Antimicrobial Activity of Selected Wild Mushrooms from Different Areas of Bosnia and Herzegovina

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Abstract

The aim of this study was to examine the antimicrobial activity of selected wild mushrooms from different areas of Bosnia traditionally used in the diet. These are the first tests of antimicrobial activity of fungi extracts growing in Bosnia and Herzegovina. Wild mushrooms *Boletus edulis* Bull. (1782) and *Cantharellus cibarius* Fr. (1821) were collected from different areas of Bosnia and Herzegovina. Nine microbial strains were tested: four Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923; Methicillin-resistant *Staphylococcus aureus*: MRSA ATCC 33591; *Bacillus subtilis* ATCC 6633; and *Enterococcus faecalis* ATCC 29212), four Gram-negative bacteria (*Salmonella abony* ATCC 6017; *Pseudomonas aeruginosa* ATCC 9027; *Escherichia coli* ATCC 25922; Extended Spectrum Beta-Lactamase producing *E. coli*: ESBL *E. coli* ATCC 35218), and one yeast *Candida albicans* ATCC 1023, through the agar well diffusion method. Investigated wild mushrooms performed antibacterial activity against three strains of Gram-positive bacteria: *S. aureus*, MRSA and *B. subtilis*. Antibacterial activity of the examined extract depends on the locality on which mushrooms grow. The broader spectrum of antibacterial activity was observed in case of *C. cibarius* extracts, and largest inhibition zones with this mushroom species were recorded against *S. aureus* (18.11 ± 0.20 mm) and *B. subtilis* (18.10 ± 0.17 mm). Extracts of *B. edulis* exhibited the largest inhibition zones against MRSA (20.03 ± 0.08 mm). In comparison to the standard antibiotic, tested extracts showed significant inhibition of this multidrug-resistant pathogen.

Keywords

Antimicrobial activity • Wild mushrooms

1 Introduction

The discovery of penicillin was followed by the discovery and commercial production of many other antibiotics. Large amounts of antibiotics used for human therapy, as well as for farm animals and even for fish in aquaculture, resulted in the selection of pathogenic bacteria resistant to multiple drugs. Indeed, some strains have become resistant to practically all of the commonly available agents. For example, the methicillin-resistant *Staphylococcus aureu* (MRSA) is resistant not only to methicillin but usually also to aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides. Such strains are also resistant to disinfectants, and MRSA can act as a major source of hospital-acquired infections. An old antibiotic, vancomycin, was resurrected for treatment of MRSA infections. However, transferable resistance to vancomycin is now quite common in *Enterococcus* and found its way finally to MRSA in 2002, although such strains are still rare [1].

The problem of multi-drug resistant microorganisms has reached an alarming level all over the world, finding new antibiotics has become an urgent need for the treatment of microbial infections. The use of natural products has been extremely successful in the discovery of new medicine [2].

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The literature describes the antimicrobial activity of various types of mushrooms [3–5]. In fact, mushrooms could be a source of natural antibiotics.

Mushrooms could be defined as macrofungi with distinctive fruiting bodies and reproductive structures, and recently they have been identified as a major source of biologically active natural products. About 10,000 known species belonging to the class basidiomycetes, of which about 5000 species are edible and almost 2000 species have medicinal properties [6]. In addition to their high nutrition content, mushrooms are rich in bioactive metabolites such as lecithin, polysaccharide, phenolic and polyphenolic compounds, polyketides, terpenoids, glycopeptides, saponin and ergosterol with high medicinal value [7–9]. Many antimicrobial compounds are isolated from mycelium and fruiting bodies of mushrooms. These secondary metabolites function just like antibiotics, and mushrooms need these antibiotics and vitamins to vegetate and reproduce [10].

The extracts of *Laetiporus sulphureus*, *Ganoderma lucidum* and *Lentinus edodes* have already demonstrated antibacterial activity [5, 11, 12]. Furthermore, *Fistulina hepatica*, *Ramaria botrytis*, and *Russula delica* extracts were promising against multi-resistant microorganisms namely MRSA, *Escherichia coli* and *Proteus mirabilis* [13].

Until now, only the compounds from microscopic fungi have been present in the market as antibiotics.

The abovementioned facts served as the basis for the study of selected wild mushrooms and their antimicrobial activity. The aim of this study was to examine the antimicrobial activity of selected wild mushrooms from different areas of Bosnia and Herzegovina traditionally used in the diet. These are the first tests of antimicrobial activity of fungi extracts growing in Bosnia and Herzegovina.

