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EVALUATION OF BACTERICIDAL ACTIVITY OF SELECTED WILD MACROFUNGI EXTRACTS AGAINST *Escherichia coli*

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Abstract

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the evaluating of novel sources for their antimicrobial potential. Nature is a generous source of compounds with antimicrobial activity. However, a large number of natural products with the potential to act as antimicrobials still await further investigation. In this study, antimicrobial activities of the extracts from four wild mushrooms: *Amanita echinocephala*, *Russula medulata*, *Ishnoderma benzoinum* and *Laetiporus sulphureus* were evaluated against Gram-negative bacterium *Escherichia coli*. The antimicrobial potential of the methanolic mushroom extracts was investigated by the microdilution method. Mild inhibitory activity was observed in 3 out of 4 mushroom species included in the study. The extracts were further tested for bactericidal activity and minimum bactericidal concentration (MBC) values were determined. The tested microorganism was most sensitive to the examined extracts of *Laetiporus sulphureus* (MBC=62.5 mg/mL).

Even though the results revealed in this study may suggest that tested wild macrofungi extracts possess mild antimicrobial activity, their antimicrobial potential against other microorganisms need to be further elucidated.

Key words: *mushroom, antimicrobial activity, microdilution method, minimum bactericidal concentration (MBC)*

INTRODUCTION

Antimicrobial resistance exhibited by pathogenic microorganisms is a significant public health concern (Walsh & Amyes, 2004). The World Health Organization presented the global report which indicates that *Escherichia coli* is among the three most resistant strains posing a real risk in the community (WHO, 2014). As a result, exploration of natural sources for novel bioactive compounds with antimicrobial properties is an emerging field of science over the last decades.

Nature is a generous source of compounds with antimicrobial activity. However, a large number of natural products with the potential to act as alternative antimicrobials still await further investigation. Previous studies have indicated that mushrooms are rich sources of natural antibiotics (De Silva et al., 2013). Various taxonomic mushroom groups have been investigated for their antimicrobial activities and many low- and high- molecular weight compounds with antimicrobial properties were identified. Many secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives, quinolines; and primary metabolites such as oxalic acid; and high- molecular weight compounds mainly peptides and proteins are among the identified antimicrobial compounds in mushrooms (Alves et al., 2012).

The aim of this study was to evaluate the antimicrobial activities of the extracts from four wild mushrooms: *Amanita echinocephala*, *Russula medulata*, *Ishnoderma benzoinum* and *Laetiporus sulphureus* against Gram-negative bacterium *Escherichia coli*.

MATERIAL AND METHODS

Fruiting body selection

Samples of the wild macromycetes *Amanita echinocephala, Russula medulata, Ishnoderma benzoinum* and *Laetiporus sulphureus* were collected from different locations and habitats in Macedonia. Geographical location and natural habitat of the mushroom specimens are shown in Table 1. Taxonomic identification was made in the Mycological Laboratory at the Institute of Biology, Faculty of Natural Sciences and Mathematics in Skopje, by implementing standard methods of microscopic and chemical techniques (colouring of fruit bodies and spores), as well appropriate literature. The representative voucher specimens were deposited at the Macedonian Collection of Fungi (MCF) at the Institute of Biology (Tab. 1).

Table 1. Geographical location and natural habitat of the mushroom species studied for antimicrobial potential.

Species	Habitat	Geographical location	Collection number
Amanita echinocephala	mycorrhizal (*on ground in park*)	Botanical garden, Skopje	MAK 10/13309
Ishnoderma benzoinum	saprotrophic (*on stump of pine trees*)	Suva Gora Mt.	MAK 11/13252
Laetiporus sulphureus	parasitic (*on living black locust trunks*)	Kozle, Skopje	MAK 11/13361
Russula medulata	mycorrhizal (*on ground in park*)	Gazi Baba, Skopje	MAK 10/13305

In vitro antimicrobial assay

Test microorganism. Antimicrobial activities of methanol extracts were tested against Gram-negative bacterium *Escherichia coli* ATCC 8739. The microorganism was provided from the collection held by the Microbiology Laboratory, Faculty of Natural Sciences and Mathematics in Skopje.

