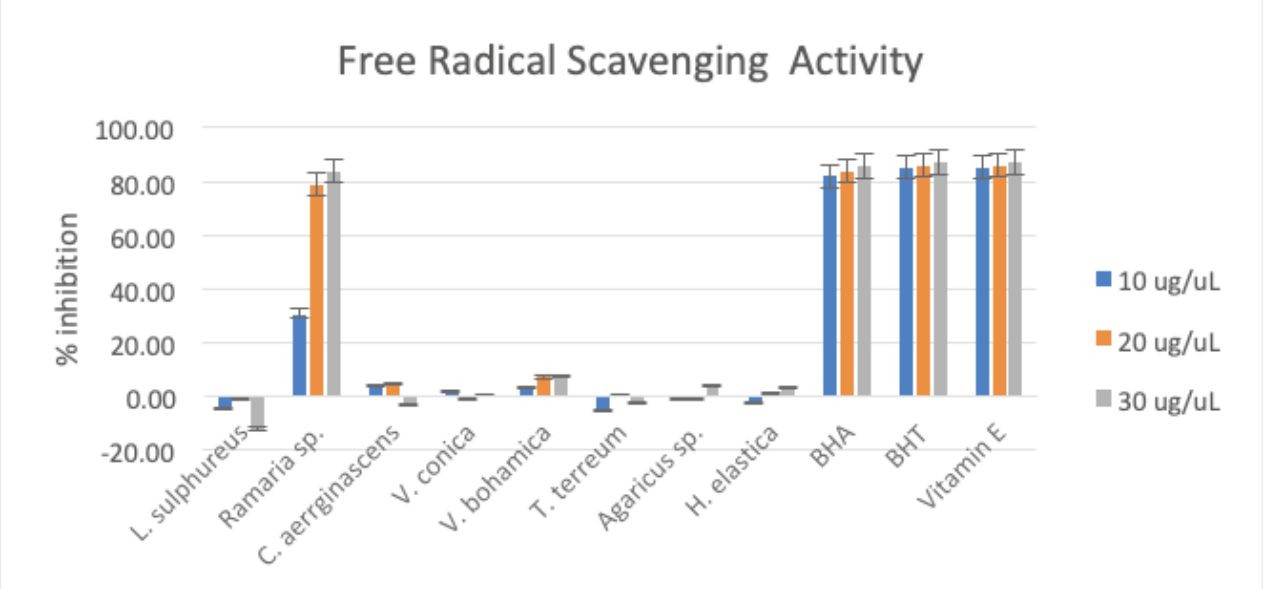

Figure 1.Comparison of DPPH activities of mushroom samples against standard BHA, BHT and α-tocopherol.

Like DPPH• free radical scavenging activity, ABTS•$^{+}$ scavenging activity is often used in the radical scavenging activities of aqueous mixtures, beverages, extracts or pure substances (Ilhami Gülçin, 2010; Miller & Rice-Evans, 1997). Figure 2 shows that *Ramaria sp.* has the highest radical scavenging activity according to the ABTS test.

