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Investigation of Antioxidant Activities of Agrocybe praecox Fungus

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Abstract: Mushrooms have been consumed as foodstuffs since time very old. From a nutritional point of view; In addition to containing low calories, it has a rich content in essential amino acids, carbohydrates, fibers, important vitamins and minerals. Antioxidants are one of the most studied topics in recent years and can be defined as a whole of systems that neutralize toxic products that occur as a result of cell metabolism. Antioxidant activities of water extracts of *Agrocybe praecox* (Pers.) Fayod mushrooms were obtained according to total phenolics, total flavonoids, metal chelation and ABTS methods. It was observed that some compounds were found in the extracts used by the analyzes performed in this study and the mushroom had antioxidant properties. In this study, the total amount of phenol contained in *A. praecox* mushroom was detected to be 52.7 mg GAE/g. The amount of quercetin was found to be 1.46 mg QE/g, catechin 23.2 mg CA/g it was found to have total flavonoid properties. The IC50 of the metal chelating activity was calculated as 5.74 mg. The ABTS+ radical scavening activity was calculated as 12.5 mg.

Creating new products by using the compounds present and detected in the fungus or isolating the enzymes with high activity in them and making them more useful in vitro will be an important medical contribution. In addition, determination of its cytotoxic feature will pave the way for new studies in pharmaceutical and medical fields.

Keywords: Agaricales, Edible mushroom, Phenolic compounds, Van

Agrocybe praecox Mantarının Antioksidan Aktivitelerinin Araştırılması

Öz: Mantarlar, çok eski zamanlardan beri gıda maddesi olarak tüketilmektedir. Beslenme açısından bakıldığında; Düşük kalori içermesinin yanı sıra esansiyel amino asitler, karbonhidratlar, lifler, önemli vitaminler ve mineraller açısından zengin içeriğe sahiptir. Antioksidanlar, son yıllarda en çok çalışılan konulardan biridir ve hücre metabolizmasının bir sonucu olarak ortaya çıkan toksik ürünleri nötralize eden bir sistem bütünü olarak tanımlanabilir. *Agrocybe praecox* (Pers.) Fayod mantarlarının su ekstraktlarının antioksidan aktiviteleri total fenolikler, total flavonoidler, metal şelasyon ve ABTS yöntemlerine göre elde edildi. Bu çalışmada yapılan analizlerde kullanılan ekstraktlarda bazı bileşiklerin bulunduğu ve mantarın antioksidan özelliklere sahip olduğu görülmüştür. Çalışmanın sonunda *A. praecox* mantarında bulunan toplam fenol miktarı 52.7 mg GAE/g olarak tespit edildi. Kuersetin miktarı 1.46 mg QE/g, kateşin 23.2 mg CA/g toplam flavonoid özelliklere sahip olduğu hesaplandı. Metal şelatlama aktivitesinin IC50'si 5.74 mg olarak belirlendi. ABTS+ radikal süpürme aktivitesi 12.5 mg olarak hesaplandı.

Mantardan tespit edilen bileşiklerin kullanılması ve yüksek aktiviteye sahip enzimlerin izole edilmesiyle elde edilebilecek yeni ürünler önemli bir tıbbi katkı olacaktır. Ek olarak, sitotoksik özelliğinin belirlenmesi farmasötik ve tıbbi alanlarda yeni çalışmaların önünü açacaktır.

Anahtar Kelimeler: Agaricales, Yenilebilir mantar, Fenolik bileşikler, Van



Introduction

Mushrooms have been consumed as foodstuffs since time very old. From a nutritional point of view; In addition to containing low calories, it has a rich content in essential amino acids, carbohydrates, fibers, important vitamins and minerals. Fungi are used effectively in the treatment of many diseases with the active ingredients found in their composition (Öztürk et al., 2009, Sevindik et al., 2021). Mushrooms are one of the sources of powerful new pharmaceutical products that are wide and have not yet been largely addressed. Mushrooms are used as medicines and foods because they have rich protein and carbohydrate content all over the world (Wasser, 2002, Eraslan et al., 2021). Active ingredients found in fungi include pheasants; they have attracted a lot of attention due to their antioxidant and anti-inflammatory effects (Cayan et al., 2018, Bal et al., 2019).

