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Study of antimicrobial properties of *Hericium* fungal extracts

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Abstract. The study aimed to investigate the antimicrobial and antimycotic properties of 14 strains of fungi of the genus *Hericium* sp. To obtain antibiotic substances, the plants were grown superficially or deeply in a liquid nutrient medium, after which alcohol and ethyl acetate extraction was performed from the dried mycelium and culture liquid; for quantitative evaluation of antibiotic activity, plant extracts were tested by the method of discs and wells on solid nutrient medium, two test cultures of micro-mycetes, three test Gram-negative bacteria and three test Gram-positive bacteria; the minimum inhibitory concentration was determined by the method of dilutions by optical density.

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None of the strains of *Hericium sp*. showed antimycotic properties of plants. The antimicrobial effect of the culture liquid was generally stronger than that of the mycelial extracts. In the surface cultivation experiment, strong growth inhibition of test cultures was observed on days 7 and 14 of *Hericium* sp. cultivation. The growth inhibition zones of *Escherichia coli* were 17.5 cm for *H. erinaceus* 977 and 19.0 cm for *H. erinaceus* 2536; sterile zones on the plate from *Micrococcus luteus* were determined by diameters of 21.2 cm for the culture fluid of *H. erinaceus* 2530, 19.2 cm for the mycelial extract of *H. erinaceus* 977, and 17.8 cm for the biomass homogenate (BH) of *H. erinaceus* 2536. Long-term deep cultivation for 60 days contributed to the accumulation of antibacterial compounds effective against *Staphylococcus aureus*: the diameter of the sterile zone at 60 days was 30 cm for extracts from the biomass of *H. erinaceus* 2530, *H. cirrhatum* 2393, *H. coralloides* 2332. The strongest antimicrobial effect was recorded in the culture fluid of *H. coralloides* 2332 on the 21st day of growth (32.1 cm). The minimum inhibitory concentrations against *S. aureus* were determined to be in the range of 50-1,900 μg/ml for *H. erinaceus* 2530, *H. coralloides* 2332, and *H. cirrhatum* 2393. In general, a higher antibiotic activity was recorded against gram-positive microorganisms (*S. aureus*), and insignificant against gram-negative microorganisms (*Pseudomonas aeruginosa*). The data obtained can be used in further laboratory experiments on the identification and quantification of new antibiotics synthesised by fungi of the genus *Hericium* sp.

Keywords: antibiotics; antimycotics; surface cultivation; deep cultivation; minimum inhibitory concentration

INTRODUCTION

A relevant issue in medicine and pharmacy is the permanent acquisition of antibiotic resistance by pathogenic microorganisms. According to M. Huemer *et al.* (2020) and E.M. Darby *et al.* (2023), the pharmaceutical, organosynthetic and biotechnology industries are constantly working on the development of new and modification of existing antibacterial agents, but pathogen cells acquire resistance mutations and transmit them via mobile genetic elements. Therefore, the study of producers and biomolecules active against pathogenic bacteria and fungi will always be relevant. The genus *Hericium* belongs to the family *Hericiaceae*, order *Russulales*, class *Agaricomycetes*, division *Basidiomycota*. Under natural conditions, these saprophytic fungi usually grow on the bark of dead or decaying trees. The fruiting bodies are in the form of spines or bulbs hanging from a branched base or a monolithic plant tissue mass. They form amyloid basidiospores. Mycelial growth rates are closely positively correlated with the activity of synthesised hydrolytic enzymes amylases, cellulases, and proteases. The temperature optimum for growth is 21-24°C, and the substrate pH range is 5.8-6.2 (Kolesnyk, 2023).

Following Y.-F. Tan *et al.* (2024) representatives of the genus *Hericium* synthesise about 230 secondary metabolites, which belong to six classes of compounds according to their chemical structure, biological action and synthesis pathway. *In vivo* and *in vitro* experiments on various extracts from this plant have shown that the biomolecules it produces have anti-cancer, antimicrobial, anti-inflammatory and antioxidant effects, as well as neuroprotective and alpha-glucosidase inhibitors. About 60 of them have antibiotic activity. Given that in many regions this genus is endangered, it is grown artificially for industrial purposes (Fisher & Mobashery, 2020). According to H. Kawagishi (2021), the main biologically active substances isolated from mushrooms of the genus *Hericium* include gericenones, ericenones, exo- and endopolysaccharides, phenols, sterols and some other compounds. Among the newly discovered compounds with biological properties are the flavonoids apigenin, luteolin, acacetin, and diomethine, which have been shown to have anti-inflammatory and neuroprotective effects. The spectrum of secondary metabolites strongly depends on the conditions of cultivation or growth (Pikovskyi *et al.*, 2023). Toxicological studies by E. Kostanda *et al.* (2024) proved that the waste products of *Hericium* plants are safe and have no side effects when used therapeutically. The most studied plant species is *H. erinaceus*. At the same time, data on the synthesis of antibiotics by other representatives of the *Hericium* genus are scarce.