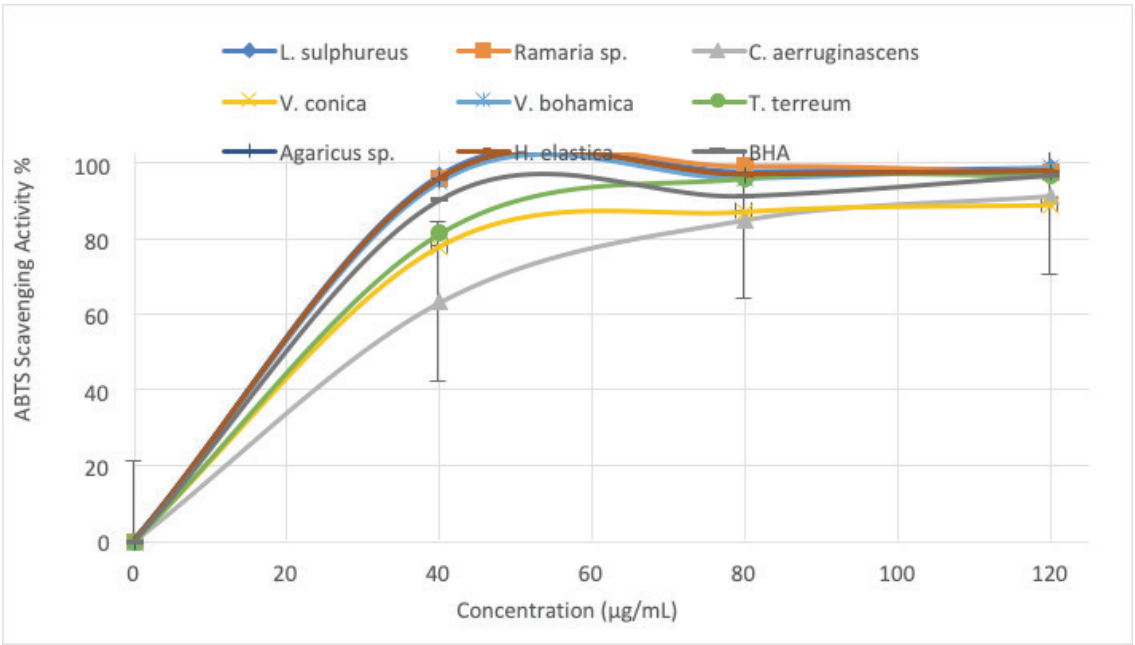

Figure 2. Comparison of ABTS•$^{+}$ radical scavenging activities of mushroom samples against standard BHA.

According to the principle of this bioanalytical method used in antioxidant studies, the yellow color of the test solution turns into green in different shades due to the reducing activities of the antioxidant substances in the medium (Ilhami Gülçin, 2006). It was found that the reducing capacity of some of the mushrooms extracts used in the study correlated with increasing extract concentrationThe reduction potential of each mushroom extract was determined by measuring the absorbance at 700 nm of solutions of different concentrations (40–120 µg/mL) (Figure 3). As can be seen from the Figure 3, α-tocopherol used as a standard showed higher reduction capacity than extracts. *Ramaria sp.* mushroom extract has the highest reduction capacity among other mushroom samples.

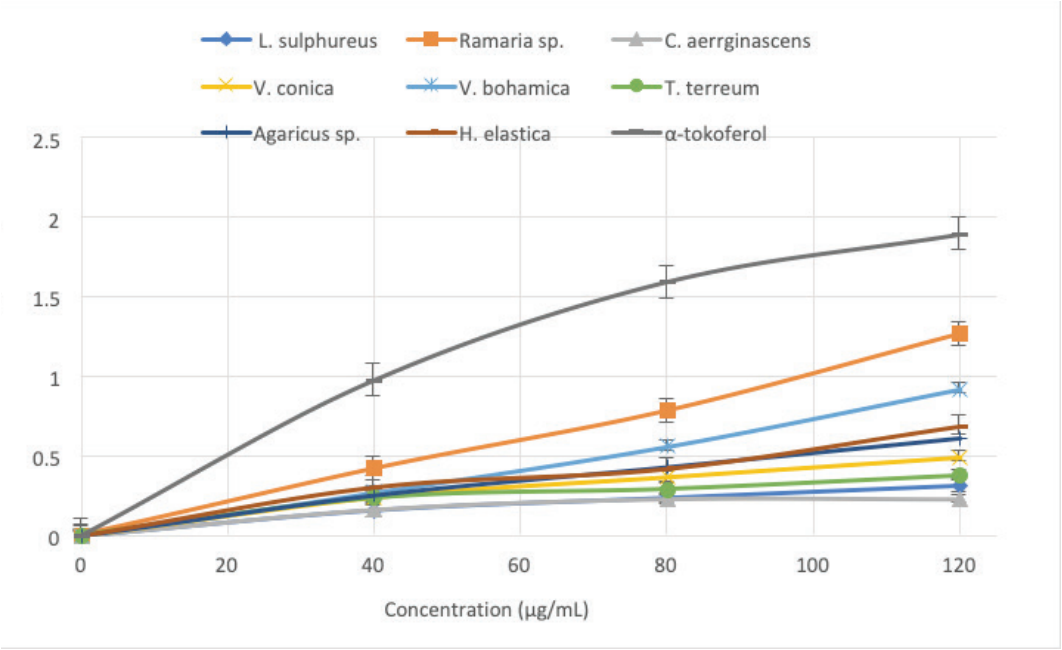

Figure 3. Comparison of total reducing power activities of mushroom samples against standard α-tocopherol.

