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Fatty acid compositions of total lipid, phospholipid and triacylglycerol fractions of the wild edible mushroom pleurotus ostreatus and russula delica with cytotoxic activities on prostate carcinoma cell lines

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Abstract
The aim of the study to investigate fatty acids (FAs) of Pleurotus ostreatus and Russula delica in Total Lipid (TL), Triacylglycerol (TG) and Phospholipid (PL) fractions. The major FAs of TL, TG, PL in both species were palmitic acid (PA), oleic acid (OLA), and linoleic acid (LA). In both species, total PUFA amounts were found to be higher than total monounsaturated fatty acids (MUFA) and total saturated fatty acids (SFA) in TL, TG and PL fractions. Also, insufficiently studied cytotoxic activity (using prostate carcinoma (PC-3) cell lines) of these mushrooms were investigated by using various solvent systems. Ethyl acetate extract of Pleurotus ostreatus and Russula delica showed significant inhibitory value at the concentrations of 520-530 µg/ml (99.45-92.82%) against PC-3 cell lines with the half-maximal inhibitory concentration (IC50); 274.53-297.77 µg/mL respectively. The present study is a guide for biochemical and nutritional values of both species and can be useful for further investigation on pharmacological applications.

Keywords: Russula delica, pleurotus ostreatus, fatty acids, cytotoxic activity, PC-3 cell lines

Introduction

Mushrooms are low-calorie foods due to their low lipid level (free fatty acids, mono-, di- and triglycerides, sterols, and phospholipids) [1-2]. Fatty acids are essential components of fungi. To prevent and treat hypertension, coronary artery disease, diabetes, osteoporosis, arthritis, cancer, another inflammatory, and autoimmune disorders, it is known that omega-3 and -6 series of fatty acids, especially polyunsaturated ones, are required [3]. The health benefits of fungal fatty acids attract the attention of scientists. Polyunsaturated fatty acids such as linoleic and oleic acid, which are essential for human basal metabolism and diet, are also present in fungi as in many plant species [4]. Linoleic acid, which plays a vital role in prostaglandin biosynthesis, is the precursor of arachidonic acid and 1-octen-3-ol, which gives fungus an aroma [5]. Ingestion of essential fatty acids by nutrients can also cause cardiovascular diseases [6].

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Several medical benefits have been attributed to the consumption of fungi, including chronic and degenerative diseases, obesity, and the treatment of cardiovascular diseases [7,8].

Some studies on the fatty acids of fungi have been reported in the literature [9-12]. The biological activity of the mushrooms growing in Turkey and some scientific studies on the phytochemical profile was made [13-14]. Essential properties of polyunsaturated fatty acids, fungus-related scientific studies have increased with each passing day. However, no data were obtained to determine the total lipid, phospholipid, and triacylglycerol fractions of fatty acid compositions. Also, there was no data about the cytotoxic activity of Russula delica used on PC-3 cell lines.

This study aimed to investigate the cytotoxic activity of Pleurotus ostreatus and Russula delica by MTT test using PC-3 cell line and to determine the total lipid, phospholipid and triacylglycerol fractions of fatty acid compositions of these mushrooms which were grown in nature when appropriate conditions were formed.

Material and Methods

Mushrooms

Pleurotus ostreatus (P. ostreatus) and Russula delica (R. delica) used in the study was obtained from the field studies conducted in April-May 2016. Macroscopic investigations were performed by using field studies and stored samples related to ecological structural features. Diagnosis of the species was made through commonly used keys and studies [15,16].

Lipid extraction and transmethylation of fatty acids of P. ostreatus and R. delica

Fatty acid analyses were performed by Folch et al. [17] 's method was modified according to Kaçar et al. [18]. Fatty acid analyses were performed on 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 inner diameter x 0.25µm film thickness). 5 g of ground mushrooms were extracted into a mixture of chloroform-methanol (2: 1 v / v). The total lipids in the sample were separated by thin-layer chromatography into phospholipids (PL) and triacylglycerol (TAG) fractions. Total lipids; 80: 20: 1 ratio was carried out in a mixture of petroleum ether-diethyl ether-acetic acid. The bands of phospholipids and triacylglycerol were determined using the standards (Sigma-Aldrich Chemicals of fatty acids). The percent 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 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 [19] 'multiple range tests were used to determine the difference between means. As a result of the statistics, $p < 0.05$ was considered statistically significant.

Preparation of extracts of macrofungi

Obtaining Mushroom Extracts

After drying at room temperature, the powdered mushrooms were extracted with 20 g of material to extract 200 ml of hexane overnight and then filtered, followed by filtration again with 200 mL of hexane. The residue was similarly extracted with 200 mL ethyl acetate and filtered. Finally, after the same process was carried out with 200 mL methanol, the hexane-ethyl acetate and methanol extracts were combined, and the solvents were evaporated in the rotary evaporator to dryness to give hexane extract, ethyl acetate extract, and methanol extracts. The resulting hexane, ethyl acetate and methanol extract were stored in the freezer for later use.