Literature sources describe the antimicrobial properties of numerous species of macromycetes, including the genus *Hericium* (Krychkovska *et al.*, 2025). R. Mishra *et al.* (2020) noted that alcohol/organic extracts from cells and culture fluid (CF) of *H. erinaceus* can inhibit the growth of many species of bacteria. J. Qi *et al.* (2024) noted the strongest bactericidal activity of *H. erinaceus* extracts against *Staphylococcus aureus*. Notably, the antimicrobial properties of *H. erinaceus* fruit bodies were studied more extensively than the antibiotic potential of the culture medium. J. Lazur *et al.* (2024) also recorded the antifungal activity of metabolites from *Hericium* sp. mycelial cultures against the growth of *Candida albicans* and *Cryptococcus neoformans*.

Researchers have been studying the properties of crested blackberry. For instance, N. Makhamrueang *et al.* (2021) confirmed that biologically active substance synthesised by H. erinaceus have an antitumour effect against gastric, liver and esophageal carcinoma; regenerate neuronal growth and counteract their apoptosis (nerve tissue regeneration stimulants NGSF); prevent the development of cognitive dysfunction

(beta-amyloid peptides); activate macrophages, the synthesis of gamma interferon and interleukins 2/10 (beta-D-glucans); have antioxidant characteristics (polysaccharides); promote wound healing; slow platelet aggregation (gericenone B). However, the antibiotic potential of *Hericium* sp. has not been actively studied in Kazakhstan.

Therefore, the objective of the experiment was to test the antibacterial and antimycotic properties of mycelium extracts and secretory compounds from the *Hericium* genus.

MATERIALS AND METHODS

Four species and 14 strains of fungi of the genus *Hericium* were used in this study. The specimens were provided by the IBK Culture Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine. The list of specimens that were used as test cultures to verify the antibiotic activity included *H. abietis* 2376; *H. cirrhatum* 2393; *H. coralloides* 1876, 2332, 2333; *H. erinaceus* 339, 963, 965, 977, 991, 992, 2239, 2530, 2536. Several strains of microfungi *Mucor globosus* N-O18, *Penicillium polonicum* URV-F 823, *Aspergillus niger* VURV-F 822 (Collection of microorganisms of crops of the Prague Research Institute); gram-negative aerobes and facultative anaerobes *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*; Gram-positive rods and cocci *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* (Collection of microorganisms of the Department of Microbial Synthesis Biotechnology, National University of Food Technologies).

For surface cultivation, *Hericium* sp. strains were cultivated in a liquid nutrient medium (50 ml per 250 ml flask) containing the following components (Sigma-Aldrich): vegetable peptone 3 g/l; yeast extract 2 g/l; magnesium sulfate heptahydrate 0.25 g/l; monosubstituted potassium phosphate 1 g/l; and dibasic potassium phosphate 1 g/l. Agar medium of the same composition with the addition of 20 g/l of bacterial agar (Sigma-Aldrich) was poured into Petri dishes. To obtain mycelium at the stage of active growth, *Hericium* sp. was inoculated onto the agar medium. Three mycelial discs cut from a cup (7-8 mm in diameter) were placed in each flask with the liquid medium and grown for 20 days at 25°C. Deep cultivations was carried out in a shaker incubator (120 rpm) in 500 ml flasks with 100 ml of similar nutrient medium at 24°C for 7-60 days, depending on the conditions of the individual experiment. The bacteria were cultured on Mueller-Hinton agar (MH) (Sigma-Aldrich) (yeast extract 0.0.5 g/l; vegetable peptone 17 g/l; starch 15 g/l; bacterial agar 20 g/l); *Penicillium* sp., *Mucor* sp. and *Aspergillus* sp. were grown on malt extract agar (MEA, Pronadisa) at 25°C for 5 days. To obtain the extracts, 20 mg of dried *Hericium* sp. mycelium was placed in 1 ml of ethanol (70%) and sonicated for 30 min at 45°C, cooled to 4°C, filtered through a paper filter and precipitated (20 min, 2,500 rpm). To concentrate the antibiotic substances secreted by the mycelium into the external environment, one volume of the culture liquid was transferred to a separating funnel, and two volumes of ethyl acetate were added, shaken (10 min) and incubated for 1 day at 4°C. Subsequently, the organic fraction was removed, and evaporated without residue on a rotary evaporator, and the dry residue was resuspended in 5 ml of 70% ethanol.

Antibiotic activity was recorded on days 7, 14, 21 and 60 of plant growth. For the antibacterial test, 10 µl of the extract (0.1 mg dry weight/disc) was applied to sterile paper discs (BioMerieux, 6 mm), dried (30 min, 40°C), placed on the surface of a dish with a pre-sown test culture and incubated at 30°C for 24 hours. After germination of the test strain, the linear dimensions of the sterile zones around the discs with antibiotics were compared. The antifungal test, as well as the repetition of the antibacterial test, was performed using the well method. A well (5 mm) was cut out in the centre of the test culture dish, into which 100 µl of ethyl acetate extract and test culture suspension were added. The suspension was prepared by resuspending micromycete spores or bacterial colonies in a sterile 0.9% sodium chloride solution. After germination, the diameters of the sterile zones were compared similarly to discs. Gentamicin sulphate solution (Kazakhstan, 40 mg/ml) served as K+. Ethanol (70%) was used as a negative control.

