



Comparison of Quorum Sensing Inhibition and Antimicrobial Properties of Some Commercial and Wild Mushrooms Extracted with Supercritical CO₂

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Abstract

Recently, bioactive properties of mushrooms have been intensively investigated, and their wealth in bioactive compounds particularly of medicinal properties have increased their consumption. In this study, quorum sensing inhibition and anti-microbial properties of some commercial and wild mushroom species were investigated. *Agaricus bisporus* species were purchased from three different commercial companies. *Laccaria bicolor*, *Bovista plumbea*, *Lactarius deliciosus* and *Boletus edulis* were collected from Trabzon, Turkey. Compounds extractions were performed using supercritical fluid extraction (CO₂) method. Quorum sensing inhibition activity was tested using *Chromobacterium violaceum* as bacterium-model. Antimicrobial potential of extracts was tested using agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Listeria monocytogenes*, *Candida parapsilosis* and *Candida albicans*. All wild mushroom extracts except for *B. plumbea* inhibited the violacein production of *C. violaceum*. *L. bicolor*, *A. bisporus* (1), *B. plumbea*, *A. bisporus* (2) extracts inhibited the bacterial growth of *S. aureus*. In addition, *L. bicolor* extract inhibited *K. pneumoniae* and *L. monocytogenes* whereas *A. bisporus* (2) extract inhibited *P. aeruginosa*. Among all mushrooms, *L. bicolor* extract showed remarkable results.

Keywords: Antimicrobial activity, cultivated mushroom, quorum sensing inhibition, wild mushroom.

1. Introduction

Mushrooms are considered as a powerful source of nutrients for human and many mushroom species are defined as functional foods. They are rich in proteins and amino acids [1], they are low-calorie because of their low fat and high-water compositions [2]. They also include β -glucan, which promotes the immune system [3] and vitamin D that is essential for bone health [4]. Mushroom contain also biological and physiologically active substances such as phenolic acids [5]. Moreover, mushrooms are cost-effective.

Collection of wild mushroom species becomes a pleasant social activity for some professional or amateur groups in Turkey. Today, the most commonly produced and consumed mushroom specie in Turkey is *Agaricus bisporus* (J.E. Lange) Imbach. During the last 10 years, mushroom production in Turkey has increased by 54% from 26.256 tons to 40.874 tons [6]. Scientific studies proved that mushroom contained bioactive compounds

with antioxidant, antimicrobial and antidiabetic properties [7, 8]. Further researches should be continued particularly to test and compare species growing in different geographies and various climatic conditions.

Recently, anti-quorum sensing properties are included to the medical properties investigated for mushrooms/plants. Quorum sensing (QS) is a cell-cell communication mechanism used by bacteria to control their community growth between many other bacterial properties. The system permits for each bacterium to perceive the bacterial population growth and to control its own gene expression and regulate its own growth in response to the perceived information [9]. Therefore, the interruption of the QS mechanism may alter the bacterial communication hence may inhibit their growth, which is an important step for the bacterial infection and adherence to surfaces as much as for biofilm formations [10].

In this study, quorum sensing inhibition capabilities and anti-microbial properties of some commercial and wild mushrooms extracts are investigated and compared with each other.

2. Materials and Methods

2.1. Mushrooms

Wild mushrooms were collected from Trabzon province located in the north eastern part of Turkey and identified by their morphological and their ecological characteristics. *Agaricus bisporus* mushrooms were purchased from three different commercial companies established in Trabzon and coded from 1 to 3. Tested mushrooms and their properties were given in Table 1.

Table 1. Tested mushrooms.

Mushroom (Code)	Source	Edibility
<i>Laccaria bicolor</i> (Maire) P.D. Orton	Wild	Edible
<i>Bovista plumbea</i> Pers.	Wild	Edible, not highly valued
<i>Lactarius deliciosus</i> (L.) Gray	Wild	Edible
<i>Boletus edulis</i> Bull.	Wild	Edible
<i>Agaricus bisporus</i> J.E. Lange) Imbach (1)	Cultivated	Edible
<i>A. bisporus</i> (2)	Cultivated	Edible
<i>A. bisporus</i> (3)	Cultivated	Edible

Mushroom samples were dried on food-dryer at 40 °C (Profilo, PFD1350W, Turkey), then were ground in a basic micro-fine grinder and passed through 1-millimeter sieve (IKA, WERKE MF10, Germany).