Gallic acid was used as the standard phenolic compound for the quantification of total phenolic compounds in mushroom extracts. For this purpose, firstly a standard graph was prepared with gallic acid. From the formula obtained from the standard graph, total phenolic compound amounts in each extract were calculated as gallic acid equivalent (GAE) (r^2: 1). The standard gallic acid graph is given in Figure 4.

$$Absorbans_{(\lambda 730\ nm)} = 0.0014 \times [Gallic\ acid]$$

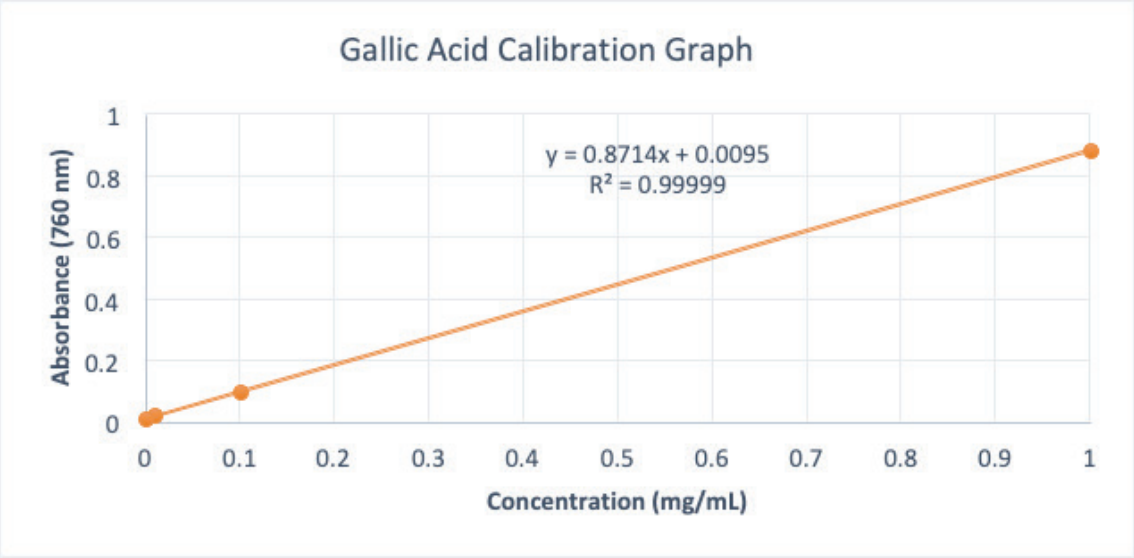

Figure 4. Standard graph using gallic acid for determination of total phenolic compound

The total amount of phenolic compounds in 1 kg of mushroom extracts is given in Table 2. When the total phenolic contents of the extracts were evaluated, *Ramaria sp.* was found to have the highest value.

Table 1. Amount of phenolic compounds in g/kg of mushroom samples

Mushroom species	**Phenolic Component (g/kg)**
Leatiporus sulpherus	0.24
Ramaria sp.	6.57
Cantharellus aerruginascens	0.81
Verpa conica	0.79
Verpa bohamica	1.79
Tricholoma terreum	0.59
Agaricus sp.	1.00
Helvella elastica	1.90

Table 2. Vitamin E content of mushroom samples in mg/kg

Mushroom species	**Vitamin E (mg/kg)**
Leatiporus sulpherus	851.2
Ramaria sp.	1204.2
Cantharellus aerruginascens	1370.8
Verpa conica	7.26
Verpa bohamica	576.8
Tricholoma terreum	1127.9
Agaricus sp.	1444.1
Helvella elastica	16.7

The artificial antioxidant α-tocopherol was used as a standard for the determination of vitamin E in mushroom samples. The amount of vitamin E contained in the samples is shown in Table 3.

