KSÜ Tarım ve Doğa Derg 24 (2): 267-277, 2021
KSU J. Agric Nat 24 (2): 267-277, 2021
https://doi.org/10.18016/ ksutarimdoga.vi.742646

Investigation of Nutritional Content, Antioxidant Anticancer and Antimicrobial Activities of *Pleurotus eryngii* (DC. Ex Fr.) Quel, Extract Obtained by Different Solvents

Hilal ACAY[1], Abdurrahman DÜNDAR[2], Semra KAÇAR[3], M. Fırat BARAN[4], Cumali KESKIN[5]
[1,3] Mardin Artuklu University, Faculty of Health Science, Department of Nutrition and Dietetics, Mardin, Turkey; [2]Mardin Artuklu University, Vocational Higher School of Health Services, Department of Medical Services and Techniques, Mardin, Turkey
[1]https://orcid.org/0000-0002-7732-106X, [2]https://orcid.org/0000-0002-7930-1054, [3]https://orcid.org/0000-0002-9869-9045, [4]https://orcid.org/0000-0001-8133-6670, [5]https://orcid.org/0000-0003-3758-0654
✉: cumalikeskin@artuklu.edu.tr

ABSTRACT

The present study related the nutritional contents, fatty acid compositions, in vitro antioxidant, anticancer and antimicrobial activities of different solvents extracts of *Pleurotus eryngii.* The nutritional contents of the *P. eryngii* were investigated in detail. Crude protein (g 100 kg-1), carbohydrate (g 100 kg-1), lipid (g 100 kg-1), ash (g 100 kg-1), dietary fiber (g 100 kg-1) and energy value (cal 100 g-1) of fruiting bodies were analysed. Fatty acid compositions of total lipid, phospholipid and triacylglycerol fractions of *Pleurotus eryngii* were investigated by Gas chromatography. Totally 13 different fatty acids constituents were identified. Antioxidant properties including total antioxidant activity, free radical scavenging activity, reducing power activity, metal chelating activity, and superoxide anion radical scavenging activity were evaluated using different tests. The highest total antioxidant, DPPH scavenging, metal chelating, and superoxide anion radical scavenging activities were determined in ethyl acetate extracts. The potential of cytotoxic activity on *PC-3* (prostate cancer) cell lines was found to depend on the concentration and the type of the tested extracts fractions. It was determined that hexane, ethyl acetate, and methanol extracts of *Pleurotus eryngii* have inhibition effect on the growth of tested microorganisms. The present results suggested that used as a daily nutrient, the *Pleurotus eryngii* could be a novel source for new drug developments and promising in some cancer treatment.

Research Article

Article History
Received : 27.05.2020
Accepted : 28.07.2020

Keywords
Antioxidant Activity
Chemical Composition
Nutritional Value
Pc-3 Cell Lines
Pleurotus Eryngii

Pleurotus eryngii (DC. Ex Fr.) Quel'in Farklı Çözücülerle Elde Edilen Özütlerinin Besinsel İçeriğinin, Antioksidan Antikanser ve Antimikrobiyal Aktivitelerinin Araştırılması

ÖZET

Bu çalışma *Pleurotus eryngii*'nin farklı çözücü ekstraktlarının besinsel içerikleri, yağ asidi bileşimleri, in vitro antioksidan, antikanser ve antimikrobiyal aktiviteleri ile ilgilidir. *P. eryngii*'nin besinsel içeriği ayrıntılı olarak araştırıldı. Meyve gövdesinin ham protein (g 100 kg-1), karbonhidrat (g 100 kg-1), lipit (g 100 kg-1), kül (g 100 kg-1), diyet lifi (g 100 kg-1) ve enerji değeri (100 g-1) analiz edildi. *Pleurotus eryngii*'nin toplam lipit, fosfolipid ve triaçilgliserol fraksiyonlarının yağ asidi bileşimleri Gaz kromatografisi ile araştırıldı. Toplam 13 farklı yağ asidi bileşeni belirlendi. Toplam antioksidan aktivite, serbest radikal süpürme aktivitesi, indirgeme gücü aktivitesi, metal şelatlama aktivitesi ve süperoksit anyon radikali süpürme aktivitesini içeren antioksidan özellikler farklı testler kullanılarak değerlendirildi. En yüksek toplam antioksidan, DPPH radikali süpürme, metal şelatlama ve süperoksit anyon radikal süpürme aktiviteleri etil asetat özütünde belirlendi. PC-3 (prostat kanseri) hücre hattı üzerindeki sitotoksik aktivite potansiyelinin, test edilen özüt fraksiyonlarının konsantrasyonuna ve tipine bağlı olduğu bulundu. *Pleurotus eryngii*'nin heksan, etil asetat ve metanol ekstraktlarının, test edilen mikroorganizmaların büyümesini inhibe

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 27.05.2020
Kabul Tarihi : 28.07.2020

Anahtar Kelimeler
Pleurotus Eryngii
Antioksidan Aktivite
Kimyasal Bileşim
Besin Değeri
PC-3 Hücre Hatları

edici etkiye sahip olduğu belirlendi. Mevcut sonuçlar, günlük besin olarak kullanılan *Pleurotus eryngii*'nin yeni ilaç gelişmeleri ve bazı kanser tedavilerinde umut vaat eden yeni bir kaynak olabileceğini düşündürmektedir.

To Cite : Acay H, Dündar A, Kaçar S, Baran MF, Keskin C 2021. Investigation of Nutritional Content, Antioxidant Anticancer and Antimicrobial Activities of *Pleurotus eryngii* (DC. Ex Fr.) Quel, Extract Obtained by Different Solvents. KSU J. Agric Nat 24 (2): 267-277. https://doi.org/10.18016/ ksutarimdoga.vi.742646