A nutraceutical is defined as a substance that provides medical or health benefits for the prevention and treatment of the disease as part of a food or food. Nutraceuticals extend from isolated nutrients and nutritional supplements to genetically modified design foods, plant products and 4 processed products such as cereals, soups and beverages. Some examples of nutritious nutraceuticals or functional foods are dietary fibers, polyunsaturated fatty acids, proteins, peptides, amino acids, ketocytes, minerals, antioxidative vitamins, and other antioxidants such as glutathione, selenium, etc. (Andlauer et al., 2002; Kruger et al., 2003). The presence of specific bioactive compounds makes funai therapeutically valuable by strengthening the immune system as well as preventing and treating life-threatening diseases such as heart diseases, hypertension, cerebral stroke and cancer. Fungi are known to exhibit antifungal, anti-inflammatory, antiviral, antibacterial, hepataprotective, antidiyabetic, hypolypydemic and hypotensive activities (Rathore et al., 2017).

The human relationship with mushrooms is fascinating as it has been used as both food and medicine for the past 20 years. The use of mushrooms has expanded not only as food, but also in the fields of pharmaceuticals, nutraceuticals and cosmetics (Rathore et al., 2017). Mushrooms have been consumed and appreciated as food for their exquisite flavors, economic and ecological values and medicinal properties for years. Overall, mushrooms contain 90% water and 10% dry matter (Sánchez, 2010). For this reason, the life expectancy is short and immediately rots. Nutritional values are comparable to eggs, milk and meat and in addition to their nutritional value, they are considered functional foods due to their health benefits (Rathee et al., 2012).

Mushrooms contain vitamins (thiamine, riboflavin, ascorbic acid, ergosterol and niasin as well as plenty of essential amino acids. Mushrooms also contain proteins, fats, ash and glucosites. Essential oils, tocopherols, phenolic compounds, flavonoids, carotenoids, folates, organic acids, etc. are other components of fungi (Sánchez, 2004; Patel et al., 2012). Mushrooms are used as a traditional drug to prevent and cure various diseases due to their lack of side effects, and their use is increasing day by day. Among natural products, mushrooms are seen as the most potential candidate in clinical trials due to the inexpensive ability to obtain easily and abundantly. Antibiotics of fungal origin are used today for bacterial infections. Research has focused on the antiquated nature of the fungus through antifungal carbohydrates, especially its effect on lung cancer. The use of fungi as a treater by various tribes from a long time ago revealed the importance of their medical potential and led researchers to emphasize their views on their modern medical potential. Mushrooms were also mixed with other fungi or herbs in societies where natural treatment was applied, increasing/reducing their bioactivity or preventing side effects (Blackwell, 2010). Many researchers have found that edible mushrooms are the source of various nutraceutical compounds, including polysaccharides (βglucan), dietary fibers, terpenes, peptides, glycoproteins, alcohols, mineral elements, unsaturated fatty acids and antioxidants such as phenolic compounds, tocopherols and ascorbic acid (Pardeshi et al., 2009).

Today many types of fungi are used for medical purposes due to their antibiotic, antiquated, immuneregulatory, cardiovascular and antumor properties. Mushrooms contain phenolic compounds, antioxidants and important in terms of specific amino acids. In addition, the protein deficit that cannot be obtained from animal foods. It is an extremely delicious food product that can be eliminated (Çavuşoğlu et al., 2018; Sevindik et al., 2018). Antioxidants are one of the most studied topics in recent years and can be defined as a whole of systems that neutralize toxic products that occur as a result of cell metabolism. Water extracts of dried wild edible mushrooms have been analyzed in different experiments in terms of antioxidant activity, ferric antioxidant reduction power (FRAP), cleansing activity on 1.1-difenil-2picrilhydrazyl (DPPH) radicals, and have been found to have the potential for natural antioxidants (Keles et al., 2011).

