Comparative analysis of antiradical and antibacterial activity of Boletus edulis basidiomycetes growing in different climatic zones

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Abstract. The aim of the study was to determine and compare the antiradical and antibacterial activity of the Boletus edulis basidiomycetes growing in different climatic zones: the temperate continental Mediterranean (Montenegro) and sharply continental eastern Siberia (Russia). The objects of study were ceps (Boletus edulis) collected in Montenegro (Durmitor National Park, harvested in August 2019) and in Russia (Siberia, Abansky district, harvested in August 2019). Mushrooms dried and crushed to a powder state were used to obtain aqueous extracts (water module 1:100). The antiradical activity of aqueous cept extracts was studied by UV and visible spectroscopy using a stable model radical of 2,2-diphenyl-1-picrylhydrazyl. For antiradical activity studies, samples were taken after 30, 60, 90 min of extraction. Antibacterial activity was evaluated by the difference in the diameter of the colonies of the test bacteria Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus 209p and Acinetobacter baumannii. It was shown that under the same conditions for sample preparation and analysis, Siberian ceps have a high antiradical activity value (the maximum value is 75% for mushrooms from Siberia against the maximum value of 33% for mushrooms from Montenegro, respectively). The results of the evaluation of antibacterial activity showed that regardless of the climatic zone of the growth of mushrooms, their aqueous extracts obtained after 30 minutes of extraction exhibit bacteriostatic effect in relation to all strains of bacteria. Longer extraction (more than 60 min) leads to the stimulating effect of Boletus edulis extracts on the growth of the same bacteria.

1. Introduction

Boletus edulis (lat. Boletus edulis), or boletus - edible mushroom of the genus Borovik (lat. Boletus), the Boletov family (lat. Boletaceae) is the most valuable natural food raw material from the mushrooms of the systematic division of basidiomycetes. Boletus grows both naturally in deciduous, coniferous and mixed forests, as well as in conditions artificially created by humans. The greatest food and biological value are characterized by ceps growing in natural biota. Boletus edulis is a source of complete protein and essential amino acids, unsaturated fatty acids, vitamins (B2, B5, C, PP) and minerals (K, P, S, Mn, Se, Cr) [1-2]. Since the water content in the composition of fresh mushroom reaches up to 90%, when dried, the concentration of useful and physiologically significant nutrients increases several times.

Clinical studies have shown that the use of ceps in food, unlike many other types of mushrooms, helps the body recover faster after surgery, illness, and exhaustion. Biologically active substances in the mushrooms increase muscle tone and endurance, stimulate metabolic processes, improve gastric secretion and intestinal motility [3-4].

A number of studies have shown the presence of antiradical and antibacterial activity of some types of basidiomycetes, including the species Boletus edulis [5-6]. However, the content and ratio of food components, biologically active substances, vitamins and minerals can vary significantly depending on the geographical zone of growth of the porcini mushroom, climatic conditions, average annual temperature and the number of sunny days. As a result, the biological activity of mushrooms and their effect on the body can change.

The purpose of this study is a comparative assessment of the antiradical and antibacterial activity of water extracts of boletus edulis species, which grow in different climatic zones, in particular, the temperate continental Mediterranean (Montenegro) and sharply continental eastern Siberia (Russia).

2. Objects and methods of research

The objects of study were ceps of the Boletus edulis species, collected in Montenegro (Durmitor National Park, harvested in August 2019) and in Russia (eastern Siberia, Abansky district, harvested in August 2019). Mushrooms that looked similar and close in size were selected for the study with cap diameter of 6-12 cm. Fresh mushrooms were cut into plates, dried in a dark ventilated room at a temperature of 20-22° C. Dried mushrooms were ground in a mill to a powder state. Dried mushroom powders were used to prepare aqueous extracts. A sample of 1.0 g of powder of dried mushrooms was poured into 100 ml of distilled water (1:100 hydraulic module), allowed to swell for 30 minutes and extracted at the boiling point of the solvent using a reflux condenser. For studies of antioxidant activity, samples were taken 30, 60, 90 min after the start of boiling. Shorter than 30 min extraction time of basidiomycetes is not effective due to the low degree of extraction of water-soluble substances.

The antiradical activity (ARA) of water extracts was studied by UV and visible spectroscopy using a stable model organic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) at a wavelength of 517 nm [7-8]. The kinetic curves of the interaction of the DPPH radical with the biologically active substances of the extract were recorded on a Shimadzu UV-1700 scanning spectrophotometer. The reaction of the interaction of the radical and the extract was carried out in quartz cuvettes with a sample layer thickness of 10 mm at a temperature of 22 ± 1° K, adding 3.9 ml 2.0×10^{-4} M solution of DPPH in 96% ethanol to 50 μl of the studied extract. The dose of the injected extract is selected empirically.