INTRODUCTION

People have consumed wild mushrooms since ancient times as part of their nutrients due to their unique taste, nutritional value, and rich mineral contents. Wild edible mushrooms have been identified as a source of many chemical and nutritional components. They are quite rich in terms of some minerals, water, protein, fibre, carbohydrates and vitamins such as Ca, Mg, K, selenium, choline, thiamine, riboflavin, ascorbic acid and ergocalciferol. On the other hand fatty acids, mono-, di- and triglycerides, sterols, and phospholipids contents are very low. Therefore, they are attractant and considered as low-calorie foods by the people for daily dietary consumption (Heleno et al. 2009; Kalac 2009; Kalac 2012). Various medicinal and nutritional properties of fungi have been notified in much research. They are including many bioactive compounds, such as phenolics. These compounds have antioxidant, antitumor, anti-inflammatory, antibacterial and antifungal activities (Dundar et al. 2013; Ferreira et al. 2010; Ren et al. 2016; Taofiq et al. 2015; Alves et al. 2013). *Pleurotus eryngii* is known as king oyster mushroom, compared to other species of *Pleurotus*, it has a unique flavour, different aromatic structure, high nutrient content, important medicinal properties, economic importance, longer shelf life, fleshy structure and other characteristics related to cookery (Rodriguez and Royse, 2007; Moonmoon et al. 2010). Recently it has become an extremely preferred species among North African, Asian and European consumers (Jeong et al. 2010). Nutritional contents of *Pleurotus* species such as chemical composition, macro and micronutrients, amino acids, proteins have been investigated (Ahmed et al. 2013; Oyetayo and Ariyo 2013; Musieba et al. 2013). But, the nutrient contents of the *Pleurotus* species may vary according to the physical and chemical differences in the habitat, substrate composition, pileus size and harvest seasons (Khan and Tania 2012). Genus *Pleurotus* contains a lot of substances that are biologically active including polysaccharides, proteoglycans, lectins, ß- and α-glucans lipids, peptide, sterols, dietary fibre and low-molecular-weight compounds such as terpenoids, fatty acid esters and polyphenols (Liu et al. 2010; Iwona et al. 2018). Hwang et al. (2003) reported that the water extract of *Pleurotus eryngii* showed inhibitory activity on the human colon cell lines (HT-29) and the human colorectal adenocarcinoma (Caco-2) cell lines. Dündar et al. (2013) reported that cultured *P. eryngii* mycelium ethanol extract showed 60.68% antioxidant activity in the ß-carotene–linoleic acid test system at a concentration of 10 mg/ml. *P. ostreatus*, a selenium accumulator, has been reported to show antitumor activity by inhibiting DNA cytosine methyltransferase (Ferreira et al. 2010). Llaurado et al. (2012) reported that the *Pleurotus* sp. water, and methanol extract had an immunomodulatory effect (on murine spleen cells) in mice, and the stimulation index was 1.90 and 1.28 respectively in 72 hours. In a study, researchers reported that the water-soluble polysaccharide extract (6.4 mg/mL) of *P.eryngii* increases the activities of antioxidant enzymes and effectively removes the DPPH free radicals (90.46%) in the CCl4-induced hepatotoxicity (Chen et al. 2012). In another study, researchers reported that the HepG-2 cells which were treated with 100 mg/mL P. eryngii polysaccharide extract showed antiproliferative effect (20% of early apoptotic cells) at 6 h (Ren et al. 2016). Also ß-glucans and α-glucans have anticancer properties (Jedinak et al. 2010; Wu et al. 2011). Furthermore, *Pleurotus eryngii* has phenolic and flavonoid compounds with antioxidant properties such as p-coumaric acid, gentisic acid, chlorogenic acid, syringic acid, caffeic acid, quercetin, rutin, kaempferol, catechin, protocatechuic acid, ferulic acid, t-cinnamic acid, vanillic acid, gallic acid, luteolin, naringenin, benzoic acid, 4-hydroxybenzoic acid, 2,5-Dihydroxybenzoic acid (Li and Nagendra 2013, Gasecka et al. 2016). However, the cytotoxic activity of the *P. eryngii* extracts on *PC-3* cell lines has not been reported yet. These studies are very important in terms of better evaluation of *P. eryngii* as an alternative nutrient source and in obtaining new comparative data for the investigation of new therapeutic alternatives. Herein, the antioxidant properties, fatty acid compositions, nutritional content, the antimicrobial and cytotoxic activity of different solvents extracts of *Pleurotus eryngii* were investigated in detail.

MATERIALS and METHODS

Material

Pleurotus eryngii samples were collected from the field study in the province of Hakkari/Türkiye between April to May 2016. Taxonomic identification of material was according to Phillips (1994), using ecological, macroscopic data.

Tested Microorganisms

Commercially purchased *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615), *E. coli* (ATCC 25922), *B. subtilis* (ATCC 11774), *P. aeruginosa* (ATCC 27853) standard bacterial strains and *C. albicans* (ATCC 10231) yeast were used for antimicrobial activity essay.

Standard antibiotic discs

Ofloxacin (OFX, 5 µg), Imipenem (IPM, 10 µg), Netilmisin (NET, 30 µg), Amoxycillin (20 µg)/Clavulanic acid (10 µg) (AMC, 30 µg), Netilmisin (NET, 30 µg) and Nystatin (N, 60 µg) (Thermo Scientific Oxoid) were purchased commercially.

Determination of nutritional contents of *Pleurotus eryngii*

Energy value, crude protein (nitrogen), ash, lipids and carbohydrate amounts of mushroom samples (of dry material%) were investigated according to the methods specified in the literature (Kjeldahl 1883; AOAC, 1990; Dundar et al. 2009). Khejdahl system was used for nitrogen determination. Energy value was determined by using the calorimeter (IKA C 200). Cauterization at 600±15 degrees C was used for the determination of ash. The dietary fibre amount was determined according to AOAC 993.21 method (AOAC 1990). Amino acid concentrations in the fungus sample were determined by using the HPLC accoutered with a combined system with an Agilent 1260 Infinity HPLC-DAD (Das et al. 2014). Each experiment was repeated 3 times and the results were given with mean and standard deviations.

Fatty Acid Compositions of Total Lipid, Phospholipid and Triacylglycerol Fractions of *Pleurotus eryngii*

The isolation and transmethylation of fatty acids were carried out as described by Kaçar et al. (2016). Shimadzu GC 2010 PLUS model Gas Chromatography device, flame ionization detector (FID) and DB-23 (Bonded 50% cyanopropyl) (J & W Scientific, Folsom, CA, USA) capillary column (30m x 0.25mm inside diameter x 0.25 µm film thickness) were used for the analysis of fatty acids methyl esters. Firstly, 5 g of ground mushrooms were extracted into a mixture of chloroform-methanol (2: 1 v/v). Secondly, the total lipids in the sample were separated by thin-layer chromatography into phospholipids and triacylglycerol fractions. Total lipids; 80: 20: 1 ratio was carried out in a mixture of petroleum ether-diethyl ether-acetic acid. Air-drying plates were sprayed with 2.7-dichlorofluorescein to make lipid fractions visible under the UV lamp. The bands of phospholipids and triacylglycerol were determined using the standards (Sigma-AldrichChemicals of fatty acids). Finally, the per cent content of the fatty acids of the hexane-extracted material was analyzed by gas chromatography, with the conversion of fatty acids to fatty acid methyl esters. Total amounts of fatty acids were obtained by the GC Solution (Version 2.4) computer program. SPSS 15 computer program was applied to compare the percentages of fatty acids of fungi. The t-test was used to compare the fatty acid percentages of the two groups. Duncan's multiple range tests were used to determine the difference between means (Duncan 1955).

Preparation of macrofungi extracts for in vitro assays

The fruiting bodies of the *P. eryngii*, dried at room temperature, were made ready for analysis by milling. The 50 g powdered mushroom samples were taken in a flask and extracted with series of organic solvents (200 ml hexane, ethyl acetate, and methanol) with different polarities in the constant stirring rate at 250 rpm for 24 hours and then filtered. This procedure was repeating three times for each solvent. Finally, solvent of hexane, ethyl acetate, and methanol extracts has been evaporated in the rotary evaporator at 40 °C under vacuum. Crude extracts were re-dissolved in methanol/DMSO (8:2) for preparing stock solution (7 mg/ml) and stored at -20°C for later use. The polarity index of hexane, ethyl acetate, and methanol were reported as 0, 4.3, and 6.6 respectively (Keskin et al. 2018).