Microbial suspension was prepared by the direct colony method. The turbidity of initial suspension was adjusted by comparison with 0.5 McFarland's standard (Andrews, 2005). The initial suspension contained about 10^8 colony forming units (CFU)/mL. Additionally, 1:100 dilutions of initial suspension were prepared into sterile 0.9% saline.

Microdilution method. The antibacterial activities of the mushroom extracts were assessed using the microdilution method with resazurin as an indicator of microbial growth (Sarker*et al.*, 2007). The antimicrobial assay was performed by using a sterile 96-well plate and the minimum inhibitory concentration (MIC) values were determined. The test plates were prepared by dispensing 50 µL of Mueller-Hinton broth into each well. A volume of 50 µL from the stock solution of tested mushroom extracts was added into the first row of the plate and then two-fold serial dilutions of extracts were performed. Each test plate included growth control and sterility control. MIC was defined as the lowest concentration of tested extracts that prevented a resazurin colour change from blue to pink. All tests were performed in triplicate and MIC values were constant.

The extracts that demonstrated inhibitory activities were further tested for bactericidal activity. A sample from each well that tested positive for inhibitory activity was inoculated on fresh sterile Mueller-Hinton agar (MHA) plates and incubated additional 24 h at 37°C. Absence of colonies was regarded as positive for bactericidal activity, while growth of colonies was regarded as negative. MBC was defined as the lowest concentration of the mushroom extract that results in microbial death. All tests were performed in triplicate and MBC values were constant.

RESULTS AND DISCUSSION

The antimicrobial activity of the tested extracts was quantitatively assessed and the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) were used as a measure of the antibacterial activity of the mushroom extracts included in the study. The antibacterial activity of the mushroom extracts is shown in Table 2.

Table 2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of methanolic extracts from mushroom samples.

Samples	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio
Amanita echinocephala	200	200	1
Ishnoderma benzoinum	125	125	1
Laetiporus sulphureus	62.5	62.5	1
Russula medulata	-	-	-

(-) No antibacterial activity was observed at the highest working concentration of 200 mg/ml.

The results of the study demonstrated that the mushroom extracts possess either relatively weak or none antibacterial activity at all. Three (*Amanita echinocephala, Ishnoderma benzoinum* and *Laetiporus sulphureus*) out of four extracts demonstrated mild inhibitory and bactericidal effects against the tested microorganism. The methanol extract of *Russula medulata* showed no antibacterial activity against *Escherichia coli* at the concentration used. Our results revealed that the examined extracts from *A. echinocephala, I. benzoinum* and *L. sulphureus* possess bactericidal activity with MBC/MIC ratio=1. However, in all cases relatively high concentrations of extracts, ranging from 62.5 to 200 mg/mL, were required to achieve the antibacterial effect.

The potent bactericidal activity of the polypores is well documented in the literature (Demiri & Yamaç, 2008; Zjawiony, 2004). In this study, the antimicrobial potential of two polypore fungi *I. benzoinum* and *L. sulphureus* has been examined. The results demonstrated that the tested microorganism was most sensitive to the extracts of the examined polypore fungi. The highest bactericidal activity was obtained in the extract from the species *L. sulphureus* with the MBC value of 62.5 mg/ml. This result is in accordance with earlier reported data which confirm moderate antimicrobial potential of *L. sulphureus* against *Escherichia coli* (Nowacka et al., 2014; Demiri & Yamaç, 2008; Turkoglu et al., 2007). Higher MBC value of 125 mg/ml and 200 mg/ml was obtained in the extracts from *I. benzoinum* and *A. echinocephala,* respectively that corresponded to lower bactericidal potential against tested *Escherichia coli*.