A. praecox (Bahar meteliği) (Sesli et al., 2020) (Basidiomycetes, Agaricales, Strophariaceae) has several features that initiate biosystematic research. Members of this group are phenotypically polymorphic and contain a variety of woody substrates. As a result, taxa identified as *A. praecox* are often confused with morphologically different taxa such as *A. molesta* (Lasch) Singer (Şen metelik) (Sesli et al., 2020) and additional taxa are often identified from a single collection. Variable criteria used to identify taxa have formed a nomenclature (Flynn,1986).

The genus *Agrocybe* produces various growth regulators such as growth stimulation and suppression on



plants. *A. praecox* is an edible mushroom species worldwide, and northern temperate is fairly common. These fungi are non-specific enzymes that grow in the soil. Enzymes make the monomers and oligomers in the plant usable. The fungus is seen as a focal point for bioremediation and hydrolytic and ligninolytic enzymatic activities (Fushimi et al., 2012).

Materials and Methods Collection of mushroom samples

The fungal samples used in the study were collected from the Campus of Van Yuzuncu Yıl University and examined in the research laboratory of the Faculty of Science, Department of Molecular Biology and Genetics. Mushroom diagnosis was made by Dr. İsmail ACAR.

Mushroom extraction

Collected mushroom samples were kept at -20°C until experiments were carried out. The mushrooms, which were then ground, weighed 10g on a delicate scale. 150 mL of hexane was placed on it and left in a magnetic mixer with a heater for 8 hours. From the samples, hexane was filtered and 150 mL of ethanol was added to the remaining dry matter and left for incubation for 48 hours. After 48 hours, ethanol was filtered and ethanol was flown, 150 mL of water was added to the extract and incubated for 48 hours. After the water was filtered from the mixture and taken into 50 mL falcon tubes, it was placed horizontally at -80 °C to stay for 1 night and left in the lyophilizer for 72 hours to remove the water from the samples. The efficiency of the dried samples was calculated by the following equation.

Extraction(w/w)=(mass of dried extract)/(total sample mass)x1

Determination total phenol capacity

Total phenol capacity was performed according to the optimized form of by using the Folin-Ciocalteu method of Singleton and Rossi (1999) technique. After the fungal extract was dissolved in pure water and mixed with the extract Folin-Ciocalteu's reagent and added %10 sodium carbonate on top, the mixture was added to the microbe wells and piped and left in the dark at room temperature for 30 minutes. The absorbances of the samples were read at 750 nm. Gallic acid was used as standard. The experiment was run/studied in 3 replicates.

Determination total flavonoid content

Flavonoid content was performed according to the optimized form of by Zhishen et al, (1999) technique. Quercetin and Catechin were used as standard. Different concentrations were obtained by serial dilution from the stock standard. After the fungal extract was dissolved in 1 mL of pure water and added to the microplate wells, pure water and sodium nitrite were added for 5 minutes and then aluminum chloride was added to it and pure water was added for 6 minutes and the wells were completed to 200 μ l and measurements were taken at

415 nm and 510 nm. Experiment 3 was studied to happen again.

Determination metal chelating activity

Metal chelating activity was performed according to the optimized form of by Dinis et al., (1994) technique. EDTA (Etilendiamintetraacetic acid) was preferred as standard. By preparing the stock concentration and obtaining different concentrations with serial dilution, extracts of this different concentration were added to the microplate wells and ferrozin, iron (II) chloride and methanol were added to it and 562 nm reading was performed after incubation at room temperature of 10 minutes.