In vitro antioxidant assays

The total antioxidant activity of mushroom extracts was determined by the ß-carotene-linoleic acid method (Dapkevicius et al. 1998). DPPH radical scavenging activities of mushroom extracts were performed spectrophotometrically according to Cu et al. (2000). Superoxide anion radical scavenging activity was performed according to the riboflavin-methionine-light-NBT method (Zhishen et al. 1999). The metal chelating activity of the mushroom extract was detected by the method of Dinis et al. (1994). The reducing power activity of the mushroom extracts was determined according to Oyaizu, (1986).

Cytotoxic activity of mushroom extracts

Cytotoxic activity of *P. eryngii* extracts was evaluated according to the method of Alley et al. (1998). MTT test was used to evaluate the PC-3 (prostate cancer) cell line. MTT solution: 5 mg MTT was dissolved in phosphate buffer solution (CMFPBS) (pH = 7.0) without 1 ml divalent cations (Ca^{++} and Mg^{++}). The solution was stored in the dark at 4 °C. Serum media for PC-3 (CRL-1435, ATCC) cell culture: 10 ml inactivated FBS (Fetal Bovine Serum) (10%), 1 ml penicillin (100 U)/streptomycin (100 mg/ml) solution (1%) DMEM-F12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12). 24-h culture phases were applied in each well for PC-3 cells in 200 µl medium

with 105 cells. The 96-well microplate was incubated for 24 hours in a humidified incubator containing 5% CO_2 at 37 °C. After 24 hours, the culture medium was removed from the wells using an 8-channel automatic pipette. 50 µl of PBS and 90 µl of fresh media was added to each well. Subsequently, 10 µl of the test substance was applied to the wells at a concentration of ½ per cent dilution to the MTT test. Cytotoxicity tests were performed 7 times, 4 replicates for each concentration and 8 repetitions in the same order for a second day.

20 µl of MTT solution was added to each well of the 96-well microplate containing the cell line incubated for 24 hours at 37 °C 5% CO_2 with *P. eryngii* extracts. After shaking at 150 rpm for 5 min, it was incubated at 37 °C for 2-3 hours. The top liquid in the wells was discarded. 100 µl of DMSO was added to the wells. Shaken at 150 rpm for 5 min. The intensity of the resulting colour was measured at 590 nm (versus 670 nm reference wavelength). By comparing the absorbance value of the tested compounds and the solvent control group, the number of cells killed % (Inhibition concentration, IC) was calculated. The absorbance values of the wells (solvent controls) containing the solution of the test substance instead of the test sample showed 100% viability. It was determined that DMSO used as 1% in the experiment had no cytotoxic effect on the cells. The absorbances at 590 nm for the MTT test were measured against the 690 nm reference wavelength. Corrected absorbance values were obtained by subtracting the blind absorbance from each solvent control and sample absorbance. Calculations were made by taking the average of absorbance values for repeats in a microplate. The relative inhibition activity (IC) was calculated according to the following formula as a percentage of the solvent control;

Inhibition%= (corrected mean Absorbance sample/corrected mean Absorbance solvent control) x100

Viability % was calculated using solvent control for MTT testing. The inhibition % curve against the exposure concentration was plotted. The concentration corresponding to 50% inhibition from the curve was determined as IC_{50}. Studies on cytotoxic activity were performed at the laboratories of Istanbul University Faculty of Pharmacy.

Determining antimicrobial activity of mushroom extracts

Antimicrobial activity of obtained extracts was determined by the disk diffusion test taking into consideration the National Committee for Clinical Laboratory Standards rules. The disk diffusion test was performed using Nutrient Agar and Sabouraud Dextrose Agar medium. Before the microorganisms were inoculated, the solid media were incubated at 35-36 °C for 30 minutes. It was sterilized and cooled to 45-50 °C, Nutrient Agar and Sabouraud Dextrose Agar (SDA Merck) were poured into 25 ml sterile petri dishes with a diameter of 9 cm. It was sterilized and cooled to 45-50 °C, Nutrient Agar and Sabouraud Dextrose Agar (SDA Merck) were poured into 25 ml sterile petri dishes with a diameter of 9 cm. The mediums were incubated at 37 degrees for overnight for contamination control. Bacteria (108 pieces / mL) and yeast (107 pieces/mL) were added to the water bath and 150 µL of them were collected and distributed to the sterile petri dishes with sterile cotton swabs. 30 mg/L (210 µg/disc) of mushroom (7 mg/mL) solutions were impregnated on 6 mm sterile blank paper discs. Discs impregnated with fungal solutions were placed on the solidified agar by lightly pressing. Petri dishes prepared in this way were incubated in the oven at 37 °C for 24/48 hours, after which the inhibition zone diameters on the medium were measured as mm using a ruler.

RESULT and DISCUSSIONS

In this study, nutritional contents, fatty acid composition, antioxidant, anticancer and antimicrobial activities of extracts obtained with solvents that vary from non-polar to polar (hexane to methanol) were investigated.

Determination of nutritional contents

Macrofungi have the essential nutrients for human health because of their structures. Mushrooms are an edible fungus that can ensure diverse major nutrients. The many varieties of mushroom have different compositions and nutritional profiles. Mushrooms are in rich with protein, vitamins, minerals, and antioxidants. These natural compounds have various health benefits. For this purpose, data on nutrients (fibre, protein, fat, carbohydrate, ash and energy amounts) of *P. eryngii* were analyzed in detail. Crude protein (Nx4.38), carbohydrate, lipids, ash, dietary fibre and energy value of *P. eryngii* fruiting bodies (% of dry substance) were determined as 15.93±0.06 g 100 kg-1, 68.4±0.59 g 100 g-1, 3.34±0.02 g 100 g-1, 9.9±0.01 g 100 g-1, 24.6±0.05 g 100 g-1 and 3888±0.23 cal 100 g-1 respectively. There are many studies on the different nutritional contents of *Pleurotus* species. Ahmet et al. (2013) investigated the nutritional content of *P. high-king* (PHK), *P. ostreatus* (PO3), *P. geesteranus* (PG1), *P. geesteranus* (PG3), and *P. ostreatus* (PO2) strains also they reported that the highest Ca (338±1.0), Mg (122±2.0), and K (14±0.0) amount was found in PG3 and the highest amount of Fe (468±11) and Cu (3.6±0.0) was in PHK type. However, they reported that the highest protein and ash content was found in strain PG1, while the highest lipid and crude fiber content was found in strains PO2 and PO3, respectively. Oyetayo et al. (2013) reported that the

rates of Mg (3.57±0.01), Ca (8.87±0.006), phosphorus (56.77±0.006), protein (20.11±0.05), fat (3.57±0.01), and dietary fiber (17.51±0.02) contents of *P. ostreatus*, which they cultivated on different wood substrates, were higher in those grown in *Pycnanthus ongoleubis*. Musieba et al. (2013) reported that ash, protein, fat and fiber contents of *P. citrinopileatus* were 9.12±1.57, 22.10±2.0, 1.32±2.03 and 20.78±2.72 g/100 g dry matter, respectively. Mazi et al. (1999) reported that ash content was between 6.9% and 10.5% and potassium and magnesium content was higher than other elements in a study they conducted on different types of edible mushrooms (P. ostreatus, P. eryngii, P. pulmunarius and Lentinula edodes). Also, they reported the most abundant amino acids in edible mushrooms, expressed as percentages of total amino acids, were glutamic acid (12.8±20.9%), aspartic acid (9.1±12.1%), and arginine (3.7±11.7%). In another study, the chemical composition of *Pleurotus ostreatus* (raw g/100g edible weight) was investigated and the protein, fat carbohydrate, ash energy, and nutritional fiber content were reported as 1.61±0.02, 0.36±0.02, 6.69±0.01, 0.89±0.01, 36 kcal and 4.10±0.06, respectively (Manzi et al. 2001).