2.2. Extracts Preparation

Supercritical CO₂ fluid extraction was applied for 10 g of mushroom powder at 250 bar, 50 °C for 3 hours (Spe-ed SFE model 7070). CO₂ flow rate was 10 g/min and ethanol was used as co-solvent with a flow rate of 0.5 mL/min. The extracts were dissolved as 10 mg/mL in dimethyl sulfoxide (DMSO) to form working solution.

2.3. Anti-Quorum Sensing Activity

Anti-quorum sensing activity was tested against *Chromobacterium violaceum* (*C. violaceum*) (Table 2). This bacterium produces a purple pigment called violacein and this production is controlled by the QS system [12]. A compound with anti-quorum sensing property will inhibit the pigment production by *C. violaceum* without altering the bacterial cells growth. Therefore, the investigation of anti-QS activity was tested for the concentrations below the MIC (Minimal Inhibitory Concentration) for each extract, knowing that MIC is the least concentration able to inhibit the bacterial growth. MIC was determined in accordance to the guidelines of the Clinical & Laboratory Standards Institute (CLSI) [11] using the microdilution method on 96 wells plate, and then the concentration next below

the MIC (SubMIC) and its next lower concentration were tested for pigment inhibition. Extract concentrations started by 5 mg/mL and bacterial final concentration was 5x10⁴ CFU/mL. After an incubation of 24 h, the 96-well plate was dried at 50 °C for 1 hour, the pigments were dissolved in 200 µL of DMSO and left to dissolve for 2 hours on shaker (225 rpm). The pigment solutions were taken to a new 96 well plate and their absorbance were read at OD₅₈₅ nm. The same test for each extract was repeated three times, twice for pigment testing and the third was used for plate count agar test to verify the bacterial growth condition. Bacterial count was realized by taking 100 µL and spreading it on Mueller Hinton agar and incubating at 37 °C for 24h.

Table 2. Tested microorganisms, and their American Type Culture Collection (ATCC) number.

Microorganism	ATCC number	Used test
<i>C. violaceum</i>	12472	Anti-quorum sensing
<i>S. aureus</i>	25923	Anti-microbial activity
<i>E. coli</i>	25922	
<i>E. faecalis</i>	29212	
<i>P. aeruginosa</i>	27853	
<i>S. Typhimurium</i>	14028	
<i>K. pneumoniae</i>	13883	
<i>P. mirabilis</i>	7002	
<i>L. monocytogenes</i>	43251	
<i>C. parapsilosis</i>	22019	
<i>C. albicans</i>	10231	

2.4. Antimicrobial Activity

Antimicrobial activity was tested using agar well diffusion method in accordance to the guidelines of CLSI on Mueller Hinton agar [11]. The tested microorganisms were given in Table 2.

Microorganisms were obtained from Karadeniz Technical University, Department of Medical Microbiology, Faculty of Health Sciences in Trabzon, Turkey. The tested microorganisms and extracts were applied as 50 µL of 10 mg/mL solutions. Luria Bertani (LB) fluid and agar medium (LABM; United Kingdom) were used for bacterial cultures. DMSO was used for negative control and ampicillin, gentamicin, tetracycline, cefotaxime and amphotericin B were used as positive controls.

Extracts with positive antimicrobial activity were tested for their minimal inhibitory concentration in accordance to the guidelines of the Clinical & Laboratory Standards Institute (CLSI) on 96 wells plate. The extract concentration started by 5 mg/mL and bacterial final concentration was 5x10⁴ CFU/mL. The last 2 well were used for growth control (bacteria without extract) and for sterility control (extract without bacteria).