Determination ABTS radical scavening activity

ABTS radical sweeping activity was performed according to the optimized form of by Re et al., (1999) technique. For the creation of ABTS radical, 7 Mm ABTS solution 2.45 Mm potassium persulfate solution is mixed in a ratio of 1:1 and reacted. It is kept in a dark environment at room temperature for 16 hours before radical use. Extract and diluted ABTS radical are added to the plate with 96 wells, mixed and incubated at room temperature for 6 minutes. After incubation, absorbance reading is performed at 734 nm.

Results

In the study, antioxidant activities of water extracts of *Agrocybe praecox* fungus were looked at. The yield of water extracts was calculated and found to be 15%.

Antioxidant activities of *A. praecox* fungus; total phenol was determined using total flavonoid, metal chelating and ABTS methods.

Gallic Acid was used as standard to determine the total phenol content. Gallic Acid Standard Curve is given in Figure 1.

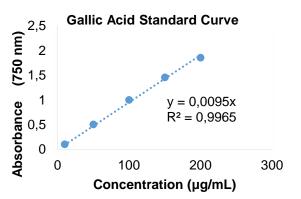


Figure 1. Gallic Acid Standard Curve, Experiments studied to be 3 repe.

The total phenolic content of *A. praecox* fungus was calculated according to 3 different concentrations. The results obtained from the fungus are given in Table 1.



For the determination of total flavonoid content Quercetin and Catechin were used as standard and standard curves were drawn. The standard curves of Quercetin and Catechin are given in Figures 2 and 3 respectively.

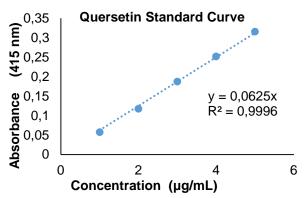


Figure 2. Quersetin Standard Curve, Experiments were studied to be 3 repe.

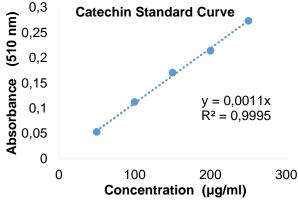


Figure 3. The Standard Curve of the Catechin was studied to be experiments 3 repe.

Total flavonoid content of *A. praecox* mushroom was calculated according to 3 different concentrations. The results obtained from mushrooms are given in Table 1.

EDTA was used as standard for determination of metal chelating activity. EDTA Standard Curve is given in Figure 4.

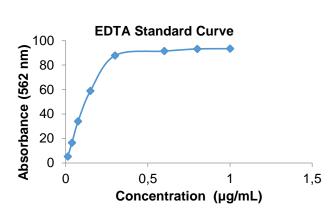


Figure 4. EDTA Standard Curve, Experiments were studied to be 3 repe. EDTA was used as standard.

Metal chelating activity of *A. praecox* mushroom was calculated according to 7 different concentrations. The results obtained from mushrooms are given in Table 1.

Trolox was used as a standard for determination of radical scavenging activity. Trolox Standard Curve is given in Figure 5.

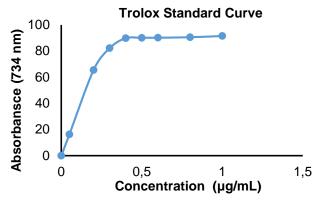


Figure 5. Trolox Standard Curve, Experiments were studied to be 3 repe another.

Radical scavenging activity of *A. praecox* mushroom was calculated according to different concentrations. The results obtained from mushrooms are given in Table 1.



Table 1. Antioxidant activities of the fu	ungus A. praecox
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Agrocybe praecox
%15
52.7 ± 1.90 mg/mL
23.2 ± 2.09 mg/mL
1.46 ± 0.3 mg/mL
5.74 ± 0.09 mg/mL
12.5 ± 0.35 mg/mL

*Values are presented as mean±SD; Experiments were made in 3 pere.