The minimal inhibitory concentration (MIC) of the obtained plant extracts was determined by the method of serial dilutions (3, 6, 12, 24, 48, 96, 192) in MH medium (with conversion of concentration to dry weight). The test tubes with MH and extract (ethanol = negative control) were inoculated with 50 µl of S. aureus test culture and grown for 24 hours at 37°C, and the optical density of the tube contents was determined at 600 nm (Specord Plus 250 spectrophotometer, Analytik Jena, Germany; blank nutrient medium). The minimum inhibitory concentration was determined in the test tube with the highest dilution, in which the absorbance of the test solution did not differ from that of the blank solution (sterile MH with extract). All experiments were repeated in quadruplicate. The data were processed using the statistical tools Microsoft Excel and SigmaPlot 9.0 (coefficient of variation, standard deviation, confidence interval). The difference was considered statistically significant with a confidence level of at least 0.95.

RESULTS

In the surface cultivation experiment, the antibiotic effect was observed in all the studied representatives of the plant under study. However, in most cultures, the initial culture liquid did not inhibit the growth of microorganisms. The extract of the medium of 5 species, concentrated tenfold, inhibited the growth of Escherichia coli and Staphylococcus aureus. *H. abietis* and *H. cirrhatum* were found to be new producers of antibiotic substances. Antimicrobial activity was absent in all strains

after the first week of cultivation. Therefore, the information on the antibacterial properties of the studied fungi, which were characterised by relatively high activity after two weeks of cultivation, is summarised in Table 1.

Table 1. *Antimicrobial effect of Hericium species (paper disc method) after 14 days of surface cultivation*

Species, strain			Diameter of the sterile zone (cm)			
			Bacillus subtilis	***Bacillus subtilis***	***Bacillus subtilis***	***Bacillus subtilis***
H. cirrhatum	2393	BH	0.96 ± 0.04	1.20 ± 0.06	1.00 ± 0.02	-
		CF	1.30 ± 0.12	-	-	1.00 ± 0.05
H. coralloides	2332	BH	0.88 ± 0.04	1.00 ± 0.05	1.20 ± 0.09	0.90 ± 0.01
		CF	1.14 ± 0.06	0.96 ± 0.02	1.20 ± 0.10	0.92 ± 0.08
H. erinaceus	963	BH	1.04 ± 0.02	1.16 ± 0.08	0.90 ± 0.01	0.96 ± 0.04
		CF	0	0.90 ± 0.02	-	1.00 ± 0.05
	977	BH	1.06 ± 0.04	0	-	1.14 ± 0.01
		CF	1.24 ± 0.10	0.90 ± 0.01	1.10 ± 0.05	0.90 ± 0.02
	991	BH	1.16 ± 0.05	1.00 ± 0.02	1.06 ± 0.04	0.94 ± 0.02
		CF	1.06 ± 0.04	0.90 ± 0.01	1.20 ± 0.06	0.90 ± 0.03
	992	BH	1.10 ± 0.05	1.00 ± 0.03	0.96 ± 0.04	1.14 ± 0.08
		CF	1.00 ± 0.03	0.90 ± 0.02	1.00 ± 0.05	0.94 ± 0.05
	2530	BH	1.10 ± 0.01	-	1.00 ± 0.04	-
		CF	1.00 ± 0.03	0	1.24 ± 0.04	0.84 ± 0.02
	2536	BH	0.83 ± 0.02	1.06 ± 0.08	-	0.88 ± 0.05
		CF	1.20 ± 0.06	1.08 ± 0.02	1.10 ± 0.03	0.90 ± 0.04

Note: *"-" – no research was conducted*
Source: *compiled by the authors*

Sterile zones on test culture plates were determined by a range of 8.3-15.0 mm. Staphylococcus growth was most strongly inhibited by concentrated extract of *H. erinaceus* 2239 medium (13.4 ± 1.3 mm); bacilli by extract of *H. cirrhatum* 2393 extract 13.2 ± 1.0 mm; Escherichia coli by *H. coralloides* 2333 medium concentrate 13.9 ± 0.8 mm; *M. luteus* with *H. erinaceus* 2530 liquid extract 12.5 ± 0.3 mm; *P. aeruginosa* with *H. erinaceus* biomass extract (977 and 992) 11.6 ± 0.2 mm and 11.6 ± 0.7 mm, respectively. In general, the antimicrobial potential of the culture fluid of the studied plant strains was significantly higher than the antimicrobial activity of the biomass. However, the activity of the liquid and mycelial extract did not differ for *H. coralloides* 2332 vs *E. coli/M. luteus/P. aeruginosa*; *H. erinaceus* 2536 vs *P. aeruginosa/E. coli*; *H. erinaceus* 992 vs *M. luteus*. Notably, *H. erinaceus* showed higher activity in the first test compared to other producer fungi. This selected *H. erinaceus* strain is promising for testing under other conditions. Even though most of the studied *Hericium* species showed antimicrobial activity, the antibiotic effect under these conditions was weak. In this regard, the experimental conditions were partially changed to ensure a higher concentration of culture fluid or homogenate in the experimental sample. For this purpose, a well diffusion test in agar was performed. The most informative data are presented in Table 2.