3. Results and Discussion

A positive anti-QS activity is defined as the least concentration able to eliminate the pigment production without altering the bacterial growth. The two concentrations next below the MIC value were tested for each extract. Optical Density (OD) at 585 nm was determined to evaluate the pigment productions, measurements were repeated twice and their average results were added to the charts. Vanilla was used as a positive control (Figure 4). Anti-QS effect of vanilla was previously optimized and shown to be positive at around 625 µg/mL. DMSO was also tested as negative control. Among the mushroom extracts, three wild mushroom extracts have shown anti-QS activity and their activity charts are given in Figure 1-3, respectively.

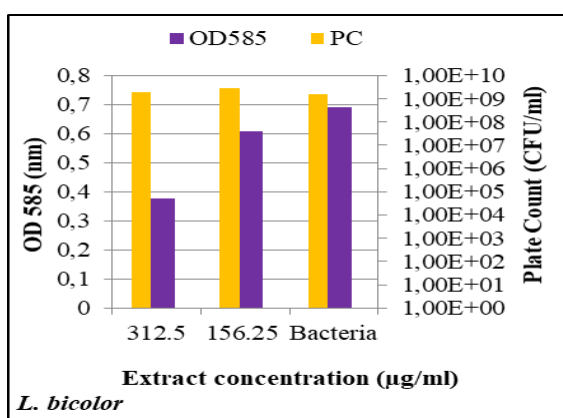


Figure 1. *L. bicolor* anti-QS activity chart.

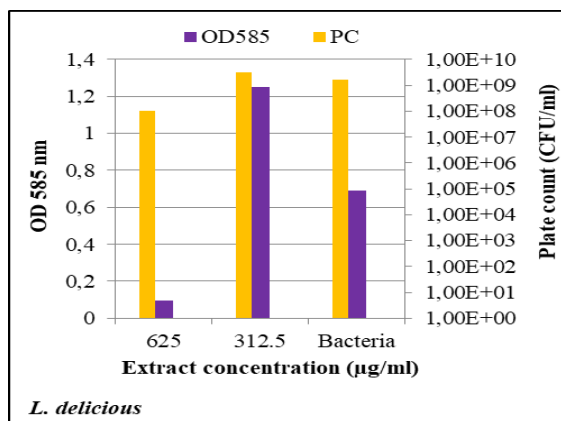


Figure 2. *L. deliciosus* anti-QS activity chart.

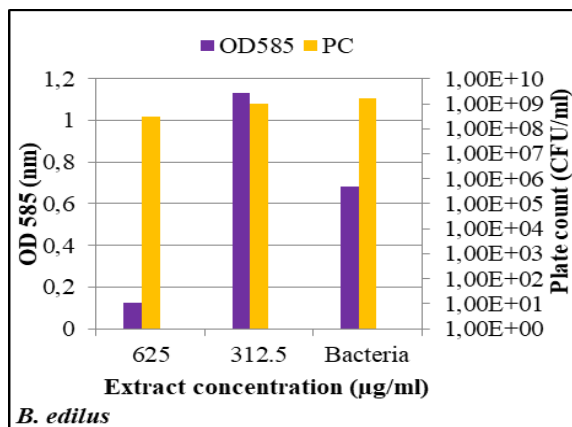


Figure 3. *B. edulis* anti-QS activity chart.

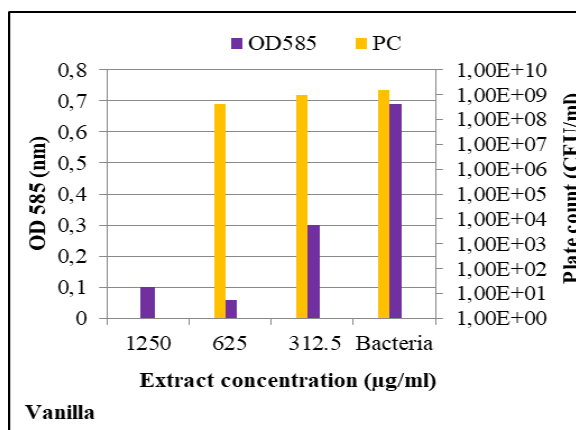


Figure 4. Vanilla anti-QS activity chart.

