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Determination of Chemical Constituents and Bioactive Properties of Alcohol Extracts of *Pleurotus sajor-caju* and *Pleurotus ostreatus*

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ABSTRACT: In this study, alcohol extracts of *Pleurotus sajor-caju* and *Pleurotus ostreatus* which are edible mushroom species were obtained and their extract contents were determined by GC-MS. Antioxidant capacity of these alcohol extracts were determined with 2-2-diphenyl, 1-picrylhydrazyl (DPPH). Antimicrobial activities of alcohol extracts was determined on *Klebsiella oxycota*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Corynobacterium diphtheria*, *Enterococcus faecalis*, *Escherichia coli*, *Serratia marcescens*, *Bacillus cereus* and *Bacillus thuringiensis* bacteria, and *Rhodotorula glutinis* and *Candida albicans* yeasts. Moreover, this study indicated the anti-quorum sensing activity of the tested mushroom extracts against to *Chromobacterium violaceum* CV026. *P. sajor-caju* and *P. ostreatus* alcohol extract compositions were determined with GC-MS. According to GC-MS results, 20 different compounds were found in *P. sajor-caju* and *P. ostreatus* alcohol extract. Hexadecanoic acid, linoleic acid, octadecanoic acid, 2,3-dihydroxypropyl ester and palmitic acid were found in both mushroom extracts. Because they contain a high content of fatty acids, alcohols, aldehydes, ketones and terpenoids, these extracts can be used both as antimicrobial, anticancerogenic, antioxidant and antiaging agents or can be consumed as food supplements.

Keywords: Mushroom extract, GC-MS, volatile compounds, antimicrobial, antioxidant

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INTRODUCTION

Fungi are rich in proteins, vitamins, minerals, essential oils and bioactive substances (Sing et al. 2014, Rezaeinan et al. 2016, Ozdal et al. 2018). Mushrooms have been used as food sources for centuries in many societies around the world. They have been used in drug development and alternative medicine in recent years (Smith et al. 2015). Medical and pharmacological researches shown that fungi have antimicrobial, antioxidant, anticancer, antiviral and anticholesterol activites (Patel et al. 2012, Alves et al. 2012, Rathee et al. 2012, Avcı et al. 2014, Ozdal 2018, Finimundy et al. 2018).

Natural antioxidants obtained from fungi reduce oxidative stress (Kamra et al.2012). Water and alcohol extracts are used in antimicrobial and antioxidant studies due to bioactive substances (polysaccharides, phenolic compounds, flavonoids and lipids) which contain fungi such as *Pleurotus*, *Lepista* and *Ganoderma* (Vamanu 2012, Ozdal 2018, Ozdal et al.2019). Also inhibit free radicals due to their high antioxidant content (Alam et al. 2011a,b).

Mushrooms belong to the genus *Pleurotus* are important sources of fiber and have high nutritional value. Thus, mycelial biomass is a cheap alternative protein source and they can be produced using waste substance. Due to the potential health benefits for the human body, mushroom products are commercially available. Furthermore, *Pleurotu*s genus mushrooms has been researched recent years because of its biologically active compounds, like polysaccharides, proteoglycans, phenolicacids, terpenes, proteins and sterols (Duru and Cayan, 2015). *Pleurotus sp.* can be easily produced in both liquid and solid media, and is a medically important fungus. Also, submerged fermentation facilitates the extraction and purification of compounds, reducing growth time and contamination, resulting in higher biomass production (Mukhopadhyay and Guha, 2015, Geyikoglu et al. 2017).

The aim of this study is to determine the antioxidant content of alcohol extracts of *P. ostreatus* (Jacq. P. Kumm.) and *P. sajor-caju* and to find their antibacterial effect on some human pathogenic bacteria. Also, anti-QS activities were decided. For these reasons, chemical composition of alcohol extracts of *P. ostreatus* and *P. sajor-caju* were investigated.

MATERIALS AND METHODS

Culture and Fermentation Condition

P. ostreatus and *P. sajor-caju* were grown at 25 ℃ for 7 days on Potato Dextrose Agar (PDA). Submerged fermentation was achieved in 250 mL Erlenmeyer flasks, containing 100 mL of liquid medium (Ozdal et al. 2019). Each flask was inoculated with one 10 mm agar plugs. Each flask was cultures at 28 ℃ and 150 rpm for 96 hours. After growth, the fungal biomasses were obtained from the aqueous medium by filtration. At the end of the incubation, the biomasses were washed 3 times with sterilized distilled water, and then dried in the oven at 60 ℃ until constant weight.

