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Antimicrobial and Anti-Quorum Sensing Activity of Some Wild Mushrooms Collected from Turkey

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ABSTRACT

Wild mushrooms are an important part of forests that have been known since the early history for their excellent medicinal properties. In this study, anti-microbial and anti-quorum sensing activities of some wild mushrooms (*Amanita rubescens, Russula delica*, Lactarius sp.) collected from the Black Sea region in Turkey were investigated. Mushroom extracts were prepared by supercritical fluid extraction method. Antimicrobial potential of extracts was tested by agar well diffusion method against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Listeria monocytogenes* ATCC 43251 and *Candida albicans* ATCC 10231. Anti-quorum sensing activity was tested on *Chromobacterium violaceum* ATCC 12472 bacteria. The results revealed that *Amanita rubescens* showed an inhibitory effect against *Staphylococcus aureus* and *Amanita rubescens* and *Lactarius* sp. extracts showed anti-quorum sensing activity against *Chromobacterium violaceum.*

Keywords: Antimicrobial, Anti-Quorum Sensing, Mushroom, Turkey

Türkiye'den Toplanan Bazı Yabani Mantarların Antimikrobiyal ve Anti-Quorum Sensing (Çoğunluğu Algılama) Aktiviteleri

ÖZ

Yabani mantarlar, tıbbi özellikleri nedeniyle çok eski tarihten beri bilinen, ormanların önemli bir parçasıdır. Bu çalışmada, Karadeniz bölgesinden toplanan bazı yabani mantarların (*Amanita rubescens, Russula delica, Lactarius* sp.) anti mikrobiyal ve anti-quorum sensing (çoğunluğu algılama) aktiviteleri incelenmiştir. Mantar ekstraktları süper kritik akışkan ekstraksiyonu metodu ile hazırlanmıştır. Ekstraktların antimikrobiyal potansiyelleri *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Listeria monocytogenes* ATCC 43251 and *Candida albicans* ATCC 10231 mikroorganizmalarına karşı agar kuyucuk difüzyon yöntemiyle test edilmiştir. Anti-quorum sensing (çoğunluğu algılama) aktivite ise *Chromobacterium violaceum* ATCC 12472 bakterisi üzerinde test edilmiştir. Sonuçlar, *Amanita rubescens* mantar ekstraktının *Staphylococcus aureus*'u inhibe ettiğini, *Amanita rubescens ve Lactarius* sp. ekstraktlarının *Chromobacterium violaceum* a karşı anti-quorum sensing (çoğunluğu algılama) aktiviteye sahip olduğunu göstermiştir.

Anahtar Kelimeler: Antimikrobiyal, Çoğunluğu Algılama, Mantar, Türkiye

INTRODUCTION

Day after day, with the progress of the biotechnology, botany and medicine, the need to come back to the natural sources for human nutrition and medicine is becoming obvious. One of the most prominent problems we are facing today is the antimicrobial resistance of bacteria toward antibiotics. Many pathogenic bacteria are gaining resistance and superbugs are quickly emerging worldwide. The main reason for this problem is the unconditional and the non-organized use of antimicrobials agents. This is why researchers are focusing on finding alternatives to the chemical antimicrobial molecules and are looking for new mechanisms to fight against infections. One of the promising alternatives is quorum-quenching mechanism, which consists of inhibiting the quorum

sensing messaging between microorganisms [1]. Quorum sensing is considered as a communication mechanism between microorganisms and is very important for their life and growth as a population. For example, bacterial divisions, nutrition, biofilm, spores' formation, pigments production and bacterial movements are essentially controlled by the quorum sensing molecules and are depending on community interactions [1]. Quorum sensing affects also bacterial virulence factors hence their pathogenicity [2] factor, which means that quorum quenching may inhibit the resistance emergence between bacteria.