P. eryngii has rich carbohydrate and protein content and has a small amount of ash and oil compared to other edible fungi. This rich nutritional composition ensures that this fungus is the perfect food for use in low-calorie diets. Some researchers have suggested that the essential amino acid content of mushrooms can be compared to animal proteins (Longvah and Deosthale 1998; Mattila et al. 2001). Figure 1 shows the essential amino acid content of *P. eryngii*. It was detected eight essential amino acids for humans, and the total contents of essential amino acids varied between 0.05 mg 100g-1 to 9.83 mg 100g-1. The major components of the essential amino acid were found as threonine, methionine and leucine. Edible mushroom, *Pleurotus* contains 40% of the essential amino acids which humans need to intake, and thus it is considered as the potential diet for individuals having malnutrition problems (Pushpa and Purushothoma 2010).

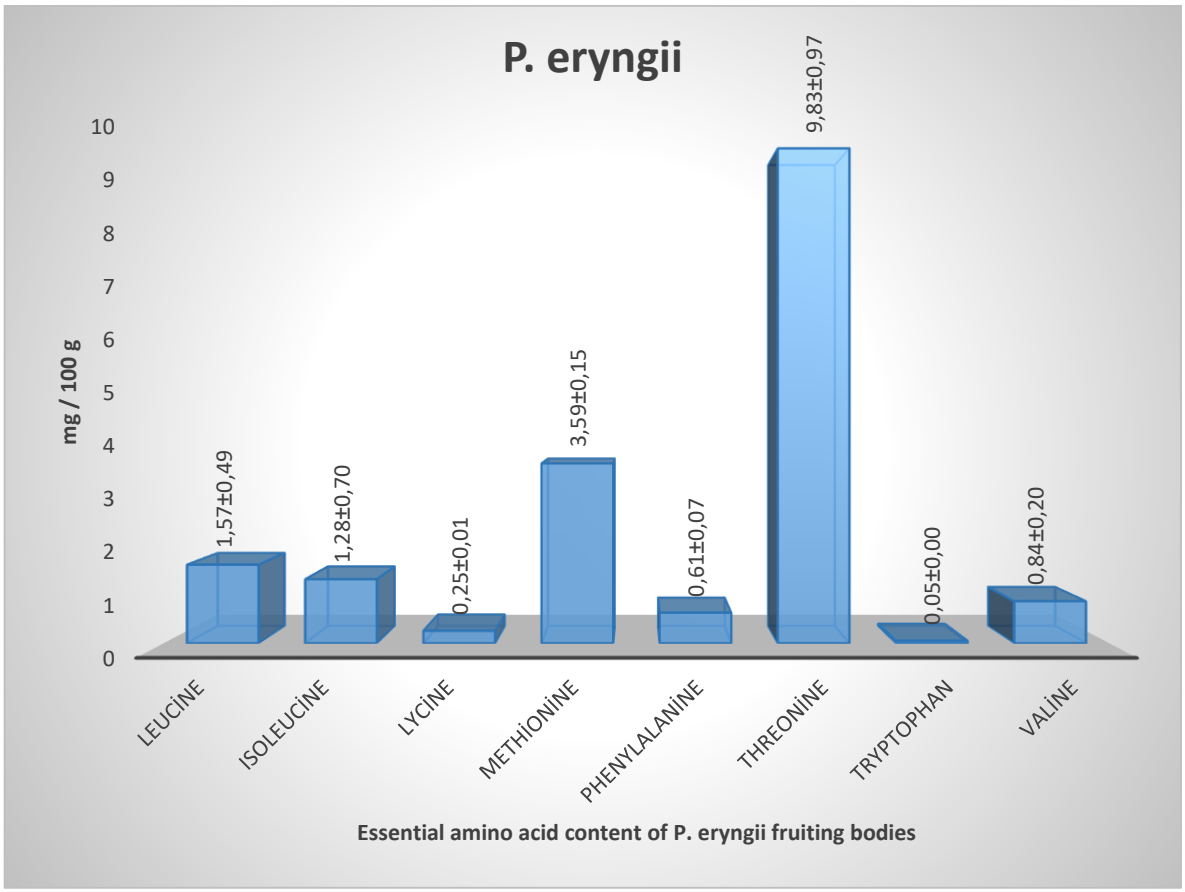

Figure 1. The essential amino acid content of *P. eryngii* fruiting bodies (mg 100 g-1) (n=3, ±SEM)
Şekil 1. P. eryngii meyve gövdesinin esansiyel amino asit içeriği (mg 100 g-1) (n=3, ±SEM)

Determination of fatty acid compositions of total lipid (TL), phospholipid (PL) and triacylglycerol (TG) fractions

Fatty acids are the main unit of lipids such as phospholipids, triglycerides, and monoglycerides. Fatty acids can be formed as saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA). Triacylglycerols (TGs) are the main neutral

lipids and they are the primary storage molecules of metabolic energy in most living organisms. Phospholipids (PLs) are the presence in nature, and they are key elements of the lipid bilayer of the cell membrane. Additionally, TGs are utilized in the food industry. PLs have favourable effects on human health (Aparicio and Aparicio-Ruiz 2000). Nevertheless, no reports have been published about the fatty acid compositions of TL, TG and PL fractions of *P. eryngii*. From this point of view, TL, TG and PL fatty acid fractions of *P. eryngii* were investigated in detail (Table 1). The following main fatty acids were identified in total lipid, triacylglycerol and phospholipid fractions: C8:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0 as saturated fatty acids (SFAs); C16:1n-7, C18:1n-9, and C20:1n-9 as monounsaturated fatty acids (MUFAs); and C18:2n-6, C18:3n-3, as polyunsaturated fatty acids (PUFAs). C 8:0 was identified only in the TG fraction at a trace amount. The results show that ΣMUFA percentages (44.5%) were higher than ΣSFA (18.25%) and ΣPUFA (37.2%) in total lipid fraction. In the phospholipid fraction, the PUFA ratio (50.14%) was the significantly highest when compared with SFA (11.85) and MUFA (30.54). The MUFA (52.95%) percentage was found higher than SFA (11.17%) and ΣPUFA (29.71%) in triacylglycerol fraction. Linoleic (C18:2n-6), oleic (C18:1n-9) and palmitic (C16:0) acids were found as major fatty acids in all fractions (Table 1). Oleic acid is the most abundant fatty acid in the human body and makes up almost half of the fatty acids. Fats with high oleic acid in its composition have been reported to have many advantages for human well-being. It is stated that oleic acid, a bioactive compound, is an effective glucosyltransferase inhibitor and inhibits high levels of human telomerase activity in a cell-free enzymatic assay (Oda et al. 2002; Won et al. 2007). Linoleic acid is an essential fatty acid because it cannot be synthesized in the human cells and it must be taken from the diet. In its insufficiency reported that retardation in growth, reproductive disorders and dermatoses in the skin, pathological changes in the liver and kidney (Dimou et al. 2002). We conclude that the presence of linoleic (C18: 2n-6) and oleic acids (C18: 1n-9) in *P. eryngii* and the abundance of essential fatty acids may create a new perspective in formulating new mushroom-based dietary supplements in further studies.