Preparation of Alcohol Extracts

Ten g of biomasses were put in 100 mL of ethanol (99 % purity) and it shaked at 150 rpm for 24 hours at 30 ℃. After shaking dried at 40 ℃. By filtration through a paper of Whatman No 1 filter (Bo et al. 2010). Dried extracts were dissolved in ethanol for in antioxidant and antibacterial studies used.

Identification of Components

Chemical constituents of mushroom extracts were determined by GC-MS (Agilent 7820A, 7673 series autosampler).

Determination of Antioxidant Activity

Antioxidant capacity was determined using the DPPH method (Reis et al. 2012; Odal et al. 2019).

Determination of Antibacterial Properties

K. oxycota, P. aeruginosa, S. aureus, C. diphteria Enterococcus faecalis, E. coli, S. marcescens, B. cereus and *B. thuringiensis* bacteria were used in the study. *R. glutinis* and *C. albicans* were used as yeast. These microorganisms were supplied by Prof. Dr. Recep Kotan, Ataturk University. These microorganisms were seeded in Nutrient agar (NA) and cultivated to incubate for 24 hours. 0.1 g extracts were dissolved in 2 mL ethanol and the zone diameters were measured by Kirby-Bauer disk diffusion method. The discs were dried for 24 hours before use. At the end of the time, the diameter of the inhibition zone formed around each disc was measured in centimeters. Ethanol impregnated disk was used as a control.

Anti-Quorum Sensing Activity

Inhibition of QS by *Pleurotus* extracts was followed by employing the indicator strain *Chromobacterium violaceum* CV026. Cultures of *C. violaceum* CV026 were grown overnight LB broth and spread onto LB plates containing a C6-HSL molecule. Sterile paper disks (6 mm diameter) impregnated with 0.5 mg, 1 mg and 1.5 mg of each extract (10, 20 and 30 µL of stock solutions) were placed onto agar medium, and then incubated at 30°C for 24 hours. Lack of purple pigmentation from *C. violaceum* in the vicinity of the test extract indicated the inhibitory effect of the *Pleurotus* extracts (Table 5). QS inhibition was detected by a colorless, opaque, but, not inhibition of cell growth, halo around the disks. As a control, ethanol impregnated disk was used.

RESULTS AND DISCUSSION

Appearance and amount of alcohol extracts

As seen in Table 1, *L. sajor caju* provided a higher extract yield (44 mg/g) and *P. ostreatus* provided a lower extract yield (39 mg/g). The appearance of the two extracts was different (*P. sajor-caju*, dark brown powder and *P. ostreatus*, light orange/brown powder).

Table 1. Appearance and amount of alcohol extracts obtained from mushroom

Mushroom species	Appearance	Yield of extract
P. ostreatus	Light orange/brown	39 mg/g
P. sajor caju	Dark brown	44 mg/g

GC-MS Profile of Volatile Compounds

Table 2. *P. sajor-caju* alcohol extract component

Retention Time	Compounds	Formula	%Component
7.69	(2,3-epoxyheptane)	$C_7H_{14}O$	0.75
7.75	(3,4-Dihydroxy-3-methylbutyl) acetate	$C_7H_{14}O_4$	0.33
7,89	2,3,4,5,6,7,8-heptahydroxyoctanal	$C_8H_{16}O_8$	0.10
8.55	2-(2-butoxyethhoxy)-Ethanol	$C_8H_{18}O_3$	0.14
9.027	Chlorozotocin	$C_9H_{16}ClN_3O_7$	0.04
9.07	1-(methoxymethoxy)-3-methyl-3- hydroxybutane	$C_7H_{16}O_3$	0.05
10.67	4-Cyclopropylcarbonyloxytridecane	$C_{17}H_{32}O_2$	0.13
11.556	Cis-9,10-epoxyoctadecanoic acid	$C_{18}H_{34}O_3$	0.35
19.907	Hexadecanoic acid	$C_{16}H_{32}O_2$	3.72
20.47	3-deoxy-estradiol	$C_{18}H_{24}O$	0.08
21.057	Oleic acid	$C_{18}H_{34}O_2$	0.08
22.089	Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	0.57
23.22	Linoleic acid	$C_{18}H_{32}O_2$	9.2
26.803	Palmitic acid etylester	$C_{18}H_{36}O_2$	0.09
28.116	6,9,12,15-docosatetraenoic acid methyl ester	$C_{23}H_{38}O_2$	0.08