Assay of cytotoxic activity of P. ostreatus and R. delica.

Cytotoxic effect of fungus was determined according to the method of Alley et al. [20]. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to evaluate PC-3 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 prepared 10 ml inactivated fetal bovine serum (FBS) (10%), 1 ml penicillin (100 U) / streptomycin (100 g / ml) solution (1%) with Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM-F12). In the study, 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 phosphate-buffered saline (PBS) was added to the wells. 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 percent dilution to the MTT test. Cytotoxicity tests were performed 7 times, 4 replicates for each concentration.

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 fungus. 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 dimethyl sulfoxide (DMSO) was added to the wells. Shaken at 150 rpm for 5 min. The intensity of the resulting color 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 that died in % (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=100-(adjusted mean A_{sample} x100/adjusted mean. A solvent/positive control) % 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.

Results

The total lipid, phospholipid and triacylglycerol fractions of the fatty acid compositions of P. ostreatus are shown in Table 1. And also the total lipid, phospholipid and triacylglycerol fractions of the fatty acid compositions of the R. delica are shown in Table 2. The total saturated and unsaturated fatty acids found in the mushrooms studied were between 22.3-53.8% and 46.2-77.6%, respectively. Linoleic (28.60 - 72.44%), oleic (13.56 -53.24%) and palmitic (9.06 -13.21%) acids were found to be mainly fatty acids in total lipid fractios. The highest amount of oleic acid was detected in the triagylglycerol fraction in R. delica with 63.95%.

The highest amount of linoleic acid was determined in P. ostreatus in phospholipid fraction with 74.26%. The amount of oleic acid in P. ostreatus was lower than the amount of linoleic acid. The highest amount of oleic acid was found in the triacylglycerol fraction. During the study, stearic acid was found to be 3.71 - 4.37% in phospholipid fractions. Normally, the fungi have low amounts of linolenic acids, however, in both species, the concentration of linolenic acid (C18: 2n-6) is quite high, and the percentages vary with both species and fractions.

Table 1. Percent Content of Fatty Acids in Total Lipid, Triacylglycerol and Phospholipid Fraction of Pleurotus osteratus

FATTY ACIDS	PL (MEANS±S.E)*	TG (MEANS ±S.E)*	Total (MEANS ±S.E)*
C8:0§	-	1.32±0.02a	-
C12:0	0.02±0.01a	-	0.03±0.01b
C14:0	0.16±0.03a	0.85±0.04b	0.25±0.02c
C15:0	2.48±0.09a	2.47±0.21a	2.25±0.33a
C16:0	9.36±0.45a	15.17±0.87b	9.06±0.94a
C17:0	0.08±0.01a	0.87±0.05b	0.10±0.01c
C18:0	0.95±0.05a	4.37±0.99b	1.40±0.56c
C20:0	0.22±0.02a	-	0.31±0.04b
∑SFA	13.27±1.22a	25.05±1.02b	13.4±1.09a
C16:1n-7	0.13±0.09a	0.36±0.04b	0.27±0.02c
C18:1n-9	12.23±1.22a	21.92±1.20b	13.56±1.07a
C20:1n-9	-	-	-
∑MUFA	12.36±1.34a	22.28±1.76b	13.83±1.06a
C18:2n-6	74.26±3.90a	52.19±2.08b	72.44±3.44a
C18:3n-3	0.06±0.01a	0.43±0.04b	0.27±0.02c
∑PUFA	74.32±3.90a	52.62±2.33b	72.71±3.45a

* 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. S.E: Standard error, 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.

Table 2. Percent Content of Fatty Acids in Total Lipid, Triacylglycerol and Phospholipid Fraction of Rusula delica

FATTY ACIDS	PL (MEANS±S.E)*	TG (MEANS ±S.E)*	Total (MEANS ±S.E)*
C8:0§	-	0.16±0.02a	
C12:0	0.03±0.01a	0.04±0.02b	0.02±0.01c
C14:0	0.23±0.05a	0.19±0.04b	0.14±0.08c
C15:0	0.61±0.06a	-	0.33±0.03b
C16:0	13.14±1.27a	13.39±1.33a	13.21±1.07a
C17:0	0.03±0.01a	-	-
C18:0	2.99±0.56a	3.71±0.45b	3.63±0.59b
C20:0	0.12±0.01a	0.13±0.02a	0.12±0.01a
∑SFA	17.15±1.48a	17.62±1.22a	17.45±1.01a
C16:1n-7	0.38±0.03a	0.24±0.05b	0.24±0.07b
C18:1n-9	21.68±2.33a	63.95±3.45b	53.24±2.06c
C20:1n-9	-	-	0.38±0.05a
∑MUFA	22.06±1.00a	64.19±2.30b	53.86±3.76c
C18:2n-6	60.12±2.88a	18.12±1.20b	28.60±2.36c
C18:3n-3	0.05±0.01a	0.02±0.01b	0.04±0.02c
C20:5n-3	0.55±0.02a	-	-
∑PUFA	60.72±2.37a	18.14±1.04b	28.64±2.33c

* 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. S.E .: Standard error, 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.