Edible mushrooms are a common food known for its benefits and that has been used in folk medicine a long time ago for its wealth in medicinal and other bioactive compounds, and especially for their low-fat content [3]. A lot of edible mushroom have been identified to harbor many medicinal activities such as antioxidant [4, 5], antimicrobial [6, 7], anti-inflammatory [8, 9] and anticancer [10, 11], and today a lot of mushroom are known for their medicinal impact and are used in the commercial scale.

In this study, three wild mushroom's extracts from Turkey are tested for their antimicrobial and anti-quorum sensing activities.

MATERIALS and METHODS

Extracts Preparation

Three wild mushrooms (*Amanita rubescens*, *Russula delica*, *Lactarius* sp.) were collected in Trabzon, Turkey (Fig 1) and identified by their morphological and their ecological characteristics. Mushrooms were then dried at 50 ºC and pulverized.

Fig 1: Study area

Supercritical CO_2 extraction was applied for 10 g of mushroom powder at 250 Bar, 50 ºC during 3 hours. CO_2 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) as working solution.

Antimicrobial Activity

Antimicrobial activity was tested by agar well diffusion method in accordance to the guidelines of The Clinical Laboratory Standards Institute (CLSI) on Mueller Hinton agar. The microorganisms tested were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Listeria monocytogenes* ATCC 43251, and *Candida albicans* ATCC 10231 and extracts were applied as 5 µl of 10 mg/ml solutions. DMSO was used as negative control and ampicillin (10 µg) gentamicin (10 µg), tetracycline (30 µg), cefotaxime (30 µg) and amphotericin B (10 µg) were used as positive control.

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 $5x10^4$ CFU/ml. The last 2 well were used for growth control (bacteria without extract) and for sterility control (Extract without bacteria).

Anti-Quorum Sensing Activity

Anti-quorum sensing activity was tested against *Chromobacterium violaceum* ATCC 12472. For this purpose, the minimal inhibitory concentration of the different extracts was first determined in accordance to the guidelines of The CLSI on 96 wells plate as mentioned before, then the SubMic and its next lower concentration were tested for pigment production inhibition. Extract concentrations started by 5 mg/ml and bacterial final concentration was $5x10^4$ CFU/ml. After an incubation of 24 h the well was dried at 50 ºC for 1 hour, then the pigments were dissolved in 200 µl of DMSO and leaved 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 OD_{585} nm. The same test for each extract was repeated three time, twice for pigment testing and the third was used for plate count agar test. Agar counting was realized by taking 100 µl and spreading it on Mueller Hinton agar and incubating at 37ºC for 24h.

RESULT and DISCUSSION

Antimicrobial test results (inhibition diameter zone in mm) was presented at Table 1 and anti-quorum sensing activity charts of positive control (vanilla), negative control (DMSO), *A. rubescens* and *Lactarius* sp. were showed at Figure 2-5, respectively.

Only *Amanita rubescens* showed antimicrobial activity against *Staphylococcus aureus* with a minimal inhibitory concentration of 78.125 µg/ml (Table 1).

Anti-quorum sensing activity test is considered positive when an extract could inhibit the quorum sensing activity (in this study pigment production measured by OD_{585}) without affecting the bacterial growth (tested on plate count agar). In this study, *Amanita rubescens* and *Lactarius* sp. extracts showed an anti-quorum sensing activity against *Chromobacterium violaceum* (Fig. 4-5)

Table 1: Antimicrobial test results (inhibition diameter zone in mm)

Material	*S. aureus*	*E. coli*	*P.aeruginosa*	*C. albicans*	*E.faecalis*	*P. mirabilis*	*K.pneumoniae*	*L.monocytogenes*
Amanita rubescens	2	0	0	0	0	-	0	0
Russula delica	0	0	0	0	0	-	0	0
Lactarius sp.	0	0	0	0	0	-	0	0
DMSO	0	0	0	0.1	0	0	0	0
Ampicillin	>30	16-17	-*	-	14	-	-	-
Gentamicin	-	-	21-22	-	-	-	-	-
Amphotericin B	-	-	-	> 30	-	-	-	-
Cefotaxime	-	-	-	-	-	42	-	-
Tetracycline	-	-	-	-	-	-	-	25

*-: Not tested.