The present study was a continuation of our previous studies in which antibacterial activity of the same mushroom species have been tested against Gram-positive bacterium *Staphylococcus aureus* (Nikolovska Nedelkoska et al., 2017) and Gram-negative bacterium *Pseudomonas aeruginosa* (Nikolovska Nedelkoska et al., 2018). Comparison of the results obtained shows that *S. aureus* and *P. aeruginosa* were more sensitive to the examined macrofungi extracts than the tested *Escherichia coli*. These findings are also in accordance with earlier reported data from our first research on antibacterial screening of the selected Macedonian wild mushrooms (Nikolovska Nedelkoska et al., 2013), which showed that the tested mushroom extracts possess either none (*Boletus lupinus, Flammulina velutypes*) or relatively weak (*Phellinus igniarius, Sarcodon imbricatus, Tricholoma aurantium, Xerocomus ichnusanus*) antibacterial activity against *E. coli* (MIC=10-50 mg/mL) compared with other tested bacteria. The cell wall structure might be the reason for the relatively high resistance towards antimicrobial agents that was observed for the microbial species tested in this study. It is known that Gram-negative bacteria possess an outer membrane and a periplasmic space, both of which are absent from Gram-positive bacteria, and that differences in the cell wall structure can produce differences in antibiotic susceptibility of the cells (Martínez de Tejada et al., 2012). Several studies have also shown that the Gram-positive bacteria are generally more sensitive to the antimicrobial effect of the macrofungi extracts compared to Gram-negative bacteria, but this relationship does not hold for every mushroom species (Alves et al., 2012; Suay et al., 2000).

Even there are many studies on phytochemical characterisation of cultivated and wild mushrooms, only little information is available on chemical characterisation of specific classes of antimicrobial compounds in tested mushrooms. According to the available literature data, few antimicrobial secondary metabolites have been identified in mushroom

extracts from *I. benzoinum* and *L. sulphureus*. The antibiotic 1-hydroxy-2-nonyn-4-one has been isolated from the submerged cultures of *I. benzoinum* (Anke et al., 1982). Another example of antimicrobial secondary metabolites is a cyclodepsipeptide, beauvericin, produced by *L. sulphureus* (Zjawiony, 2004).

CONCLUDING REMARKS

The present study was undertaken to quantitatively assess the antimicrobial potential of methanolic extracts from fruiting bodies of four wild macromycetes (*Amanita echinocephala, Russula medulata, Ishnoderma benzoinum* and *Laetiporus sulphureus*) against the tested *Escherichia coli*. Even though the results revealed in this study may suggest that tested wild macrofungi extracts possess mild antimicrobial activity, their antimicrobial potential against other microorganisms need to be further elucidated.

The evaluation of the mushroom extracts against other microorganisms may demonstrate a stronger effect and will be a promising field for assessing their potential as novel antibiotics. Considering that the extracts used in the study were crude mixtures rather than pure substance, further studies are needed for the chemical characterization of specific classes of antimicrobial compounds of the selected mushrooms.

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ЕВАЛУАЦИЈА НА БАКТЕРИЦИДНАТА АКТИВНОСТ НА СЕЛЕКТИРАНИ ДИВИ МАКРОФУНГИ ВО ОДНОС НА *Escherichia coli*

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Резиме

Зголемената неефикасност на медикаментната терапија и евидентната резистентност на патогените микроорганизми ја наметна потребата од евалуирање на нови извори за алтернативни антимикробни соединенија. Природата е дарежлив извор на компоненти со антимикробна активност. Сепак, голем број природни продукти со антимикробен потенцијал сè уште не се доволно истражени. Во оваа студија антимикробната активност на екстрактите добиени од четири видови на диви печурки: *Amanita echinocephala*, *Russula medulata*, *Ishnoderma benzoinum* и *Laetiporus sulphureus* беа евалуирани во однос на Грам-негативната бактерија *Escherichia coli*, со примена на микродилуциониот метод (метод на последователни разредувања). Умерена антимикробна активност беше утврдена кај три од четирите анализирани видови. Исто така, беше испитана бактерицидната активност на екстрактите и беа определени минималните бактерицидни концентрации (МБЦ). Тест-микроорганизмот покажа најголема сензитивност кон екстрактот добиен од габата *Laetiporus sulphureus* (МБЦ=62,5 mg/mL).

И покрај тоа што резултатите од оваа студија укажуваат на релативно слаба антимикробна активност на видовите печурки опфатени во истражувањето, сепак за конечна антимикробна евалуација на овие видови потребно е да се испита нивниот антимикробен потенцијал и во однос на други микроорганизми.

Клучни зборови: *печурки, антимикробна активност, микродилуционен метод, минимална бактерицидна концентрација (МБЦ).*