Table 2. *P. sajor-caju* alcohol extract component (continued)

Retention Time	Compounds	Formula	%Component
30.262	Linoleic acid ethylester	$C_{20}H_{36}O_2$	0.42
30.968	Palmitic acid	$C_{16}H_{32}O_2$	1.63
33.361	Octadecanoic-acid-2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	1.98
34.144	9,12,15-octadecatrienoicacid,2,3-dihydroxypropyl ester	$C_{21}H_{36}O_4$	0.17
38.586	Ergosterol	$C_{28}H_{44}O$	2.12

P. sajor-caju alcohol extract compounds are given in Table 2. As shown in the Table 2, as a result of GS-MS, 20 different compounds were determined in the ethanol extract. Linoleic acid (9.2%), palmitic acid (1.63%), ergosterol (2.12%), octadecanoic acid, 2,3-dihydroxypropyl ester (1.98%) and hexadecanoic acid (3,72%) were found at high rates. The effect of contact and equilibrium times and dye concentration factor.

Table 3. *P. ostreatus* alcohol extract component

Retention Time	Compounds	Formula	%Component
7.047	Linalool	$C_{10}H_{18}O$	0.296
7.874	Dodocene	$C_{12}H_{24}$	0.448
8.118	2-(2-butoxyethhoxy)-Ethanol	$C_8H_{18}O_3$	0.264
8.763	1-((1-butoxypropan-2-yl)oxy)propan-2-ol	$C_{10}H_{22}O_3$	0.128
9.388	Phenylacetaldehyde	$C8H_8O$	0.824
10.515	Isoeugenol	$C_{10}H_{12}O_2$	0.264
10.689	3-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	0.848
11.297	Caryophyllene	$C_{15}H_{24}$	0.336
11.605	Acetic acid, cinnamyl ester	$C_{11}H_{12}O_2$	0.168
12.58	2,4-di-tert-butylphenol	$C14H_{22}O$	0.416
16.47	3-Chloropropionic acid, 3-pentadecyl ester	$C_{18}H_{35}ClO_2$	0.6
17.95	1,2-benzenedicarboxylic acid bis(2-methylpropyl)ester	$C_{16}H_{22}O_4$	0.872
19.61	2,3-dimethyl-5-(trifluoromethyl)benzene-1,4-diol	$C_9H_9F_3O_2$	0.272
19.81	Hexadecanoic acid	$C_{16}H_{32}O_2$	1.32
23.017	Linoleic acid	$C_{18}H_{32}O_2$	7.26
28.11	Benzyl butyl Phthalate	$C_{19}H_{20}O_4$	0.288
30.957	Palmitic acid	$C_{16}H_{32}O2$	2.408
31.795	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	0.04
33.367	Octadecanoicacid,2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	2.42
34.145	1-Monolinolenoyl-rac-glycerol	$C_{21}H_{36}O_4$	1.016

Antioxidant assay

To determine antioxidant activity, DPPH free radical scavenging technique was applied (Figure 1). In the presence of 312-1562 µg/mL extract, *P. sajor-caju* (97.01%) and *P. ostreatus* (98%) showed the radical scavenging activity. As the extract concentration increased, the scavenging activity was also increased. This means that there is a dose dependent DPPH scavenging efficiency of the mycelium ethanol extracts.

Antimicrobial Activity

The alcohol extracts of the mycelia cells of two different mushroom species were performed against five Gram-positive (*S. aureus, C. diphtheria, E. faecalis, B. cereus* and *B. thuringiensis*) and four Gram-negative (*P. aeruginosa*, *K. oxycota, E. coli* and *S. marcescens*) bacteria and two yeasts (*R. glutinis* and *C. albicans*) by the disc diffusion technique. When the extracts were applied to 20 µl, antibacterial and antifungal activities were not determined, but zone diameters between 0.5 cm and 2 cm were measured when 40 and 60 µl of extract were applied. As shown in Table 4, the concentration of the extracts changes the antibacterial effect. Among all bacteria, *P. aeruginosa* was the most non-sensitive to extracts. The extracts of *P. ostreatus* and *P. sajor-caju* were highly antibacterial against *Corynobacterium diphtheria*. It also found that *P. sajor-caju* extract more effective than *P. ostreatus.*

The extracts of *P. ostreatus* and *P. sajor-caju* were antifungal against *Rhodotorula glutinis* and *C. albicans.* The extract of *P. sajor-caju* had very highly antimicrobial activity compared to *P. ostreatus.*