In this study, three out of four wild mushroom extracts showed anti-QS activity against *C. violaceum* pigment production, they were *L. bicolor*, *L. deliciosus* and *B. edulis*. As shown Figure 1-3, These mushrooms notably decreased the pigment production in comparison to the bacterial growth, which remains invariable or slightly affected. Based on these charts (Figure 1-3), the activities of the mushrooms can be compared with each other as follows; *L. deliciosus* > *B. edulis* > *L. bicolor*. Anti-QS activity was not observed in any of the mushroom samples cultivated in the laboratory. Whereas in other study previously conducted, laboratory-grown mushroom species have shown positive anti-QS activity [13]. However, in the mentioned study, all the conditions were different starting with the grown mushroom species (*Pleurotus ostreatus*), to the cultivation conditions (the mushrooms were cultivated using different substrates), till the extraction method where organic extraction using methanol was applied [13].

Therefore, it can be concluded that mushroom specie, the substrate used as growth nutrient, the type of extraction and the concentration of studied extracts are highly determining factors concerning anti-QS activity.

Looking to the results of other studies, *Auricularia auricular* mushroom pigments showed anti-QS activity on *C. violaceum* [14]. Similarly, *Tremella fuciformis* mushroom's 75% (v/v) aqueous methanol extract inhibited violacein production of *C. violaceum* [15].

Moreover, anti-quorum sensing activity of *Inonotus obliquus* mushroom's aqueous and ethanolic extracts was tested on *Pseudomonas aeruginosa* [16]. It was reported that both extracts showed positive anti-QS activity. In addition, another study conducted by Soković et al. [17] investigated anti-QS activity of hot water extracts of *Agaricus blazei* on *P. aeruginosa* and positive activity was observed [17]. It can be concluded that wild mushrooms constitute a promising source of compounds with anti-QS activity and that more investigations are in need to reveal the properties of these compounds and the conditions needed for their isolation.

In the last decades, significant success was achieved in the treatment and the prevention of microbial infections and this is due to the development of antimicrobial drugs. However, the development of resistance against antimicrobial drugs by some microorganisms has led to the declination of these achievements [18] and day after day new antimicrobial resistance profiles are generated and superbugs are quickly spreading. Therefore, the research for new antimicrobial compounds has become compulsory. Mushrooms, with their wealth in chemical and biological constituents and with their wide presence in the natural environments between all the microbial communities either in competition or in symbiotic

interactions make them one of the promising natural resources for antimicrobial compounds. Antimicrobial potential of extracts was tested by agar well diffusion method in this study. In the agar diffusion method, a suitable medium containing the test organism is used with a pit system in which the sample to be tested is present. At the end of the incubation period, if the tested sample is effective, inhibition zones are formed around the pits, where no microorganism reproduction (Figure 5).



Figure 5. Agar well diffusion assay.

Antimicrobial assay results (mm) of all tested mushroom extracts and positive controls (antibiotics) are given in Table 3. Taken that the well's diameter (7 mm) was subtracted after measuring the inhibition zone diameter.

Table 3. Agar Well Diffusion Results (mm).

Sample	Inhibiton zone (mm)									
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>S. Typhimurium</i>	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>L. monocytogenes</i>
<i>L. bicolor</i>	2	0	0	0	0	0	0	0	2	1
<i>A. bisporus</i> (1)	0	0	0	0	0	0	0	0	0	0
<i>L. deliciosus</i>	0	0	0	0	0	0	0	0	0	0
<i>A. bisporus</i> (2)	2	0	0	0	0	0	0	0	0	0
<i>B. plumbea</i>	2	0	0	0	0	0	0	0	0	0
<i>B. edulis</i>	0	0	0	0	0	0	0	0	0	0
<i>A. bisporus</i> (3)	2	0	2	0	0	0	0	0	0	0
Ampicillin	> 30	16 - 17	...*	> 30	27
Gentamicin	21-22	21	...
Amphotericin B	30	0**
Tetracycline	25
Cefotaxime	37

* Not tested, **May have acquired bacterial resistance to the used control. The tests were not repeated, as no extract had a positive effect.