The cytotoxic activities of P. ostreatus and R. delica on PC-3 cell lines are given in Table 3. Ethylacetate extract of P.ostreatus showed significant inhibition against PC-3 cell sequence at a concentration of 530 µg / ml (92.82%). However, the maximum inhibition at the concentration of 670 µg / ml was found to be 28.55% when methanol extract was used. Russula delica showed significant inhibition value against PC-3 cell line at a concentration of 520 µg / ml (99.45%) in ethylacetate extract . The IC50 values of Pleurotus ostreatus and Russula delica were determined as 274.5 and 297.7 µg / ml for the extraction of ethylacetate, respectively.

Table 3. Cytotoxic Activities of Extracts from Mushroom Species

Mushrooms Species	Exposure concentration (µg/mL)		% Inhibition		IC50 (µg/mL)	
	EtOAc	MeOH	EtOAc	MeOH	EtOAc	MeOH
Rusula delica	520	520	99.452	15.989	274.53	-
	260	-	46.790	-		
	130	-	16.372	-		
	65	-	9.485	-		
	32.5	-	4.558	-		
	16.25	-	0.745	-		
Pleurotus osteratus	530	670	92.828	28.556	297.77	-
	265	-	41.119	-		
	132.5	-	22.226	-		
	66.25	-	8.303	-		

EtOAC: Ethyl acetate extracts; MeOH: Methanol extracts ;(-) Unable to determine

Discussion

The lipid levels of the mushrooms are generally low and constitute about 2-8% of the dry fungus. Undoubtedly, mushrooms that are eaten after being fresh and processed are an important source of food for many people, especially for vegetarians [21]. Triacylglycerol acts primarily as a depot for fatty acids that are catabolized in energy metabolism, while TGs are used in the food industry [22]. Phospholipids act as components of cell membranes and structures and have positive effects on human health [23]. Therefore, it is necessary to determine the phospholipid and triacylglycerol fractions of fatty acids. . Üstün [24] states that there are in the range of 56.83-90.43% unsaturated fatty acids and total saturated fatty acids in the range of 9.57 to 43.07% in macrofungi. Oleic acid is a major component of monounsaturated fatty acids and is effective in lowering blood cholesterol levels [9]. Zengin et al [11], in their study, stated that oleic acid is the most common fatty acid in 5 mushroom samples (S. crispa, R. roseouls, S. collinitus, R. delica and H. lacunosa). Also, oleic acid was previously reported as main fatty acid in H. lacunosa (43.82%) [25] S. crispa (49%) [26] and R. delica (41.20%). The presence of high content of linoleic acid in fungi has been mentioned in previous studies [1,11,27,28]. However, R. delica contained less linoleic acid (13.17%). Contrary to literature, the higest level of linoleic acid (60.12%) was obtained from the posfolipid fraction of R. delica in our study.

Some studies on the fatty acids of fungi have been reported in the literature [11-14]. Maftoun et al. [29] reported that, in the large compilation data on the nutritional composition of Pleurotus

mushrooms. Oleic acid (C18: 1) is the main monounsaturated fatty acid, while linoleic acid (C18: 2n - 6c) is the major polyunsaturated fatty acid. In this study, among the saturated fatty acids (20.2%) the main ingredients were palmitic acid (C16: 11.2%), pentadecanoic acid (C15: 0; 2.55%) and stearic acid (C18: 0; 2.53%). Among the polyunsaturated fatty acids (69.1%), linoleic acid was the most common and abundant (68.1%). In our literature review, we did not find any data on the fatty acid fractions of mushrooms. The study is compatible with the data obtained in the literature based on the percentage of lipid. However, percentage values vary according to both species and fractions.

The anticancer properties of P. ostreatus were investigated in various studies [30-34]. A large amount of compound such as polysaccharides, polysaccharide-peptides, polysaccharide protein complex and lectins were isolated from fungi. These compounds were found to have antioxidant, immunomodulatory, anticancer, antimicrobial, antidiabeticand antihyperperestrolemic properties [30,35] Gu and Sivam [36] reported that water-soluble extract of P. ostreatus was effective against PC-3 cell line, but the literature on the subject is quite limited. And also, there is no study on cytotoxic effect of R. delica on PC-3 cell line.

Conclusions

In the study; the potential of the cytotoxic activity was found to depend on the concentration and solvent type of the extract used. It is clear that these species have potential for cytotoxic activity. Therefore, studies on this subject should be increased.

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Conflict of interest
The authors declare that there are no conflicts of interest.

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