Cept extract ARA was evaluated by reducing the absorbance of the radical at 517 nm for 2.5,10,15,30 min after mixing DPPH and the extract (exposure time), it was calculated by the formula:

$$ARA(\%) = \frac{D_{517}I - D_{517}II}{D_{517}I} \cdot 100$$

where $D_{517}I$ – control, absorbance value for DPPH solution without extract; $D_{517}II$ – with extract.

For comparison, an aqueous solution of ascorbic acid was used as a standard, as a substance with high ARA. The experiment was performed in triplicate. The statistical measurement error did not exceed 5%.

Antibacterial activity was evaluated by the difference in the size of the diameter of the colonies of test bacteria in the experiment and control [9]. The control was test bacteria combined with 0.5 ml of distilled water. Standard type cultures of microorganisms were used as test strains: Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus 209p and Acinetobacter baumannii, provided by the Krasnoyarsk Regional Clinical Microbiological Laboratory.

The effect of aqueous extracts of ceps on bacterial strains was evaluated as positive (stimulating) or negative (bactericidal or bacteriostatic) when the size of the test culture colonies in the experiment was correspondingly significantly increased or decreased compared to the control. If the size of the

colonies in the experiment did not significantly differ from the control, the effect of the aqueous extract was evaluated as zero. All experiments to determine antibacterial activity were carried out in 5-fold repetition. Statistical data processing was performed according to G.F. Lakinu [10].

3. Results and discussion

Appearance of water extracts of ceps from Montenegro was light yellow in color, in contrast to a slightly darker yellowish-brown tint of cep extracts from Siberia. With an increase in extraction time, the color of the hoods did not fundamentally change, but became a little more saturated.

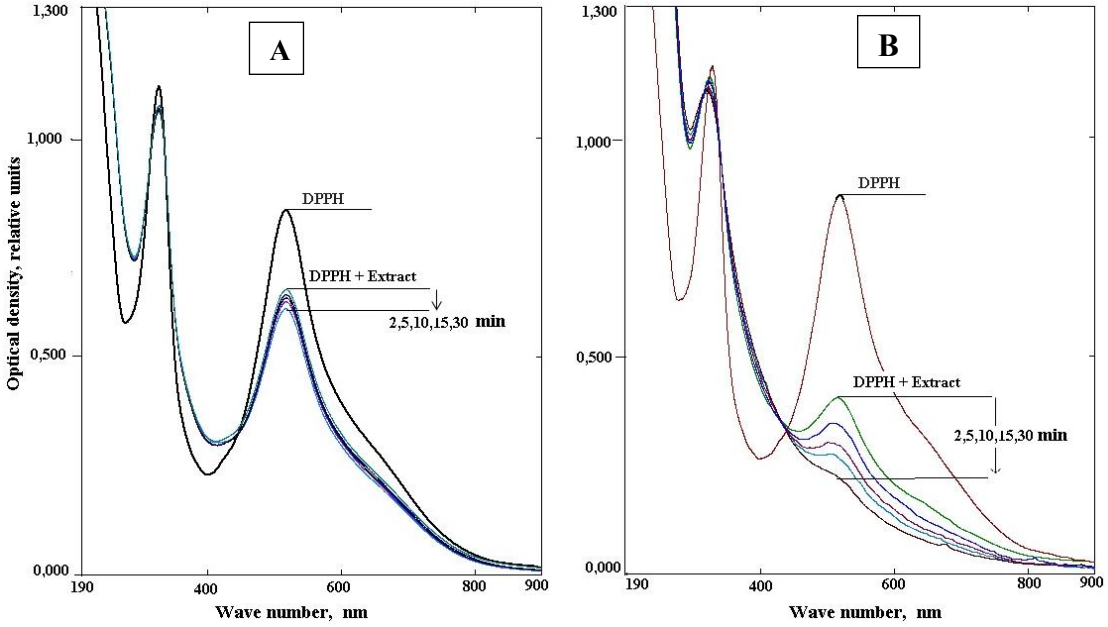

Figure 1. Electronic spectra of the radical DPPH and DPPH in a mixture with aqueous cep extracts; extraction time - 60 minutes A - From Montenegro; B - from Siberia.

A spectral study showed ARA presence of water extracts for both ceps from Montenegro and Siberia. But the values of ARA in % for these samples were quite different.

Figure 1 shows electronic spectra reflecting the interaction of the DPPH radical and compounds of a reducing nature in cep extracts from Montenegro (figure 1, A) and Siberia (figure 1, B). A decrease in the optical density for the peak of a pure solution of the DPPH radical at 517 nm after adding the extract to it indicates the antiradical activity of the studied sample.