The highest value of vitamin E, was found in *Agaricus sp. (1444.1 mg/kg), C. aerruginascens (1370.8 mg/kg)* and *Ramaria sp. (1204.2 mg/kg)* respectively (Table 3).

CONCLUSIONS

In this study, methanol-dichloromethane extract of 8 different mushroom species were studied. In our study, antioxidant capacity measurements and vitamin E determination were performed. When the experimental results are evaluated, especially *Ramaria sp.* was found to have high activity in all tests. According to the results of our experiments, it is predicted that the *Ramaria sp.* species has a strong antioxidant effect and therefore may have a good therapeutic effect. But further studies should be done on the isolation and characterization of new compounds from mushrooms, which are responsible for antioxidant and activity.

REFERENCES

Elmastaş, M., Gülçin, I., Işildak, Ö., Küfrevioğlu, Ö. I., Ibaoğlu, K., & Aboul-Enein, H. Y. (2006). Radical scavenging activity and antioxidant capacity of bay leaf extracts. *Journal of the Iranian Chemical Society*. https://doi.org/10.1007/BF03247217

Elsayed, E. A., El Enshasy, H., Wadaan, M. A. M., & Aziz, R. (2014). Mushrooms: A potential natural source of anti-inflammatory compounds for medical applications. *Mediators of Inflammation*. https://doi.org/10.1155/2014/805841

Friedman, M. (2016). Mushroom Polysaccharides: Chemistry and Antiobesity, Antidiabetes, Anticancer, and Antibiotic Properties in Cells, Rodents, and Humans. *Foods*. https://doi.org/10.3390/foods5040080

Gallego, P., Rojas, Á., Falcón, G., Carbonero, P., García-Lozano, M. R., Gil, A., … Del Campo, J. A. (2019). Water-soluble extracts from edible mushrooms (: Agaricus bisporus) as inhibitors of hepatitis C viral replication. *Food and Function*, *10*(6), 3758–3767. https://doi.org/10.1039/c9fo00733d

Gülçin, I., Sat, I. G., Beydemir, S., & Küfrevioglu, Ö. I. (2004). Evaluation of the in vitro antioxidant properties of broccoli extracts (Brassica oleracea L.). *Italian Journal of Food Science*.

Gülçin, Ilhami. (2006). Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology*. https://doi.org/10.1016/j.tox.2005.09.011

Gülçin, Ilhami. (2010). Antioxidant properties of resveratrol: A structure-activity insight. *Innovative Food Science and Emerging Technologies*. https://doi.org/10.1016/j.ifset.2009.07.002

Miller, N. J., & Rice-Evans, C. A. (1997). Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. *Free Radical Research*. https://doi.org/10.3109/10715769709097799

Oktay, M., Gülçin, I., & Küfrevioğlu, Ö. I. (2003). Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. *LWT - Food Science and Technology*. https://doi.org/10.1016/S0023-6438(02)00226-8

Oyaizu, M. (1986). Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*. https://doi.org/10.5264/eiyogakuzashi.44.307

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. https://doi.org/10.1016/S0891-5849(98)00315-3

Sharma, D., Singh, V. P., & Singh, N. K. (2018). A review on phytochemistry and pharmacology of medicinal as well as poisonous mushrooms. *Mini reviews in medicinal chemistry*, 18(13), 1095-1109.

Singh, R. S., Walia, A. K., & Kennedy, J. F. (2019). Mushroom lectins in biomedical research and development. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2019.10.180

Slinkard, K., & Singleton, V. L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American Journal of Enology and Viticulture*, *28*(1), 49–55.

Su, X., Liu, K., Xie, Y., Zhang, M., Wang, Y., Zhao, M., … Wang, J. (2019). Protective effect of a polyphenols-rich extract from Inonotus Sanghuang on bleomycin-induced acute lung injury in mice. *Life Sciences*, *230*, 208–217. https://doi.org/10.1016/j.lfs.2019.05.074

Wu, D., Pae, M., Ren, Z., Guo, Z., Smith, D., & Meydani, S. N. (2007). Dietary Supplementation with White Button Mushroom Enhances Natural Killer Cell Activity in C57BL/6 Mice. *The Journal of Nutrition*, *137*(6), 1472–1477. https://doi.org/10.1093/jn/137.6.147