Table 2. *Antibacterial activity of Hericium erinaceus strains on the 7th and 14th day of cultivation (cylinder method)*

Zone of inhibition (mm) obtained using bacterial test cultures					
Strains					
H. erinaceus		***H. erinaceus***	***H. erinaceus***	***H. erinaceus***	***H. erinaceus***
		Growth time of H. erinaceus strains, days			
		7	14	7	14
Hericium erinaceus 977	BH	8.0 ± 0.1*	17.5 ± 0.4*	9.5 ± 0.2*	4.8 ± 0.2*
	CF	12.3 ± 0.2*	13.0 ± 0.2*	10.7 ± 0.7*	7.0 ± 0.5*
Hericium erinaceus 2530	BH	7.8 ± 0.0*	14.3 ± 0.3*	7.3 ± 0.2*	12.8 ± 0.2*
	CF	16.0 ± 0.4*	7.8 ± 0.2*	14.0 ± 0.0*	8.7 ± 0.4*
Hericium erinaceus 2536	BH	19.0 ± 0.5*	16.7 ± 0.3*	13.0 ± 0.3*	5.3 ± 0.4*
	CF	8.7 ± 0.2*	12.8 ± 0.1*	11.0 ± 0.5*	7.3 ± 0.6*

Note: *"-" – data not available; *p ⩾ 0.95*
Source: *compiled by the authors*

The activity of *H. erinaceus* relative to test cultures varied: the inhibition area ranged from 4.8 mm to 21.2 mm in the moon test. In addition, a dependence of the intensity of the antibacterial action on the cultivation time of the fungi was observed. The following trend emerged: with increasing cultivation time of the fungi, the antibacterial effect against the test cultures *E. coli* and *M. luteus* predominantly increased but decreased against *P. aeruginosa*. On the seventh day of growth, the homogenised mycelium of *H. erinaceus* 2536 showed the best activity against *E. coli*, while liquid 2530 exhibited antibacterial properties against *P. aeruginosa*. In addition, homogenised biomass and extract of strain 2530 significantly inhibited *M. luteus* after seven days of plant cultivation. The maximum diameters of sterile zones on plates with *M. luteus* were recorded in samples with culture medium extract 2350 and homogenised mycelium 977 after two weeks of cultivation. *M. luteus* was the least resistant to the activity of the tested strains of the genus *Hericium*, and P. aeruginosa was the least sensitive.

Strains *H. erinaceus* 977 and 2536 showed antimicrobial activity against several microorganisms: *B. subtilis/P. aeruginosa* and *B. subtilis/E. coli*, respectively. Furthermore, strain *H. coralloides* 2332 showed high (compared to other studied species) activity against the only staphylococcal microorganism among the test cultures of *S. aureus* bacteria. Therefore, this test culture was used in further experiments. The analysis of the data obtained determined that with the extension of the cultivation period of the studied strains, the antimicrobial activity against *E. coli* and *M. luteus* increases but decreases concerning *P. aeruginosa*. The strongest antimicrobial effect against *E. coli* was shown by liquid 2530 and homogenate of strain 2536 after two weeks of cultivation. At the same time, concerning *M. luteus*, homogenate 2530 and culture liquid 2536 showed the most effective effect after a week's cultivation. The strongest antibiotic effect was observed in *H. erinaceus* strains 2530 and 2332.

Under the conditions of deep cultivation, the antibiotic activity of fungal extracts of mycelial biomass and plant culture fluid was absent in all studied species of the genus *Hericium* against such test cultures as *Aspergillus niger*, *Penicillium polonicum*, *Mucor globosus*, *Pseudomonas aeruginosa* and *Escherichia coli* (except for extracts of *H. coralloides* 2332 against *E. coli* after three weeks of cultivation). Cultures of *Bacillus subtilis* were moderately sensitive to these concentrations of extracts, with growth retardation recorded on day 14, and was maximal in H. erinaceus 2530 (28 ± 0.1 mm). A weak effect against *Klebsiella pneumoniae* bacteria was recorded in fungi of IPC 2332 and IPC 2393 on the 14th-21st day of cultivation. The antimicrobial effect of the obtained extracts on the Gram-positive bacterium *S. aureus* was found to be a promising area among the studied fungi of the genus *Hericium*. The most active strains are presented in Table 3 and Figure 1. Since the plant medium extracts obtained after one week of deep cultivation of fungal biomass did not show activity against the studied microorganisms, the effect of biomass extracts was determined after two and three weeks of cultivation of strains in deep culture and 60 days of growth in stationary culture. Importantly, prolonged cultivation of biomass (60 days) increased the antibacterial properties of the studied fungal species.

Table 3. *Antimicrobial effect of ethyl acetate extracts of culture fluid and 70% alcohol extracts of biomass of the studied strains of the genus Hericium against Staphylococcus aureus*

Species, strains	Days of cultivation	Diameter of sterile area, mm
Ethyl acetate extract of culture fluid		
Hericium cirrhatum IBK 2393	7	0 ± 0
	14	30.0 ± 0.5
	21	28.3 ± 0.2
	K+	18.8 ± 0.1
	K-	0 ± 0
Water-alcohol extract of mycelial mass		
Hericium cirrhatum IBK 2393	14	18.5 ± 0.2
	21	21.0 ± 1.0
	60*	30.0 ± 0.5
	K+	18.8 ± 0.1
	K-	0 ± 0
Hericium coralloides IBK 2332	14	12.5 ± 0.4
	21	15.5 ± 0.5
	60*	30.0 ± 1.0
	K+	18.8 ± 0.1
	K-	0 ± 0
Hericium erinaceus IBK 2530	14	14.2 ± 0.3
	21	14.0 ± 0.0
	60*	30.0 ± 1.0

