DOI: https://doi.org/10.55302/JAFES18723067nn

ANTIBACTERIAL ACTIVITIES OF SOME WILD MUSHROOM EXTRACTS AGAINST *PSEUDOMONAS AERUGINOSA*

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Abstract

Mushrooms synthesize a multitude of low-molecular-weight secondary metabolites that have an important role as communication signals, to defend mushroom habitat or to inhibit the growth of competitors. Investigations have shown that some of these metabolites have potent antimicrobial activity and could be beneficial for humans. In this study, antimicrobial potential of the extracts from six wild mushrooms: *Amanita echinocephala, Russula medulata, Cerena unicolor, Hericium erinaceus, Ishnoderma benzoinum* and *Laetiporus sulphureus* was evaluated against Gram-negative bacterium *Pseudomonas aeruginosa*. The antimicrobial activities of the methanolic mushroom extracts were investigated by the microdilution method. All the extracts that demonstrated inhibitory activities were further tested for bactericidal activity and minimum bactericidal concentration (MBC) values were determined. Antimicrobial activity was observed in all species included in the study, while the activities depended on the type and concentration of extract. The tested microorganism was more sensitive to the examined extracts from the polypore fungi (*C. unicolor, H. erinaceus, I. benzoinum* and *L. sulphureus*). The highest antibacterial activity was obtained in the extracts from polypores *I. benzoinum* and *L. sulphureus* (MBC=15.625 mg/mL). This study demonstrated that the analysed wild macrofungi have the potential to accumulate bioactive metabolites that possess antimicrobial activity.

Keywords: Microdilution method, minimum bactericidal concentration (*MBC*).

Introduction

Pseudomonas aeruginosa is a common pathogen associated with a spectrum of infections in humans. The organism is intrinsically resistant to many antimicrobials and can develop resistance during anti-pseudomonal chemotherapy that causes infections with a high mortality rate (Poole, 2011). Interest in natural products as antimicrobial agents has waned in recent years, but the frequency of antibiotic resistance without new antibiotic classes on the horizon suggests the need of re-evaluation of natural products as a route to identify novel chemical skeletons with antibacterial activity (Moloney, 2016). Previous studies have indicated that macrofungi, as a specific response to the natural hostile environment, synthesize a multitude of metabolites with antimicrobial properties (Alves et al. 2012). Considering that humans and animals share common microbial pathogens with fungi, fungal defensive strategies against microorganisms could be of benefit for humans. Hence, various taxonomic mushroom groups have been investigated for their antimicrobial activities (Suay et al. 2000, Yamac and Bilgili 2006, Nikolovska Nedelkoska et al. 2013). Numerous studies have shown the pharmaceutical potential of Basidiomycetes, especially polypores, which are considered by many authors as major sources of bioactive natural products among species of the diverse fungal phylum Basidomycota (Zjawiony, 2004). According to antimicrobial evaluation of 204 mushroom species, more than 75% of screened polypores exhibited strong antimicrobial potential (Suay et al. 2000). With an increasing number of bacteria developing resistance to commercial antibiotics, a rich

diversity of higher fungi, including polypores, provide a wide range of good cadidates for critically needed new antibiotics. Therefore, the aim of this study was to evaluate the antimicrobial activity of the extracts from six wild mushrooms: *Amanita echinocephala, Russula medulata, Cerena unicolor, Hericium erinaceus, Ishnoderma benzoinum* and *Laetiporus sulphureus* against Gram-negative bacterium *Pseudomonas aeruginosa*.

Material and methods

Fruiting body selection

The fruiting bodies of the wild macromycetes *Amanita echinocephala, Russula medulata, Cerena unicolor, Hericium erinaceus, Ishnoderma benzoinum* and *Laetiporus sulphureus* were collected from different locations and habitats in Macedonia. Geographical location, natural habitat and collection number in the Macedonian Collection of Fungi (MCF) 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, as well appropriate literature.

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

Species	*Habitat*	*Geographical location*	*Collection number*
A. echinocephala	mycorrhizal (*on ground in park*)	Botanical garden, Skopje	MAK 10/13309
R. medulata	mycorrhizal (*on ground in park*)	Gazi Baba, Skopje	MAK 10/13305
C. unicolor	saprotrophic (*on living beech trunks in conifer forest*)	Suva Gora Mt.	MAK 11/13368
H. erinaceus	saprotrophic (*on living oak trunks in deciduous forest*)	Sk. Crna Gora Mt.	MAK 11/13360
I. benzoinum	saprotrophic (*on stump of pine trees*)	Suva Gora Mt.	MAK 11/13252
L. sulphureus	parasitic (*on living black locust trunks*)	Kozle, Skopje	MAK 11/13361

Preparation of methanolic extracts of mushrooms

Samples were cleaned and subsequently air-dried in the oven at 40^{o}C. Dried specimens were ground to fine powder and extracted by stirring with 80% (v/v) methanol in ultrasonic bath for 30 min at 4ºC, and then centrifuged at 12000 rpm for 15 min. Supernatants were used for the evaluation of antimicrobial potential of the samples. The organic solvent in the extracts was evaporated to dryness under vacuum. The yields of methanolic extracts of the fruiting bodies are presented in Table 2. The tested extracts were dissolved in 10% (v/v) DMSO in sterile water. A solvent control test was performed to study the effect of DMSO on the growth of microorganisms. The test approved that DMSO had no inhibitory effect on the tested organisms.