Table 1. Fatty acid composition of TL, PG and TG fractions of *P. eryngii fruiting* bodies
Çizelge 1. *P. eryngii* meyve gövdelerinin TL, PG ve TG fraksiyonlarının yağ asidi bileşimi

Fatty acid	TL (Mean±S.E.M.)*	PL (Mean±S.E.M.)*	TG (Mean±S.E.M.)*
C8:0§	-	-	0.04±0.01[a]
C12:0	-	0.04±0.01[a]	0.03±0.01[b]
C14:0	0.17±0.03[b]	-	0.25±0.02[a]
C15:0	1.75±0.08[b]	2.67±0.09[a]	-
C16:0	11.58±0.90[a]	11.85±1.01[a]	11.17±1.00[a]
C17:0	0.12±0.07[a]	-	0.13±0.08[a]
C18:0	4.31±0.90[c]	1.74±0.87[a]	5.27±0.98[b]
C20:0	0.31±0.04[b]	1.57±0.56[a]	0.39±0.06[b]
∑SFA	18.25±0.97[a]	17.87±1.21[a]	17.28±1.29[a]
C16:1n-7	0.54±0.01[c]	1.41±0.09[a]	0.29±0.06[b]
C18:1n-9	43.57±2.09[c]	30.54±2.21[a]	52.66±2.34[b]
C20:1n-9	0.39±0.02[a]	-	-
∑MUFA	44.5±2.45[c]	31.95±2.30[a]	52.95±1.88[b]
C18:2n-6	37.03±2.67[c]	50.01±2.09[a]	29.63±1.90[b]
C18:3n-3	0.17±0.03[c]	0.13±0.02[a]	0.08±0.01b
∑PUFA	37.2±2.05[c]	50.14±2.45[a]	29.71±1.66[b]

* Each data is the average of 3 replicates. Three injections were performed per repeat. § The data determined by the same letters in each line are not different from each other at the level of p> 0.05 probability. SEM.: Standard error mean, SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, PUFA: Unsaturated Fatty Acids. The percentages of fatty acids in total, triacylglycerol and phospholipid fusion were evaluated in their own right.

Determination of antioxidant properties

In living organisms, free radicals are formed in different ways. They react with membrane lipids, nucleic acids, proteins, enzymes and other small molecules to cause cellular damage. Low doses of synthetic antioxidants were able to inhibit cancer but some studies with experimental animals, indicate that they caused carcinogenesis and liver damage when used in high doses. This has led to increased interest in natural antioxidant sources. Mushrooms contain antioxidant substances to reduce oxidative damage in humans' cell (Kosanić et al. 2012; Khatua et al. 2013; Adebayo et al. 2014). Radicals scavenging activity, reducing power activity, metal chelating activity, total antioxidant activity and superoxide anion radical scavenging activity methods were applied to determine antioxidant properties. The highest results (DPPH,

metal chelating, total antioxidant and superoxide radical extinguishing activity) were determined in ethyl acetate extract, except for reduction power activity. It was determined that the activities increased with the concentration and the results changed with the used solvents. The data obtained from ethyl acetate extract was quite close to the standards used as the positive control (Table 2). *P. eryngii* methanol extract had the highest reducing power activity, but it was found that they had moderate activity in comparison with positive controls (BHT, BHA, Ascorbic acid, Trolox). These differences might be due to chemical alteration in the active compounds present in this mushroom caused by using the organic solvent extraction process. Similar results have been reported in different research (Le et al. 2007; Tan et al. 2015).

Table 2. Antioxidant activities of *Pleurotus eryngii* extracts*
Çizelge 2. Pleurotus eryngii ekstraktlarının antioksidan aktiviteleri

Pleurotus eryngii extracts/ standards	DPPH scavenging activity % (5 mg/mL	Reducing power activity (A562 nm) (10 mg/mL)	Metal chelating activity % (4 mg/mL	Total antioxidant activity % (0.1 mg/ mL-48th h)	Super oxide anion radical activity % (2 mg/mL)
HEG	74.16±0.56	1.57±0.28	80.10±3.11	62.69±0.89	77.41±1.28
EtOAc	88.82±1.02	1.39±0.09	88.84±3.04	57.97±0.06	83.24±3.08
MeOH	78.82±0.09	1.78±0.09	83.60±2.63	63.62±0.09	73.11±2.09
BHA	89.85±0.38	3.23±0.00	-	69.49±0.35	70.34±0.00
BHT	89.16±2.04	3.26±0.08	-	69.04±0.52	82.93±3.08
Askorbik asit	92.54±2.55	3.76±0.03	-	63.71±1.26	68.58±2.06
Trolox	92.14±1.23	3.58±0.08	-	59.43±0.18	82.74±3.22
EDTA	-	-	91.18±0.04	-	

*Only the results of the highest activities are given. Each value three times (n=3, ±SEM) are given by calculating; BHA: Butylated hydroxyanisole; BHT: Butylated hydroxytoluene; HEG: Hexane extracts; EtOAc: Ethyl acetate extracts; MeOH: Methanol extracts

Determination of cytotoxic activity

The result of the cytotoxic activity of *P. eryngii* extracts on the PC-3 cell lines and 50 % growth inhibition (IC_{50}) shown in Table 3. In recent studies reported that different types of the extract obtained from fruit bodies or mycelium of Pleurotus mushrooms may have been as potential anticancer agents on different cancer cell lines (Hwang et al. 2003; Sun et al. 2017). However, no studies have been set against the PC-3 cell line. Cytotoxic activity (against PC-3 cell lines) of *P. eryngii was* investigated by using the various solvent extract. Ethyl acetate extract of *P. eryngii* showed significant inhibitory activity at the concentration of 530 µg ml-1 (86.203 %) against PC-3 cell lines with IC_{50}; 109.89±2.01 µg mL-1 (Figure 2). Methanol extracts did not show any cytotoxic activity. It has been found that the potential of cytotoxic activity is depended on the concentration and solvent type of extracts. There has been increasing attention regarding the minimal toxicity fungal metabolites and potent bio pharmacological activities in cancer therapy. In particular, it can be said that naturally occurring anticancer compounds with low toxicity and high potency to normal cells should be in human diets. The positive results obtained from the ethyl acetate extract may have positive consequences for other cancer cells.