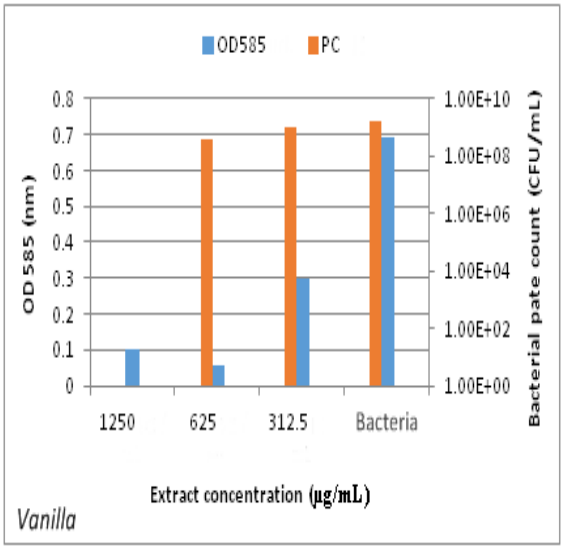

Fig 2. Vanilla anti-QS activity chart

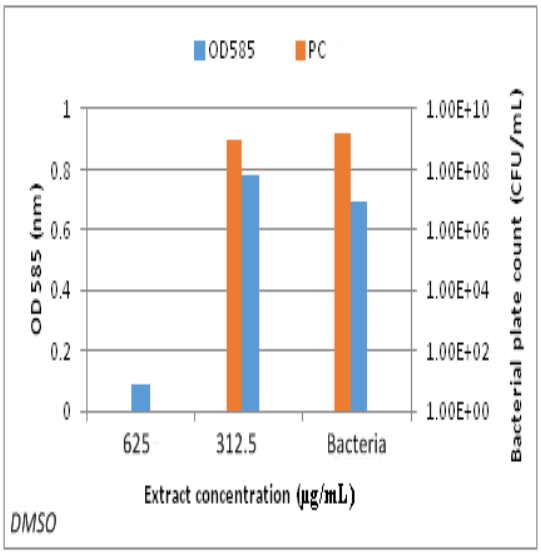

Fig 3. DMSO anti-QS activity chart

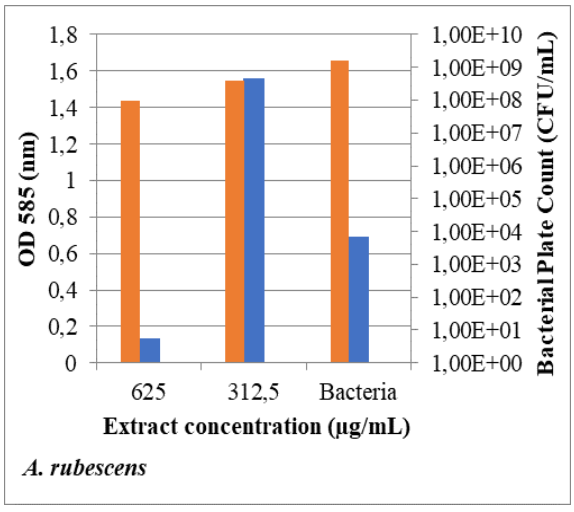

Fig 4. *A. rubescens* anti-QS activity chart

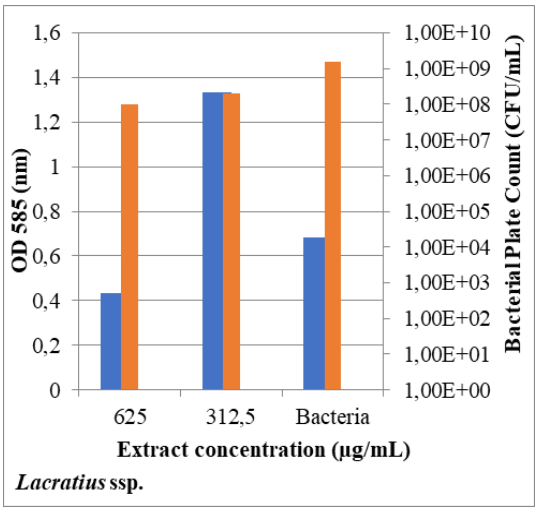

Fig 5. *Lactarius* sp. anti-QS activity chart

Since mushrooms has been known for a longtime for their wealth in medicinal compounds they have been intensively investigated for their antimicrobial activities. Similar study was driven on the wild mushroom of Khartoum showed positive antimicrobial effect of ethanol and water extracts against *C. albicans, S. aureus, P. aeruginosa*, and *E. coli* [12]. Other study that worked on the methanolic and water extracts of wild mushroom and showed antimicrobial effects of *Fistulina hepatica*, *R. botrytis* and *R. delica* against clinical bacterial strains with resistant traits [13]. The same study tested *Russula delica* and some *Lactarius* sp. Extracts and showed no effective antimicrobial activity against the tested bacterial strain. *Amanita* strain was not studied. As for Turkish mushroom strains, a study was driven by Akyüz *et al*., [14] on wild edible mushroom collected from Anatolia region of Turkey showed antimicrobial activity of methanolic extracts from *Pleurotus* spp., *Terfezia boudieri* and *Agaricus bisporus* against many bacterial strains. *Amanita, Lactarius* and *Russula* were not included in the study. Another Polish study showed antimicrobial activities of some *Amanita, Lactarius* and *Russula* species (differents from the species studied here) with MIC around 600 µg/ml and 2.5 mg/ml [15]. Furthermore, acetone and methanol extracts of *A. rubescens, L. piperatus* and *R. cyanoxantha* also showed antimicrobial activity with MIC in scale of mg/ml against many bacteria [16]. Our study showed an antimicrobial activity of methanolic extract of *A. rubescens* against *S. aureus* with MIC activity around 78,125 µg/ml, which means that the Black Sea region may be harboring some high value mushroom strains that should be more investigated and studied.