Table 2. Yield of mushroom methanolic extracts

Mushroom species	*Yield of extracts* [a] *(g/100 g of dry mushroom)*
A. echinocephala	33,082 ± 3,356
R. medulata	4,167 ± 0,577
C. unicolor	20,800 ± 1,131
H. erinaceus	17,333 ± 0,764
I. benzoinum	15,000 ± 1,323
L. sulphureus	33,833 ± 4,254

[a]Each value is the mean of three replicate determinations ± standard deviation

Test microorganism
Antimicrobial activities of methanol extracts were tested against Gram-negative bacterium *Pseudomonas aeruginosa* ATCC 9027. The microorganism was provided from the collection held by the Microbiology Laboratory, Faculty of Natural Sciences and Mathematics in Skopje.

Suspension preparation
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
In vitro 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 broth microdilution test is a standard reference method for quantitative assessment of an antimicrobial agent against a given bacterium. The antimicrobial assay was performed by using a sterile 96-well plate and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined (Nikolovska Nedelkoska et al. 2007). Each test plate included growth control and sterility control. MIC was defined as the lowest concentration of tested extracts that prevented a resazurin color change from blue to pink. All tests were performed in triplicate.
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.

Results and discussion
The minimum bactericidal concentration (MBC) values were determined among the tested extracts and are presented in Table 3. The results showed that all selected mushroom species exhibited the bactericidal activity against tested bacterium, with MBC values ranging from 15.625 to 200 mg/mL. In this study the methanolic extracts from polypores *Ishnoderma benzoinum* and *Laetiporus sulphureus* demonstrated the strongest antimicrobial potential with the same MBC value of 15.625 mg/mL, followed by the extracts of *Hericium erinaceus* and *Cerena unicolor* (18.75 mg/mL and 25 mg/mL, respectively). Higher MBC values were obtained in the extracts from *Amanita echinocephala* and *Russula medulata* (50 mg/mL and 200 mg/mL, respectively), that corresponded to lower bactericidal potential against tested *Pseudomonas aeruginosa.*

Table 3. Minimum bactericidal concentration (MBC) of methanolic extracts from mushroom samples

MBC values [a]					
A. echinocephala	*C. unicolor*	*H. erinaceus*	*I. benzoinum*	*L. sulphureus*	*R.medulata*
50	*25*	*18.750*	*15.625*	*15.625*	*200*

[a] Minimum bactericidal concentration (MBC) values given as mg/mL

The present study was a continuation of our previous study in which antibacterial activity of the same mushroom species have been tested against Gram-positive bacterium *Staphylococcus aureus* (Nikolovska Nedelkoska et al. 2017). According to obtained results the mushroom extracts included in the study showed bactericidal activities against Gram-positive *S. aureus* that were equal to (*Laetiporus sulphureus*) or stronger than the activities observed against Gram-negative *Pseudomonas aeruginosa*. The only exception was the extract from *Ishnoderma benzoinum*, which exhibited lower bactericidal activity against *S. aureus* (MBC=31.250 mg/mL). Those results are in accordance with earlier reported data which confirm 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 (Yamac and Bilgili 2006, Pala and Wani 2011, Alves et al. 2012).

In the current study the examined extracts from the polypore fungi *Ishnoderma benzoinum* and *Laetiporus sulphureus* demonstrated the most potent bactericidal activity against tested *Pseudomonas aeruginosa*. Several previous studies have shown the antimicrobial potential of these macrofungi species (Turkoglu et al. 2007, Teplyakova et al. 2012). Turkoglu et al. (2007) evaluated the antimicrobial effect of the ethanolic extract of *L. sulphureus* and observed strong inhibition of the growth of the Gram-positive bacteria tested. Teplyakova et al. (2012) investigated the antiviral activity of aqueous extracts from mycelium of 11 basidial fungi species and found that *I. benzoinum* is among the most perspective strains for producing antiviral medicines. In general, there is little information available on chemical characterisation of specific classes of antimicrobial compounds in tested polypores *I. benzoinum* and *L. sulphureus*. Based on the evidence reported in the literature, few antimicrobial secondary metabolites have been identified in mushroom extracts from *I. benzoinum* and *L. sulphureus.* For instance, an antibiotic, 1-hydroxy-2-nonyn-4-one, has been isolated from submerged cultures of several strains of *I. benzoinum* (Anke et al. 1982). Another example of antimicrobial secondary metabolites is a cyclodepsipeptide, beauvericin, produced by the polypore *L. sulphureus* (Zjawiony, 2004). All these observations were in accordance with the bactericidal activity reported here.

Conclusions

The present study was undertaken to quantitatively assess the antimicrobial potential of methanolic extracts from fruiting bodies of six wild macromycetes (*Amanita echinocephala, Russula medulata, Cerena unicolor, Hericium erinaceus, Ishnoderma benzoinum* and *Laetiporus sulphureus*). Results from this study showed that all analyzed mushroom extracts exhibit bactericidal activity against *Pseudomonas aeruginosa*. Especially, extracts from the species *I. benzoinum* and *L. sulphureus* demonstrated most promising bactericidal activity against *P. aeruginosa* that may serve as potential candidates for the development of novel antibiotics. Concerning the development of natural antimicrobials, further work is needed toward the elucidation of the structure of the active compound and possible mechanism of action.

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