In this study, *A. praecox* fungus samples were collected and powdered and the antioxidant activities of the obtained water extracts were examined. Water extract yield is given in Table 1. Antioxidant activity of *A. praecox* fungus was investigated using total phenol, total flavonoid, metal chelation and ABTS methods.

The total phenolic activity of the fungus used in the study was determined as 52.7 GAE/mg crude extract and the phenolic compounds it contained were found to be quite high. Total flavonoid activity was calculated as 1.46 QE/mg and 23.2 CE/mg. It may be where it is more effective than the standard catechin quercetin. It is well known that fungi absorb or store heavy metals in the environment. Based on this, the metal chelating activity of A. praecox fungus was examined and by calculating the IC₅₀, the percentage inhibition of the fungus was determined to be 5.74 mg when the chelating activity of heavy metals was high. The damage of free radicals to the body has been supported by research and the presence of antioxidants must be present in order to cleanse the radicals mushroom. Write with the study that we have antioxidant properties of A. praecox fungus.

Discussion and Conclusion

Mushrooms are an easily digestible protein source that contains less than animal foods but more protein than many plant foods (Andlauer et al., 2002; Kruger et al., 2003). Fungi contain all the essential amino acids but small amounts of methionine, cysteine and sulfuric amino acids. Fungi contain all minerals at all stages of their development including abundant P and K to a lesser extent Fe and Ca (Andlauer et al., 2002; Kruger et al., 2003). They are also a source of vitamins such as thiamine, riboflavin, niacin, biotin and ascorbic acid (Andlauer et al., 2002; Kruger et al., 2003). In addition, since fungi are rich sources of folic acid they can be used in the treatment of anemia (Schwartz., 1950). Some types of fungi contain high levels of β -carotene and ergosterol. These compounds are converted to active vitamin D when exposed to UV rays (Breene, 1990). In addition, chanterelle (2.9-5.8 μ g / 100 μ g) contains high amount of vitamin D2 (Mattila et al., 2000). Mushrooms; fatty acids, glycerides (mono-, di- and tri-), sterols, sterol esters and phospholipids etc. It contains all the main lipid components. However, their oil content is generally low, about 2-8% in dried mushrooms (Öztürk et al., 2009). Fungi contain many biochemical substances with therapeutic activity such as protein polysaccharide compounds (polysaccharide-K, polysaccharide peptide and lentinan), secondary metabolites (terpenes, alkaloids and lactones) and enzymes (lactase, glucose oxidase and peroxidase (Smith et al., 2002).

Fungi can synthesize some phenolic compounds, flavanoids, tocopherols, ascorbic acid, quinones, terpenoids and phenyl propanoid derivative compounds with antioxidant effect. The compounds calvacin, volvotoxin, flammutoxin, lentinan and poricin, which have antitumor effect are very important antioxidant substances isolated only from macro fungi. These compounds also show antiviral properties (Smith et al., 2002).

Numerous medicinal effects of commercial mushrooms have been discovered as well as their nutritional properties (Türkoğlu et al., 2006). Recently, a large number of fungi used in scientific studies on new treatment methods; It has been found to have many therapeutic effects such as antitumor, anti-inflammatory, immunosupporting and antibiotic effects. In the literature study, there is no information showing that the mushroom sample used in this study has antioxidant properties. Only a few studies have noted that this mushroom has antitumor properties (Rathore et al., 2017).

Thanks to our study, it has been proven that *A.* praecox edible fungi have antioxidant properties and it has been one of our aims to add new information to the literature and to enable researchers to benefit from this information. It is obvious how important fungi are used as food supplements among the people. It reveals the importance of their use and importance in terms of the demands placed on them since ancient times. With further studies a more comprehensive study will be conducted by making antitumor, antibacterial and cytotoxicity analyzes of this species. Gallic acid, quercetin, catechin, chelating activity and radical scavenging activity values of the study were found to be quite high. Similar results were obtained in other studies

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