Determination of antimicrobial activity

There are several reports in the literature regarding the antimicrobial activity of extracts from the genus of *Pleurotus* (Mondal et al. 2013; Neelam and Singh 2013; Rahman et al. 2013). *P. eryngii* extracts have inhibitory activity against a group of bacterial reference strains were stated in some studies (Shang et al. 2013; Schillaci et al. 2013; Akyüz and Kırbağ

Table 3. Cytotoxic activity of *P. eryngii* extracts and 50% growth inhibition (IC_{50})[a]
Çizelge 3. P. eryngii ekstrelerinin sitotoksik aktivitesi ve %50 büyüme inhibisyonu (IC50)[a]

Mushroom	Exposure Concentrations (µg/mL)		% İnhibition		IC_{50} (µg/mL)	
	EtOAc	MeOH	EtOAc	MeOH	EtOAc	MeOH
P. eryngii	530	680	86.203	51.868	109.89±2.01	_
	265	-	61.131	ND		
	132.5	-	52.909	ND		
	66.25	-	36.578	ND		
	33.13	-	29.954	ND		
	16.56	-	18.555	ND		
	8.28	-	8.103	ND		

[a]IC_{50}values were expressed as the mean±SEM, determined from the results of MTT assay in triplicate experiments. EtOAc: Ethyl acetate extracts; MeOH: Methanol extracts, ND: Not Detected

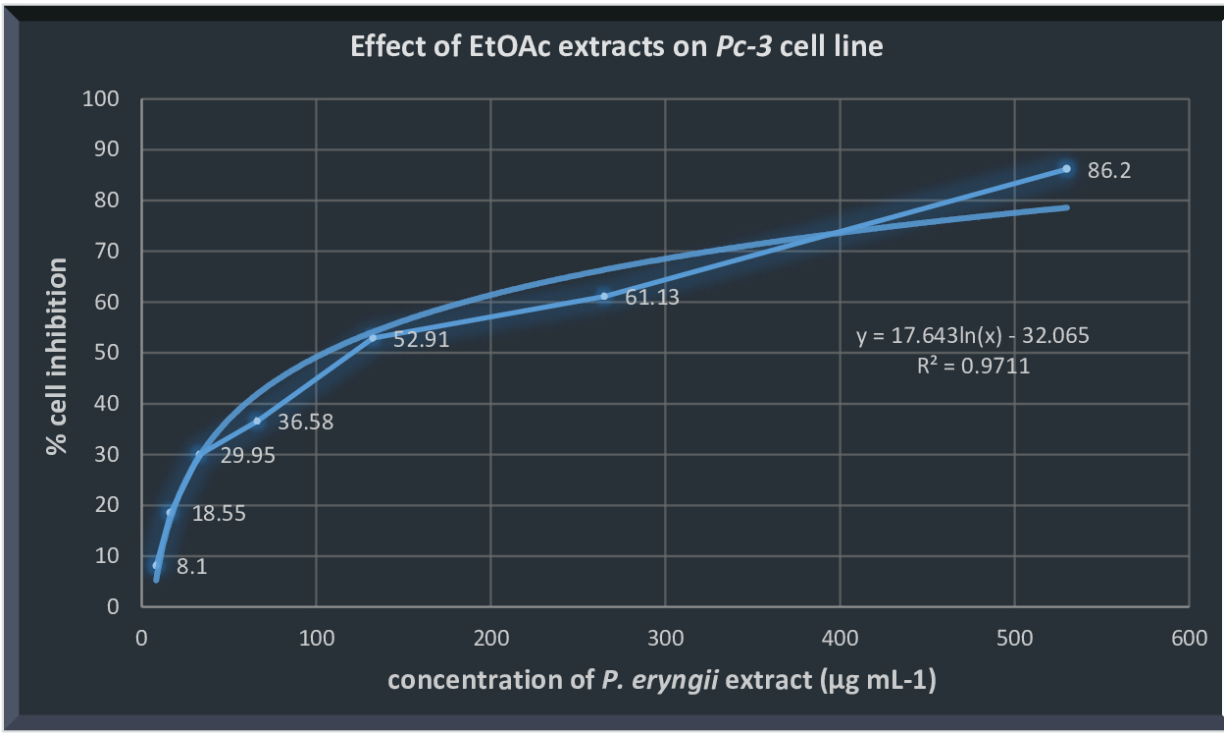

Figure 2. Cytotoxic activity of ethyl acetate extract
Şekil 2. Etil asetat ekstresinin sitotoksik aktivitesi

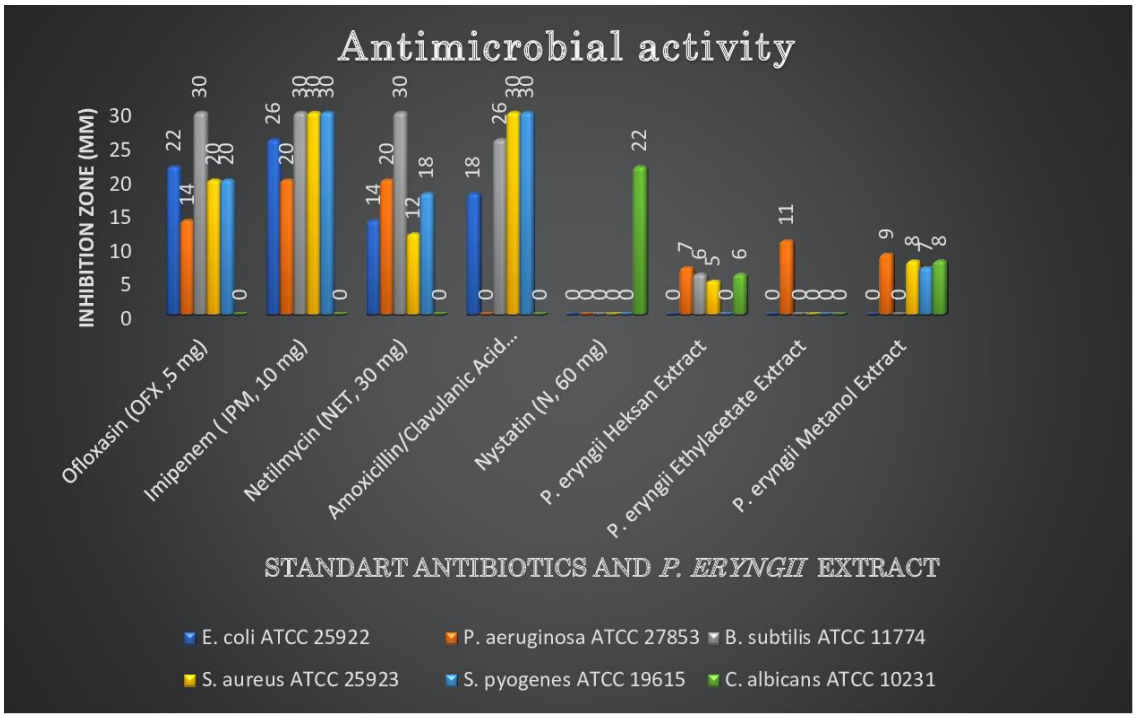

Figure 3. Antimicrobial activity assay
Şekil 3. Antimikrobiyal aktivite deneyi

2009). In the current study, the highest antimicrobial activity was obtained against *Pseudomonas aeruginosa* ATCC 27853 (11 mm) in ethyl acetate extract. *P. eryngii* hexane extract showed weak growth inhibition on *P. aeruginosa*, *B. subtilis*, *S. aureus* bacterial strains and *C. albicans* yeast. The methanol extract showed moderate growth inhibition against *P. aeruginosa*, *S. aureus*, *S. pyogenes* bacterial strains and *C. albicans* yeast. The ethyl acetate extracts only inhibited *P. aeruginosa* growth. However, none of the tested extracts showed an inhibitory effect on the growth of *E. coli* bacteria strain (Figure 3). In this regard, the *Pleurotus eryngii* extracts were not showed strong antimicrobial activities when compared with standard antibiotics but we believe that the values may change when solvent differences considered.