2 Materials and Methods

Wild mushrooms *Boletus edulis* Bull. (1782) and *Cantharellus cibarius* Fr. (1821) were collected from different areas of Bosnia and Herzegovina (Table 1).

In vitro antimicrobial activity of lyophilized mushrooms was evaluated against four Gram-positive (*Staphylococcus aureus* ATCC 25923; Methicillin-resistant *Staphylococcus aureus*: MRSA ATCC 33591; *Bacillus subtilis* ATCC 6633; and *Enterococcus faecalis* ATCC 29212), four Gram-negative bacteria species (*Salmonella abony* ATCC 6017; *Pseudomonas aeruginosa* ATCC 9027; *Escherichia coli* ATCC 25922; Extended Spectrum Beta-Lactamase producing *E. coli*: ESBL *E. coli* ATCC 35218), and one yeast *Candida albicans* ATCC 1023. For antimicrobial assays, lyophilized material was dissolved in 5% dimethyl sulfoxide (DMSO) to the final concentration of 1 mg/mL. Antimicrobial effects of investigated wild mushrooms were determined by agar well diffusion method [14]. Standard antibiotic Ampicillin (10 µg; HiMedia Laboratories Pvt. Ltd., India), and antimycotic Nystatin (100 units; Oxoid Ltd., England) were used as positive control, while DMSO was used as negative control. Bacterial species were cultured overnight at 37 °C in Mueller Hinton medium (*Fluka* Biochemica; Buchs, Switzerland), while *C. albicans* in Sabouraund Glucose Agar (*Fluka* Biochemica; Buchs, Switzerland). According to Wayne [15], the sterile saline solution was used for inoculums adjusting to the final density of 0.5 McFarland standards ($\sim 1.5 \times 10^8$ CFU/mL). Microbial inoculums were spread over the entire surface of plates with growth medium and left for 15 min at room temperature to achieve a total absorption. Dissolved material of wild mushrooms and control samples (100 µl) were then

Table 1 Geographic origin of investigated wild mushrooms

Sample code	Mushrooms species	Locality	Coordinates
1	*Boletus edulis* Bull. (1782); Boletaceae	Bužim	45.05°N, 16.05°E
2		Cazin	44.97°N, 15.94°E
3		Olovo	44.13°N, 18.58°E
4		Ključ	44.53°N, 16.77°E
5		Foča	43.51°N, 18.78°E
6	*Cantharellus cibarius* Fr. (1821); Cantharellaceae	Gračanica	44.71°N, 18.31°E
7		Podvinjci	44.06°N, 18.23°E
8		Rudo	43.62°N, 19.37°E
9		Ključ	44.53°N, 16.77°E
10		Foča	43.51°N, 18.78°E

Leg. et det.: M. Salihović, 2017

transferred into the wells of inoculated agar plates. Plates with bacteria were incubated for 18–24 h at 37 °C, and those containing fungi were incubated for 24–48 h at 37 °C. Antimicrobial activity of tested mushrooms was evaluated based on inhibition zones diameter (mm). All tests were performed in three replications and the mean values (±SD) were calculated.

3 Results and Discussion

Overall results showed that investigated wild mushrooms extracts exhibited various antibacterial activity against three strains of Gram-positive bacteria: *S. aureus*, MRSA and *B. subtilis* (Table 2; Fig. 1). Extract of *B. edulis* collected at the locality Ključ caused the largest inhibition zones, especially in the case of MRSA (20.03 ± 0.08 mm). It is known that fruiting bodies of *B. edulis* are an important source of carbohydrates (mannose, rhamnose, glycans), lectins (boledulin A, B, C), organic acids (malic, oxalic, quinic, ketoglutaric acids), amino acids (glutamine, alanine, serine, proline) and microelements. Polysaccharides isolated from *B. edulis* are responsible for many biological activities [16]. Furthermore, extracts of *C. cibarius* collected in almost every site were also effective against MRSA (Table 2). Standard antibiotic Ampicillin, used as positive control, did not show any inhibition of this MDR pathogen.

DMSO as solvent control showed no inhibition.