Table 3. Continued

Species, strains	Days of cultivation	Diameter of sterile area, mm
Hericium erinaceus IBK 2530	K+	18.8 ± 0.1
	K-	0 ± 0

Note: *K+ – solution of the antibiotic gentamicin sulfate, positive control. K-ethyl – acetate (for the culture liquid test) or 70% ethanol (for the fungal mass test, negative control; 60* – biomass grown under stationary conditions on day 60 of growth*
Source: *compiled by the authors*

Figure 1*. Antimicrobial effect of ethyl acetate extract of culture fluid of Hericium species vs Staphylococcus aureus*
Note: *control (negative) – ethyl acetate; 7, 14, 21 – days of cultivation*
Source: *compiled by the authors*

Diffusion tests revealed the presence of significant antibiotic activity of *H. coralloides* 2332, *H. cirrhatum* 2393, *and H. erinaceus* 2530 extracts against bacterial test cultures. For the further use of antimicrobial substances obtained from blackberry mycelium in the fight against bacterial infections, it was advisable to establish the minimum inhibitory concentrations of the studied samples. The minimum inhibitory concentration (MIC) is the limiting amount of extract that will inhibit optically detectable bacterial germination. The results for the most active strains (in terms of MIC against Staphylococcus aureus) are shown in Table 4. Since in the previous studies, representatives of the genus *Hericium* showed the maximum activity of mycelium and culture fluid extracts mainly against the Gram-positive bacterium *S. aureus*, this test culture was used to determine the MIC. The quantitative MIC test made it possible to state the fact that the antibacterial effect of one of the extracts of *H. cirrhatum* 2393 was close to that of the control with the extractant (70% ethanol). Therefore, it is possible to assume that this sample has moderate antibacterial properties. With subsequent chromatographic purifications and simultaneous concentration of the ethanol extract of *H. erinaceus* 2530, the antimicrobial effect of the extracts will increase. The MIC for biomass is within 130 µg/ml, and for culture fluid 50 µg/ml against *S. aureus*.

Table 4*. Results of the antibacterial effect of the studied Hericium extracts against Staphylococcus aureus (two weeks of cultivation of the producer)*

Species, strain	Extraction object	The maximum dilution for which inhibition is set	MIC^, µg/ml
H. cirrhatum 2393	Mycelium	6	1.900
H. coralloides 2332	Mycelium	96	130
H. erinaceus 2530	Mycelium	96	130
	Cultural fluid	96	50

Note: *^ - concentration calculated according to the dry matter content of the extracts*
Source: *compiled by the authors*

When processing the data on the antibacterial activity of the ethanol extract of *H. coralloides* and *H. erinaceus* biomass using the microdilution method, inhibition of *S. aureus* growth was detected at 96 dilutions, which corresponds to an extract concentration of 130 µg/ml. Importantly, the antimicrobial activity of fungi can be affected by the biological characteristics of the fungal species and strain, growing conditions, method of extracts, method of evaluation and interpretation of results, and other factors. Therefore, changes in the growth conditions of a given plant genus, for example, cultivation on a substrate or cultivation in a liquid nutrient medium (surface or submerged), and changes in the composition of the medium can alter the number of secondary metabolites synthesised. The chemical composition of extracts extracted with different solvents may differ and cause changes in their antimicrobial properties. In addition, an important

factor is the extraction temperature, which may increase the extraction efficiency but may lead to the degradation of thermolabile antimicrobial substances. Even though the obtained results of inhibition of microbial growth are less than those reported by W.B. Suleiman *et al.* (2022) and M. Sevindik *et al.* (2024), the antimicrobial activity of the studied fungi can be increased by selecting the most optimal cultivation parameters of the strains, as well as by applying various methods of treatment of the culture fluid and mycelium, as a result of which the concentration of biologically active substances in the extracts will increase and, consequently, the antimicrobial properties of the strains of the genus *Hericium* will be improved.

Comparing the average diameter of the sterile zone at the wells or around the discs, based on the test culture, under the influence of extracts of different strains of the genus *Hericium* grown under different conditions, for *S. aureus* it was 21.4 mm; for *M. luteus* 21.2 mm; for *E. coli* 19 mm; for *B. subtilis* 11.1 mm; for *P. aeruginosa* 9.5 mm. Selecting the most optimal cultivation conditions for each strain (the experiment in which growth inhibition was maximal) and expressing the data in percentage terms, the studied fungi under conditions close to the optimum inhibit the growth of *S. aureus* (100%), quite actively inhibit *B. subtilis* (87%), worse for *M. luteus* and *E. coli* (66% and 59%, respectively) and weakest in *P. aeruginosa* (Fig. 2a, b).