CONCLUSIONS

Mushrooms are very rich in classes of phenolic compounds such as coumaric acid, chlorogenic acid, syringic acid, caffeic acid, quercetin, rutin, catechin, protocatechuic acid, ferulic acid, gallic acid, which are considered as secondary metabolites. These compounds are very important in terms of antioxidant metabolism. They eliminate the effects of free radicals caused by cellular activities. Also, by directing the cells to apoptosis, they prevent cancer cell proliferation and tumour generation. Furthermore, *Pleurotus* mushrooms are noted as one of the richest and well-balanced sources in terms of human nutrition and commonly human food and nutraceutical industries. Moreover, due to high nutritional values, these mushrooms can help significantly against malnutrition disease and can have positive effects on general health. In this respect, the further chromatographic and structural study needs to be conducted to know which compounds are in charge of from which activity. Also, in vivo studies are needed to understand how mushrooms are efficacious in living creatures. Following these studies, we suggest that usage of these potentials of mushrooms in the pharmaceutical and food industry may be beneficial to reduce the utilization of synthetic compounds. The believe that results will provide new baseline information for further study on *Pleurotus eryngii*.

ACKNOWLEDGEMENTS

This study was a part of the project having the financial support of the coordination Unit of Scientific Research Project of Mardin Artuklu University. Project no. MAÜ -15- SYO- 04.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

Adebayo EA, Oloke JK, Aina DA, Bora TC 2014. Antioxidant and Nutritional Importance of some *Pleurotus* species. Journal of Microbiology, Biotechnology and Food Sciences, 3: 289-294.

Ahmed M, Abdullah N, Ahmed KU, Bhuyan MHMB 2013. Yield and nutritional composition of oyster mushroom strains newly introduced in Bangladesh. Pesquisa Agropecuária Brasileira, 48(2): 197-202.

Akyüz M, Kırbağ S 2009. Antimicrobial activity of *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *eryngii* which obtained from the various culture medium. Ecological Life Sciences, 4(2): 61-68.

Alley MC, Scudiere DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbot BJ, Mayo JG, Shoemaker RH, Boyd MR 1998. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Research, 48: 589-600.

Alves MJ, Ferreira ICFR, Froufe HJC, Abreu RMV, Martins A, Pintado M 2013. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. Journal of Applied Microbiology, 115: 346–357.

AOAC 993.21. 1990. Total Dietary Fiber in Foods and Food Products J. AOAC İnt. 77,687 AOAC, Official methods of analysis (15th ed.). Virginia DC, USA: Association of the agricultural chemist. (1994): 746-780.

Aparicio R, Aparicio-Ruiz R 2000. Authentication of vegetable oils by chromatographic techniques. Journal of Chromatography A. 881(1-2): 93–104.

Chen JJ, Mao D, Yong YY, Li JL, Wei H, Lu L 2012. Hepatoprotective and hypolipidemic effects of water-soluble polysaccharide extract of *Pleurotus eryngii*. Food Chemistry, 130: 687–694.

Chu YH, Chang CL, Hsu HF 2000. Flavonoid content of several vegetables and their antioxidant activity. Journal of the Science of Food and Agriculture, 80: 561-566.

Dapkevicius A, Venskutonis R, Van-Beek TA, Linssen JPH 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. Journal of the Science of Food and Agriculture, 77: 140–146.

Das AJ, Khawas P, Miyaji T, Deka SC 2014. HPLC and GC-MS analyses of organic acids, carbohydrates, amino acids and volatile aromatic compounds in some varieties of rice beer from northeast India. Journal of the Institute of Brewing, 120: 244–252.

Dimou DM, Georgala A, Komaitis M, Aggelis G 2002. Mycelial fatty acid composition of Pleurotus spp. and its application in the intrageneric differentiation. Mycological Research, 106: 925–999.

Dinis TCP, Madeira VMC, Almeida MLM 1994. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics, 315: 161-169.

Duncan DB 1955. Multiple ranges and multiple F tests. Biometrics, 11:1-42.

Dundar A, Acay H, Yildiz A 2009. Effect of using different lignocellulosic wastes for the cultivation of *Pleurotus ostreatus* (Jacq.) P. Kumm. on mushroom yield, chemical composition and nutritional value. African Journal of Biotechnology, 8:662–666.

Dundar A, Okumus V, Ozdemir S, Yildiz A 2013. Antioxidant properties of cultured mycelia from four *Pleurotus* species produced in the submerged

medium. International Journal of Food Properties, 6: 1105-1116.

Ferreira ICFR, Vaz JA, Vasconcelos MH, Martins A 2010. Compounds from wild mushrooms with antitumor potential. Anti-cancer Agents in Medicinal Chemistry, 10: 424-436.

Gasecka M, · Mleczek M, Siwulski M, Niedzielski P 2013. Phenolic composition and antioxidant properties of Pleurotus ostreatus and Pleurotus eryngii enriched with selenium and zinc. European Food Research and Technology, 242: 723–732.

Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira ICFR 2009. Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high-performance liquid chromatography. Microchemical Journal, 93:195–199.

Hwang YJ, Nam HK, Chang MJ, Noh GW, Kim SH 2003. Effect of *Lentinus edodes* and *Pleurotus eryngii* extracts on proliferation and apoptosis in human colon cancer cell lines. Journal of the Korean Society of Food Science and Nutrition, 32: 217–222.

Iwona GK, Kałużewicz A, Spiżewski T, Siwulski M, Sobieralski K 2018. Bioactive compounds and medicinal properties of Oyster mushrooms (Pleurotus sp.). Folia Horticulturae, 30(2): 191-201.

Jeong YT, Jeong SC, Gu YA, Islam R, Song CH 2010. Antitumor and immunomodulating activities of endo-biopolymers obtained from a submerged culture of *Pleurotus eryngii*. Food Science and Biotechnology, 19: 399–404.

Kaçar S, Başhan M, Oymak AS 2016. Effect of seasonal variation on lipid and fatty acid profile in muscle tissue of male and female *Silurus triostegus*. Journal of Food Science and Technology Mysore, 53(7): 2913–2922.