Quorum quenching mechanism appeared to be a promising alternative to classical antibiotics and had been intensively studied in the last few years. Quorum sensing certainly isn't only important for bacteria, but also is a primary factor in the symbiosis relationships between the different microorganisms and constitute and general language for the cross talking between even the eukaryotic kingdom all over the natural earth, among all fungi, plant and even algae [17].

Many studies have worked on investigating anti quorum sensing activities in natural resources but few were driven on mushroom species. In our work we aimed to scan an anti QS activity in the tested 3 mushrooms and the results showed a positive effect for *A. rubescens* and *Lactarius* sp. at concentration of 625 µg/ml. Another study showed ant anti quorum sensing activity of *Agrocybe aegerita* methanolic extracts against *P. aeruginosa* [18] and other work showed anti QS activity of *Agaricus blazei* water extracts against clinical *P. aeruginosa* strains biofilm formation [19].

CONCLUSION

In this study, anti-microbial and anti-quorum sensing activities of some wild mushrooms (*Amanita rubescens, Russula delica, Lactarius* sp.) collected from the Black Sea region in Turkey were investigated. The analysis results revealed that *Amanita rubescens* showed an inhibitory effect against *Staphylococcus aureus* and *Amanita rubescens* and *Lactarius* sp. Extracts showed an anti-quorum sensing activity against *Chromobacterium violaceum*. This is a simple study to show that the Black Sea region harbors valuable bioactive mushroom species which should be investigated and used on a bigger scale. On the other hand, anti-quorum sensing activity should be more studied as mean of mushroom species and mechanism since these regions may include powerful active species against pathogenic bacterial species.

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