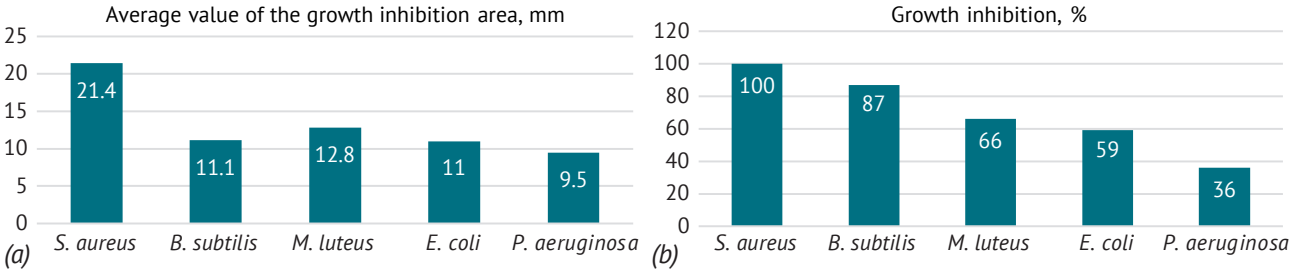

***Figure 2**. Comparative growth inhibition of test cultures by different strains of the genus Hericium under different experimental conditions*

***Note:** (a) the mean value (mm) of the sterile area on the plate with each microorganism tested due to the diffusion of Hericium sp.; b) the relative growth inhibition of test cultures by Hericium sp. extracts, assuming 100% inhibition of S. aureus*

***Source:** compiled by the authors*

According to Figure 2, *H. erinaceus* has a stronger antibiotic effect against Gram-positive bacteria compared to Gram-negative bacteria. The maximum inhibition of *S. aureus* was observed under the influence of ethyl acetate extract of *H. coralloides* 2332 culture fluid after three weeks of cultivation of the producer (deep cultivation). The most effective against *B. subtilis* and *M. luteus* was the medium after incubation of *H. erinaceus* 2530 in it for 14 days of deep (for bacillus) and surface cultivation (for micrococcus). The growth of *E. coli* was most strongly inhibited by the homogenate of *H. erinaceus* 2536 biomass, which was surface cultured for 7 days. *P. aeruginosa* was the most resistant. The sterile zone of the largest diameter concerning this bacillus was produced by extracts from the mycelium of *H. erinaceus* 977 and 2530, obtained after two weeks of surface fermentation of the producers.

Thus, a pronounced antimicrobial effect was observed for both the biomass homogenate and the culture fluid of fungi of the genus *Hericium* on representatives of *Firmacutes* and *Gracilicutes*. *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, indicate the significant potential of using biomass and exometabolites of fungi of this genus for the development of new safe biopreparations for agricultural and pharmaceutical requirements. The advantages of the research are the food and environmental safety, and the absence of side effects observed in most modern synthetic drugs.

DISCUSSION

The study proved the antimicrobial effect of *Hericium* metabolites. The level of synthesis of antibiotic compounds correlated with the duration and conditions of cultivation. However, mycelial extracts did not affect fungi of the genera *Mucor*, *Penicillium*, *and Aspergillus*. The culture liquid and extracts of dried mycelium showed various antimicrobial properties. After surface cultivation (in 50 ml of medium), the largest zones of inhibition were found with samples of culture fluid of *H. cirrhatum* 2393 and *H. erinaceus* 977 and test culture of *B. subtilis*, as well as *H. erinaceus* 2530 and test culture of *M. luteus*. Under these growing conditions, the biomass homogenate inhibited bacterial growth less than the culture liquid. The cylinder method revealed a significant inhibition of *E. coli* and *M. luteus* growth by secretory metabolites of *H. erinaceus* 2530, *H. erinaceus* 2536; cell-containing compounds of *H. erinaceus* 977 on 7 and 14 days of incubation. In the experiment on deep cultivation (in 100 ml of medium), the maximum antibacterial effect was found against *S. aureus* in the extracts of culture fluid of *H. cirrhatum* 2393,

H. coralloides 2332 and *H. erinaceus* 2530. negative *E. coli* and *P. aeruginosa*.

The cell wall of the *Firmacutes* group is composed of peptidoglycan (50-90% of the cell's dry weight). Proteins, teichoic and teichuronic acids are associated with murein. The latter is absent in bacteria of the *Gracilicutes* group. The wall of Gram-negative microorganisms is more complex. Although the content of peptidoglycan reaches no more than 10%, it is located between the cytoplasmic membrane and the outer wall. The outer membrane, formed by phospholipids, lipoproteins, proteins and lipopolysaccharides, is the first barrier against the environment (Skliar *et al.*, 2020). According to J.F. Fisher and S. Mobashery (2020) and S. Chen *et al.* (2023), it limits the penetration of detergents, proteins, antibiotics, and fatty acids into the cell. Perhaps, due to this resistance, antibacterial compounds synthesised by *Hericium* sp. fungi do not penetrate *E. coli*, *K. pneumoniae* and *P. aeruginosa* cells, so the antibiotic effect is more effective against *S. aureus*, *B. subtilis* and *M. luteus*. However, S. Darmasiwi *et al.* (2022) obtained somewhat contradictory data: phenolic secondary metabolites synthesised by *H. erinaceus* effectively inhibited the growth of Gram-negative bacteria (*Proteus mirabilis*, *Salmonella typhimurium*) and the formation of biofilms. The concentration of antimicrobial protocatechuic and p-coumaric acids in ethanol extracts was 1.7 μg/ml.