Kalac P 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chemistry, 113: 9–16.

Kalac P 2012. Chemical composition and nutritional value of European species of wild growing mushrooms. In S. Andres, & N. Baumann (Eds.), Mushrooms: Types, properties and nutrition, Nova Science Publishers, 130–151.

Keskin C, Özen HÇ, Toker Z, Kızıl G, Kızıl M 2018. Determination of in vitro antioxidant and antimicrobial properties of the shoot and root extracts of *Astragalus diphtherites* FENZL var. *diphtherites* and *Astragalus gymnalopecias* RECH. FIL. obtained by different solvents. Journal of Agriculture and Nature, 21(2): 157-166.

Khan MA, Tania M 2012. Nutritional and Medicinal Importance of Pleurotus Mushrooms: An Overview. Food Reviews International, 28(3): 313-329.

Khatua S, Paul S, Acharya K 2013. Mushroom as the potential source of new generation of antioxidant: a review. Research Journal of Pharmacy and Technology, 6: 496-505.

Kjeldahl Johan Z 1883. A new method for the determination of nitrogen in organic bodies." Analytical Chemistry, 22: 366.

Kosanić M, Ranković B, Dašić M 2012. Mushrooms as possible antioxidant and antimicrobial agents, Iranian Journal of Pharmaceutical Research, 11: 1095-1102.

Lee YL, Huang GW, Liang ZC, Mau JL, 2007. Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. Food Science and Technology, 40: 823–833.

Li S, Nagendra PS 2013. Effects of Various Heat Treatments on Phenolic Profiles and Antioxidant Activities of Pleurotus eryngii Extracts. Journal of Food Science, 78(8): 1122-1129.

Liu XN, Zhou B, Lin RS, Jia L, Deng P, Fan K, Wang G, Wang L, Zhang J 2010. Extraction and antioxidant activities of intracellular polysaccharide from *Pleurotus* sp. mycelium. International Journal of Biological Macromolecules, 47:116–119.

Longvah T, Deosthale Y 1998. Compositional and nutritional studies on edible wild mushroom from northeast India. Food Chemistry, 63(3): 331-334.

Manzi P, Aguzzi A, Pizzoferrato L 2001. Nutritional value of mushrooms widely consumed in Italy. Food Chemistry, 73: 321–325.

Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L 1999. Nutrients in edible mushrooms: An inter-species comparatives study. Food Chemistry, 65: 477-482.

Mattila P, Kanko K, Earola M, Pihlava JM, Astola J, Vahterist L 2001. Contents of vitamins, mineral elements, some phenolic compounds in cultivated mushrooms. Journal of Agricultural and Food Chemistry, 49: 2343–2348.

Mondal T, Some R, Dutta S 2013. Studies on antioxidant and antimicrobial properties of some common mushrooms. Journal of Today's Biological Sciences: Research and Reviews, 2: 60-67.

Moonmoon M, Uddin MN, Ahmed S, Shelly NJ, Khan MA 2010. Cultivation of different strains of king oyster mushroom (*Pleurotus eryngii*) on saw dust and rice straw in Bangladesh. Saudi Journal of Biological Sciences, 17: 341–345.

Musieba F, Okoth S, Mibey RK, Wanjiku S, Moraa K 2013. Proximate Composition, Amino Acids and Vitamins Profile of Pleurotus citrinopileatus Singer: An Indigenous Mushroom in Kenya. American Journal of Food Technology, 8(3): 200-206.

Neelam S, Singh S 2013. Comparative *in vitro* studies on Phytochemical and Antibacterial Properties of Ethanolic Extracts of *Pleurotus florida* and *Pleurotus ostreatus*. International Journal of Pharmacy and Biological Sciences, 4: 396–400.

Oda M, Ueno T, Kasai N, Takahashi H, Yoshida H, Sugawara F, Sakaguchi K, Hayashi H, Mizushina Y 2002. Inhibition of telomerase by linear-chain fatty acids: a structural analysis. Biochemical Journal, 367: 329-334.

Oyaizu M 1986. Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, 44: 307-315.

Oyetayo VO, Ariyo OO 2013. Micro and macronutrient properties of *Pleurotus ostreatus* (Jacq: fries) cultivated on different wood substrates. Journal of Biological Sciences, 6: 223-232.

Phillips R 1994. Mushrooms and Other Fungi of Great Britain & Europe, New Interlitho S.P. A., Milan: 288.

Pushpa H, Purushothoma KB 2010. Nutritional analysis of wild and cultivated edible medicinal mushrooms. World Journal of Dairy & Food Sciences, 5(2): 140-144.

Rahman MM, Asiqur R, Taslima N, Borhan U, Mafroz AB, Shahdat H 2013. Antioxidant and Antimicrobial Activity of *Pleurotus florida* Cultivated in Bangladesh. Journal of Medicinal Plants Studies, 3:166-175.

Ren D, Wang N, Guo J, Yuan L, Yang X 2016. Chemical characterization of Pleurotus eryngii polysaccharide and its tumour-inhibitory effects against human hepatoblastoma HepG-2 cells. Carbohydrate Polymers, 138: 123–133.

Rodriguez EAE, Royse DJ 2007. Yield, size and bacterial blotch resistance of *Pleurotus eryngii* grown on cottonseed hull/oak sawdust supplemented with manganese, copper and whole ground soybean. *Bioresource Technology,* 98:1898–1906.

Schillaci D, Arizza V, Gargano ML, Venturella G 2013. Antibacterial activity of Mediterranean oyster mushrooms, species of genus *pleurotus* (higher basidiomycetes). International Journal of Medicinal Mushrooms, 15: 591-594.

Shang X, Tan Q, Liu R, Yu K, Li P, Zhao GP 2013. *In vitro* anti–*Helicobacter pylori* effects of medicinal mushroom extracts, with special emphasis on the lion's mane mushroom, *Hericium erinaceus* (Higher Basidiomycetes). International Journal of Medicinal Mushrooms, 15:165-174.

Sun Y, Hu X, Li W 2017. Antioxidant, antitumor and immunostimulatory activities of the polypeptide from *Pleurotus eryngii* mycelium. International Journal of Biological Macromolecules, 97: 323-330.

Tan YS, Baskaran A, Nallathamby N, Chua KH, Kuppusamy UR, Sabaratnam V 2015. Influence of customized cooking methods on the phenolic contents and antioxidant activities of selected species of oyster mushrooms (*Pleurotus* spp.). International Journal of Food Science Technology, 52:3058–3064.

Taofiq O, Calhelha RC, Heleno SA, Barros L, Martins A, Santos-Buelga C 2015. The contribution of phenolic acids to the anti-inflammatory activity of mushrooms: screening in phenolic extracts, individual parent molecules and synthesized glucuronated and methylated derivatives. Food Research International, 76: 821-827.

Won SR, Hong MJ, Kim YM, Li CY, Kim JW, Rhee HI 2007. Oleic acid: an efficient inhibitor of glucosyltransferase. FEBS Letters, 581: 4999–5002.

Zhishen J, Mengcheng T, Jianming W 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64: 555–559.