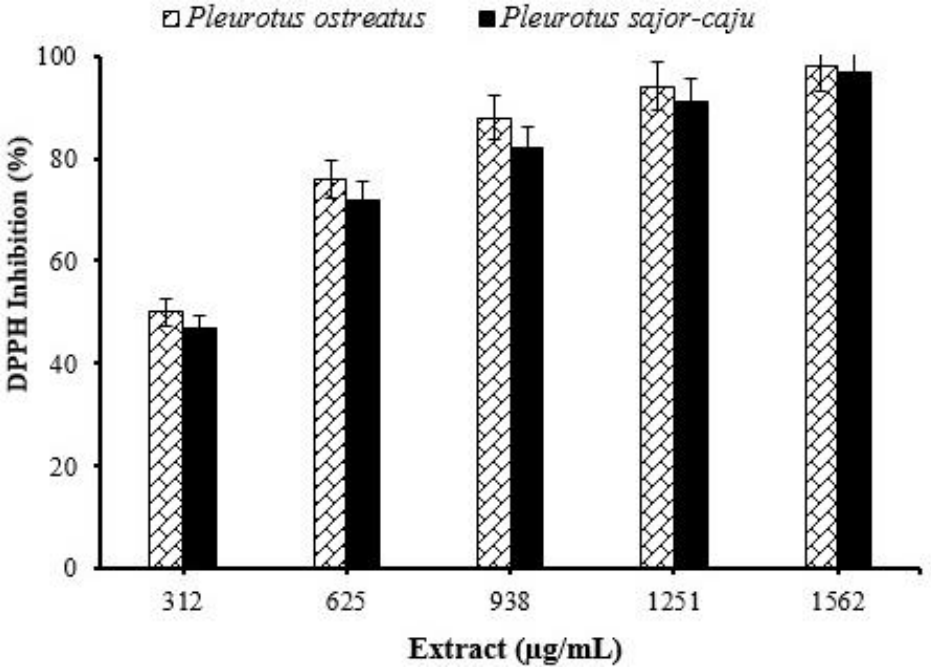

Figure 1. DPPH activity results

Table 4. Inhibition zones (cm) of alcohol extract of *Pleurotus* species against bacteria

Microorgaanisms	*P. sajor-caju*			*P. ostreatus*		
	20µl	40 µl	60 µl	20µl	40 µl	60 µl
P. aeruginosa	-	0.7	1.0	-	-	0.9
S. aureus	-	0.9	1.3	-	1.0	1.2
C. diphtheria	-	1.5	2.0	-	0.9	1.3
K. oxycota	-	0.8	1.5	-	0.8	1.0
Enterecoccus faecalis	0.67	1.0	1.1	-	0.7	1.0
E. coli	-	0.5	1.0	-	0.5	1.0
S. marcescens	0.7	0.8	1.4	-	0.9	1.3
B. cereus	-	0.8	1.5	-	-	0.7
B. thuringiensis	-	1.1	1.7	-	0.85	1.3
R. glutinis	-	1.0	1.3	-	-	1.0
C. albicans	-	1.0	1.5	-	-	0.8

Quorum sensing inhibition tests

Extracts exhibited Anti-QS effect at concentrations 0.05–0.15 mg/disc in range of 0.7–1.3 cm as transparent zones around discs. The aqueous extract of *Pleurotus* extracts showed Anti-QS effect in all tested concentrations (Table 5). Extracts of *Inonotus obliquus* (Glamoclija et al. 2015), *Agaricus bisporus, Clitocybe nuda, Lactarius volemus, Macrolepiota procera, Xerocomellus chrysenteron* (Strapáč et al. 2019) and Agaricus blazei (Soković et al. 2014) were reported that extracts showed positive anti-QS activity**.**

Table 5. Anti- QS zones in cm induced by Pleurotus extracts in the disc-diffusion method

	P. sajor-caju			*P. ostreatus*		
Concentration	**10µl**	**20 µl**	**30 µl**	**10µl**	**20 µl**	**30 µl**
Anti-QS zones (cm)	0.7	0.9	1.2	0.8	0.9	1.3

With the adoption of the healthy lifestyle, both natural and antioxidant rich substances became the focus of interest and started to be used (Li et al. 2015). Fungi with high natural and nutritious properties are rich in antioxidant and antimicrobial substances. Both the fruiting bodies and mycelia are high important structures due to biological and pharmacological activities (Vamanu 2012; Ozdal et al. 2019).