The minimum inhibitory concentrations against Staphylococcus aureus for extracts from mycelium and culture fluid also differed significantly: 1.9 mg/ml (biomass of *H. cirrhatum* 2393), 130 μg/ml (biomass of *H. corraloides* 2332 / *H. erinaceus* 2530), 50 μg/ml (culture medium of *H. erinaceus* 2530, the most active extract). The tested producers of antibiotic compounds were obtained from a collection of microorganisms. Similar data are published by S. Ghosh *et al.* (2021) anf F. Brandalise *et al.* (2023) for the natural strain of *H. erinaceus* CUHAM 713, isolated from the soil litter of a temperate subalpine forest (Munsari, India). The species was identified by morphological characters by macroscopic and microscopic screening and confirmed by sequences of internal transcribed non-coding spacers (ITS). The mycelium was dried, and ethanol extraction was performed. The obtained extracts were characterised by MIC values of 2.75 μg/ml for *S. aureus*, 1.8 μg/ml for *E. coli* and 1.65 μg/ml for *B. subtilis*. Therefore, the new representative isolated from the soil was ten times more active against pathogens and opportunistic pathogens than the producers used in the present study.

The identification and quantification of antimicrobial compounds require mass spectrometry analysis and high-performance chromatography. Therefore, it is only possible to assume that the antimicrobial substances obtained from mycelium and culture fluid belong to a particular class. W.C. Sum *et al.* (2023) and A. Gravina *et al.* (2023) studied the antimicrobial properties of *H. coralloides* (Biotech, Finland). The study identified meroterpenoid compounds (named corallocins D and E), which were purified from ethyl acetate extracts of dried fruiting bodies. *In vitro* experiments showed that corallocins, in addition to their cytotoxic effect on NeLa cell culture, have antimicrobial effects against *B. subtilis* (MIC = 67 μg/ml) and are antimycotics against *Mucor hiemalis* (MIC = 67 μg/ml), but do not affect *S. aureus* and *E. coli*. These metabolites are strain-specific since *H. coralloides* 1876, 2332 and 2333 studied in the experiment did not act on the representative of the genus *Mucor* and were effective against *E. coli* and *S. aureus*. The antimycotic effect of representatives of the genus *Hericium* is not limited to one species. X. Song *et al.* (2020) and S. Khatib *et al.* (2025) visolated several new secondary metabolites from the fruiting bodies of *H. americanum*. Four of them belong to the class of chlorinated orcinol derivatives. 2-chloro-dimethoxymethylbenzene was found to be an antifungal agent against yeasts of the genera *Candida* and *Cryptococcus* (*C. neoformans*, *C. albicans*) with MIC values of 31-63 μg/ml. A concentration of about 8 μg/ml destroyed the synthesis of biofilms by these test cultures. The antimycotic was obtained by methanolic/ethyl acetate/acetone extraction from dried mycelium and culture fluid. At MICs above 0.5 mg/ml, the antibiotic inhibited *E. coli* and *K. pneumoniae*. The supernatant and mycelial extract obtained after cultivation on various organic substrates (soya, rice, malt) at concentrations of about 1 mg/ml inhibited Staphylococcus aureus. It is possible that by changing the extraction solvents and the culture medium, it will be possible to isolate the antifungal compounds mentioned above from the present strains (if they are not strain-specific) (Shuvar *et al.*, 2022).

The waste products of *H. erinaceus* can not only inhibit but also stimulate the growth of microorganisms. This phenomenon has a therapeutic effect. In particular, L. Wu *et al.* (2024) demonstrated that polysaccharides synthesised by blackberries provide indirect hepatoprotection in a laboratory animal model. These compounds were shown to promote the active reproduction of *Lactobacillus* sp. in the gastrointestinal tract. Under the influence of lactobacilli growth, less hepatotoxic lipopolysaccharides are transferred from the intestine to the liver, the LPS/TLR4/MyD88/NF-κB metabolic cascade is blocked in hepatocytes, the pool of inflammatory mediators is reduced, and the rate of apoptosis is slowed down. The experiment on faecal microbiota transplantation confirmed the hepatoprotective effect of *Lactobacillus* sp. Studies conducted by W. Cui *et al.* (2023) identified the therapeutic effect of polysaccharides secreted by blackberries in the prevention of type 2 diabetes mellitus. Low molecular weight β-D-glucose phosphate polymers, the HEP-1, were isolated from fruit bodies. In addition to regulating the biochemical mechanisms of glucose accumulation in hepatocytes, these sugars promoted the growth of intestinal microflora (Zubtsova *et al.*, 2019).

The bacteria synthesised "hepatic metabolites" that regulate glucose balance. If the stimulation of lactobacillus growth is proven in clinical trials, *H. erinaceus* could be used in medicine to indirectly treat liver failure and diabetes. Immunoregulatory polysaccharides of *H. erinaceus* increased the titre of bacteria of the families *Akkermansiaceae* and *Lachnospiraceae* and simultaneously inhibited the growth of *Bacteroidaceae* and *Rikenelaceae* (Turk *et al.,* 2021). The positive effect of the intestinal microflora is also supported by the pool of its metabolites of branched-chain fatty acids, butyrate and propionate, additionally synthesised by the crested blackberry.