Considering the geographic origin of *C. cibarius*, generated results indicate that the most potent extracts were made from the plant material collected in the locality Podvinjci (Table 2). Study of Aina et al. [6] revealed the antimicrobial

Table 2 Inhibition zones (mm) obtained through the agar well diffusion method

Investigated sample	Bacterial species with achieved growth inhibition		
	Staphylococcus aureus	MRSA	*Bacillus subtilis*
1	12.01 ± 0.21	NI	18.06 ± 0.13
2	10.12 ± 0.33	NI	15.06 ± 0.14
3	11.08 ± 0.13	NI	13.96 ± 0.18
4	19.03 ± 0.08	20.03 ± 0.08	19.03 ± 0.16
5	10.96 ± 0.16	NI	14.08 ± 0.19
6	14.05 ± 0.13	13.03 ± 0.17	15.07 ± 0.13
7	18.11 ± 0.20	14.11 ± 0.12	18.10 ± 0.17
8	14.11 ± 0.10	14.05 ± 0.23	14.06 ± 0.13
9	12.00 ± 0.11	12.96 ± 0.38	15.04 ± 0.17
10	9.96 ± 0.16	NI	13.00 ± 0.21
Positive control (Ampicillin; 10 μg)	33.03 ± 0.09	NI	47.98 ± 0.23

The results are the mean ± SD (n = 3). NI = No inhibition zone. DMSO = NI
Sample numbers 1–10 corresponds to the designations listed in Table 1

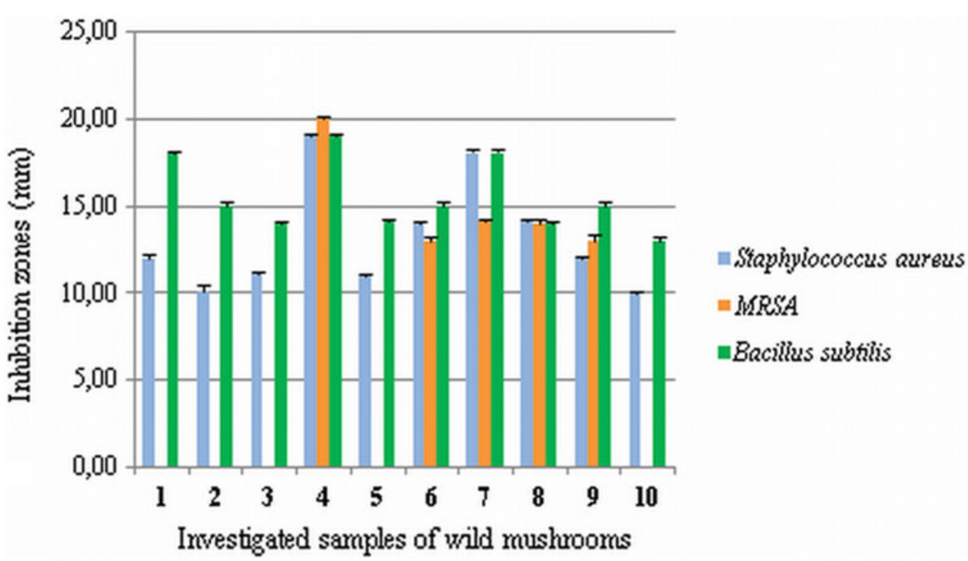

Fig. 1 Detected zones of inhibition (mm) caused by the activity of *B. edulis* and *C. cibarius* extracts (Sample numbers 1–10 corresponds to the designations listed in Table 1)

and antioxidant potency of extracts derived from *C. cibarius*, consequently suggesting its potential use in the pharmaceutical industry in making drugs with little or no side effect as it is produced from a natural source when compared to synthetic drugs. Constituent phytochemicals and nutraceuticals such as pleuromutilin and chanterellins present in *C. cibarius* have a synergistic effect in its antimicrobial and antioxidant activities [17].

Growth inhibition of *E. faecalis*, Gram-negative bacteria, and *C. albicans* was not recorded in this investigation. Different antimicrobial effects of investigated mushroom extracts could be explained by the differences in the microbial cell wall structure [18, 19]. The cell wall of Gram-positive bacteria is composed of several layers of peptidoglycans, and Gram-negative bacteria cell wall consists of one peptidoglycan layer and an outer membrane containing phospholipids and lipopolysaccharides, whereas the fungal cell wall contains chitin and other polysaccharides [20].

4 Conclusion

Investigated extracts of wild mushrooms collected in different areas of Bosnia and Herzegovina have a significant antibacterial property illustrated primary against Gram-positive bacteria. The interesting finding was the possibility of tested extracts to inhibit the growth of MDR pathogen. In the era characterized by the search for any new sources of antibiotics, these results of MRSA inhibition are invaluable. Further investigations should be focused on the evaluation of chemical composition and pharmacological activities of both *B. edulis* and *C. cibarius*. Such studies are necessary to broaden the therapeutic applications of these popular edible mushrooms.

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