As shown in Table 3, *L. bicolor*, *A. bisporus* (2), *B. plumbea* and *A. bisporus* (3) supercritical CO₂ extracts had positive antimicrobial activity. Noticeably, *L. bicolor* showed antimicrobial effect against *K. pneumoniae* and *L. monocytogenes* microorganisms. Many studies have reported the antimicrobial activity of fungi. For example, ethanol extracts and four fractions of *Inonotus sanghuang* inhibited *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* [19]. *A.*

lanipes showed antimicrobial activity against *Micrococcus luteus*, *Proteus vulgaris*, *Yersinia enterocolitica*, *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans* microorganisms [20]. Minimum inhibition concentrations (MIC) of the antimicrobial active extracts are given in Table 4.

Table 4. Minimum Inhibition Concentration (µg/mL).

Sample	Minimum Inhibition Concentration (µg/mL)						
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>L. monocytogenes</i>
<i>L. bicolor</i>	156.25	1250	625
<i>A. bisporus</i> (2)	39.1
<i>B. plumbea</i>	78.125
<i>A. bisporus</i> (3)	156.25	...	1250
Ampicillin	78.125	2500
Gentamicin	1250	156.25	...
Amphotericin B	78.125
Cefotaxime	9.76
Tetracycline	78.125

MIC means the lowest concentration that inhibits bacterial growth. Therefore, a low MIC affirm a better antimicrobial activity of the tested substance. MIC value is also dependent of the purity of the tested substance. In this study, the lowest MIC value was obtained from *A. bisporus* (2) extract with 39.1 µg/mL. The MIC value of *L. bicolor* is higher than the value of *A. bisporus* (2) MIC value, although it exhibited antimicrobial activity against 4 microorganisms. In a previous study, MIC values of aqueous extracts obtained from 21 wild basidiomycete mushrooms and cultivated mushroom (*Pleurotus ostreatus*) were reported between 10 and 1524 µg/mL against microorganisms included *Listeria innocua*, *B. cereus*, *Campylobacter jejuni*, *E. coli*, *C. albicans* and *Aspergillus ochraceus* [21].

4. Conclusion

In this study, QS inhibition and anti-microbial properties of some commercial and wild mushrooms were investigated. Except for *B. plumbea*, all wild mushroom extracts significantly decreased the violacein production of *C. violaceum*. *L. bicolor*, *A. bisporus* (1), *B. plumbea* and *A. bisporus* (2) extracts inhibited the growth of *S. aureus*. In addition, *L. bicolor* extract inhibited *K. pneumoniae* and *L. monocytogenes* while *A. bisporus* (2) extract inhibited *P. aeruginosa*. Among all mushrooms, *L. bicolor* showed remarkable results. The evaluation of our results and their comparison with other studies results prove the importance of mushroom as resource for new compounds as mean of antimicrobial and anti-QS activities. These works should be continued with the aim to isolate the active

compounds and to test their efficacy with emphasis on the importance of the different conditions surrounding the mushrooms cultivation, growth environment and extraction methods.

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Author's Contributions

Sibel Yıldız: Drafted the manuscript, conducted the project of this study.

Ayşenur Gürgen: Drafted and wrote the manuscript, obtained the mushrooms and performed the biological experiments.

Sana Tabbouche: Drafted the manuscript, performed anti-microbial and anti-quorum sensing experiments.

Gönül Serdar: Performed Supercritical CO₂ extraction of mushrooms.

Münevver Sökmen: Performed Supercritical CO₂ extraction of mushrooms.

Ali Osman Kılıç: Performed anti-microbial and anti-quorum sensing assay experiments.

Ethics

There are no ethical issues after the publication of this manuscript.

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