In our study, linoleic acid, dodecene, dodeconic acid, hexadecene, hexadecanoic were found in both of the *Pleurotus* and *Lentiudes* alcohol extracts. These compounds have been found both in body structures and in extracts of fungi. These substances have been shown that high antioxidant capacity and antibacterial activity (Avcı et al. 2014, Razla et al. 2016).

Antioxidant substances such as ß-carotene, ascorbic acid, vitamin B and ergosterol are found in most fungi. They are used in cancer treatments due to antitumor activities (Aisya et al.2010, Muna et al. 2015, Razeian et al. 2016). In addition, it has antioxidant and antimicrobial effect in the chitin which is one of the polysaccharides found in nature (Benhabiles et al. 2012).

GC- MS results showed that compounds identified in ethanol extracts of *Pleurotus sp. and Lentiudes* which represented the bioactive fractions. Three compounds were identified as the major bioactive compounds (Table 2 and 3). The fatty acids determined in high percentages were linoleic, palmitic and oleic acid. 1-octene-3-ol is the precursor of aromatic compounds and known as the alcohol of fungi (Barros et al. 2008). Linoleic acid was found in fungal samples analyzed (Fogarai et al. 2018) and our study showed similar results. For this reason, it can be considered as a source of linoleic acid, the precursor of other long chain fatty acids in the human body.

The volatile compounds from mushroom extracts could be divided in several groups: alcohols, aldehydes, ketones, terpenoids, acids and others. According to Zhang et al. (2008), aldehydes and ketones were also the dominant compounds in the mushrooms varieties. The main aldehydes present in all samples were 2-methyl-pentanal, hexanal and benzaldehyde yet their concentration varied depending on the mushroom variety. As shown in Table 2 and 3, our study results are close to previous studies and may be the cause of bioactive properties of extracts (Table 4 and Figure 1).

Volatile compounds which form the unique odor of fungi are 1-Dodecanol, 1-Hexanol, 2-Methyl-1-butanol, Benzaldehyde, 2,3-Dimethylcyclopentanone and dodecanoic acid (Fogarasi et al. 2018). In our study, it is similar to the literature studies but contains different contents.

Fungi themselves and extracts have antimicrobial effect. It has been reported to have higher activity, especially against Gram-positive bacteria (Reis, 2017; Ozdal et al. 2019). In our study, *Pleurotus* and *Lentiudes* extracts were found their antibacterial effect on the bacterial species (*P. aeuriginosa, S. aureus, E. coli, B. cereus,* B. *thuringiensis C. diphteria, K. oxycota, S. marcescens* and *E. faecalis*). It also found that *Pleurotus* ethanol extracts have antifungal activity against *C. albicans* and *R. glutinis*. Many researchers showed that extracts from the mycelial biomass of Pleurotus species exhibited antifungal activity against *C. albicans, C. alabrata, Aspergillus flavus* (Akyuz et al. 2010; Cilerdzic et al. 2015).

Quorum sensing can control different activities, such as pathogenicity factor, bioluminescence, sporulation, and biofilm generation (Mulya and Waturangi, 2021). Many researchers have reported an anti-QS activity for different mushroom extracts. Extracts of *Inonotus obliquus* (Glamoclija et al. 2015), *Agaricus bisporus, Clitocybe nuda, Lactarius volemus, Macrolepiota procera, Xerocomellus chrysenteron* (Strapáč et al. 2019) and *Agaricus blazei* (Soković et al. 2014) were reported that extracts showed positive anti-QS activity. These results supported the use of the mushroom extracts as alternative agents to treat infections caused by microorganısms and to prevent quorum sensing signaling.

CONCLUSION

In the present study the analysis ethanol extracts from *P. sajor-caju* and *P. ostreatus* was determined for volatile compounds. This study showed the presence of antimicrobial, anti-quorum sensing and antioxidant activities in two species of Pleurotus. Therefore, *P. ostreatus* and *P. sajor-caju* ethanol extracts can be used as antitaging and anticancer as they are rich in nutrients and antioxidant. By using the Anti-Qs properties of the extracts, their potential for use as antibacterial and antifungicide has been demonstrated. The studied Pleurotus extracts can be used in food supplement and medicinal products for health promotion.

Conflict of Interest

The authors declare no conflict of interest

Author's Contributions

The authors declare that they have contributed equally to the article.

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