The results of the study showed that Siberian ceps exhibit a greater importance of ARA: with an extraction time of 60 min and a 30-minute exposure, ARA for the aqueous extract of boletus from Siberia was 75% - compared with 27% for mushrooms from Montenegro the same selected conditions (table 1). For comparison, a solution of $5 \cdot 10^{-4}$ M ascorbic acid, used as a standard as a very strong antiradical agent, reduces the absorption of a DPPH solution by 90% for 30 minutes.

It was also found that for samples from Montenegro, an increase in the time of extraction of mushrooms from 30 to 90 min leads to a consistent decrease in ARA by 11% (calculation for the exposure time is 30 min), while for extracts of Siberian boletus, the ARA value passes through a maximum of 60 min of extraction - at the same 30 min exposure (table 1).

A decrease in ARA with an increase in the duration of extraction indicates that biologically active substances in the composition of ceps from Montenegro, providing antiradical activity, are most likely more thermolabile compounds in comparison with the biologically active substances of mushrooms from Siberia and are destroyed by prolonged temperature exposure.

Table 1. Comparative analysis of the antiradical activity of aquatic extracts of ceps from Montenegro and Siberia depending on the duration of extraction and exposure time (time from the moment of mixing the DPPH radical solution and the extract).

Exposure time, min	Antiradical activity, %					
	Water extracts of ceps from Montenegro			Water extracts of ceps from Siberia		
	The duration of the extraction, min					
	30	60	90	30	60	90
2	23.80±1.19	21.84±1.09	19.68±0.98	35.70±1.78	53.38±1.60	39.42±1.48
5	27.50±1.37	23.24±1.16	20.18±1.01	40.11±1.6	60.32±1.81	43.46±1.50
10	29.46±1.47	24.11±1.21	20.95±1.05	42.80±1.71	66.01±1.98	48.06±1.45
15	31.08±1.55	25.15±1.26	21.25±1.06	44.15±1.77	69.26±1.95	49.66±1.49
30	33.53±1.67	27.13±1.36	22.28±1.14	47.02±1.88	74.75±1.86	50.25±1.51

The results of the evaluation of antibacterial activity showed that, regardless of the climatic zone of the growth of mushrooms, their aqueous extracts obtained after 30 minutes of extraction show a significant bacteriostatic effect with respect to all bacterial strains Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus 209p and Acinetobacter baumannii. Longer extraction (more than 60 min) leads to extracts that have a significant stimulating effect on the growth of the same bacteria. This fact indicates that an increase in the extraction time of Boletus edulis biosubstrates for more than 60 min, regardless of their place of growth, leads to an increase in the concentration of protein-carbohydrate substances (glycoproteins, phosphoproteins) - an excellent nutrient medium for bacterial strains.

A number of studies associate the antiradical and antibacterial properties of basidiomycetes with the presence of volatile compounds in the composition of the fruit body of mushrooms, as well as a high content of phenolic substances [11-13]. For the species Boletus edulis, several types of volatile compounds were determined by chromatographic mass spectrometry - N-containing cyclic volatile substances (pyrazine and pyrrole and their derivatives), aliphatic alcohols, aldehydes, and ketones with 8 carbon atoms [14]. Obviously, the climatic zone of growth determines the accumulation and ratio of different classes of volatile components in the chemical composition of Boletus edulis.

Thus, this study showed that weather and climate conditions can affect the manifestation of the antiradical activity of the Boletus edulis culture, but almost no effect on the manifestation of antibacterial properties by the culture.

4. Conclusion

A comparative study of ARA of aquatic extracts of Boletus edulis species, growing in different climatic zones (the temperate continental Mediterranean (Montenegro) and sharply continental climate of eastern Siberia (Russia)) was carried out by UV and visible spectroscopy using a stable model organic 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). It was shown that under the same conditions of sample preparation and analysis, Siberian ceps have a high ARA value (the maximum value is 75% for mushrooms in Siberia against the maximum value of 33% for mushrooms from Montenegro, respectively). The results of the evaluation of antibacterial activity showed that, regardless of the climatic zone of fungal growth, their aqueous extracts obtained after 30 minutes of extraction exhibit a similar bacteriostatic effect in relation to the strains of bacteria Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus 209erannobacteri bacterbacteri bacterobacteri bacteri bacteracterbacteri bacteri bacterius 209 p and Acinet. Longer extraction (more than 60 min) leads to the stimulating effect of Boletus edulis extracts on the growth of the same bacteria.

It was shown that an increase in the extraction time of more than 60 min leads to a decrease in both the antiradical and antibacterial activity of ceps, regardless of the geography of the culture. The

growth of ceps in a certain climatic zone can affect the value of antiradical activity, but practically do not affect the manifestation of antibacterial properties by Boletus edulis basidiomycetes.

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