Beta-glucans from *H. erinaceus* can have a wider range of practical applications. They exhibit antibacterial properties. By adjusting the solvent composition and temperature, the rate of self-assembly of this biomolecule can be influenced. In combination with a synthesised photosensitiser made of tannic acid and ferric cations, the above polysaccharides form a hydrogel. It is characterised by a strong antibacterial effect, the ability to generate a local temperature increase in infrared light, suppress the accumulation of inflammatory initiators (tumour necrosis factor-alpha, interleukin-6), activate angiogenesis markers, promote vascularisation and collagen accumulation in injuries. Such a hydrogel with *H. erinaceus* metabolites is promising to be used for healing infected wounds (Szućko-Kociuba *et al.,* 2023). Given that *Hericium* sp. synthesises many classes of secondary metabolites with different biological properties, it is difficult to predict the structure of antibiotics contained in cells and secreted by *H. erinaceus*, *H. coralloides*, and *H. cirrhatum*. Therefore, the identification and quantification of these biomolecules by high-performance chromatography and mass spectrometry is a promising research area.

CONCLUSIONS

The antibacterial and antimycotic properties of extracts from fungi of the genus *Hericium* sp. were studied. No antifungal activity was found against micromycetes of the genera *Mucor*, *Penicillium*, *and Aspergillus*. However, all the plant strains used in the study demonstrated inhibition of bacterial growth (*H. cirrhatum*, *H. coralloides*, *H. abietis*, *H. erinaceus*). In general, ethyl acetate extracts of the culture fluid were characterised by a stronger antibiotic effect than ethanol extracts from dried mycelium. The synthesis of secondary antimicrobial metabolites was directly proportional to the duration of cultivation of the producers. In the well hole test method, bioactive molecules diffused into the thickness more actively than in the disc test. After surface cultivation of plants, the most effective sample against *E. coli* was the biomass homogenate of *H. erinaceus* 2536 (diameter of the inhibition zone 19 mm); against *P. aeruginosa*, the culture fluid of *H. erinaceus* 2530 (14 mm); against *M. luteus*, the mycelium extract of *H. erinaceus* 977 (19.2 mm); against *B. subtilis*, the extract of the culture medium of *H. cirrhatum* 2393 (13 mm).

All strains showed a stronger growth inhibition against Firmacutes microorganisms compared to *Gracilicutes*: the growth of *S. aureus* was most actively inhibited (21.4 mm), and *M. luteus* and *B. subtilis* were less active (12.8 mm and 11.1 mm, respectively). The most resistant was *P. aeruginosa* (9.5 mm). After in-depth cultivation, metabolites were extracted and tests with *S. aureus* were performed. The zones of inhibition were determined with a diameter of 30 cm after 14-60 days of cultivation for samples from biomass and the growth medium of *H. cirrhatum* 2393, *H. coralloides* 2332, and *H. erinaceus* 2530. The minimum inhibitory concentration (MIC) for *S. aureus* was 50-1900 μg/ml, depending on the producer and the material used to produce the antibiotic. The lowest MIC value was obtained for the culture fluid, indicating high antibiotic activity. Therefore, the identification and quantification of antibiotic substances by more precise methods, such as high-performance chromatography and mass spectrometry, is a prospect for further research.

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CONFLICT OF INTEREST

None.

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Вивчення антимікробних властивостей екстрактів грибів *Hericium*

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Анотація. Метою роботи було дослідження протимікробних та антимікотичних властивостей 14 штамів грибів роду *Hericium* sp. Для отримання антибіотичних речовин рослини вирощували поверхнево або глибинно в рідкому поживному середовищі, після чого проводили спиртову та етилацетатну екстракцію з висушеного міцелію та культуральної рідини; для кількісної оцінки антибіотичної активності екстракти рослин перевіряли методом дисків та лунок на твердому поживному середовищі, двох тест-культур мікроміцетів, трьох тестових грамнегативних бактеріях та трьох тестових грампозитивних бактеріях; мінімальну інгібуючу концентрацію встановлювали методом розведення за оптичною ділянкою, що дозволяє виявити антимікотичні властивості гриба. Жоден зі штамів *Hericium* sp. не виявив антимікотичних властивостей рослин. Антимікробна дія культуральної рідини загалом виявилася сильнішою, ніж екстрактів із міцелію. В експерименті з поверхневого культивування сильне пригнічення росту тест-культур спостерігали на 7 і 14 добу вирощування *Hericium* sp. Зони пригнічення росту *Escherichia coli* становили 17,5 см для *H. erinaceus* 977 та 19,0 см для *H. erinaceus* 2536; стерильні зони на чашці з *Micrococcus luteus* визначалися діаметрами 21,2 см для культуральної рідини *H.erinaceus* 2530, 19,2 см для міцеліального екстракту *H. erinaceus* 977 та 17,8 см для гомогенату біомаси (ГБ) *H. erinaceus* 2536. Тривале глибинне культивування протягом 60 діб сприяло накопиченню антибактеріальних сполук, ефективних проти *Staphylococcus aureus*: діаметр стерильної зони на 60 добу становив 30 см для екстрактів із біомаси *H. erinaceus* 2530, *H. cirrhatum* 2393, *H. coralloides* 2332. Найсильнішу антимікробну дію зафіксовано в культуральної рідини *H. coralloides* 2332 на 21 добу росту (32,1 см). Мінімальні інгібуючі концентрації щодо *S. aureus* детермінувалися діапазоном 50-1,900 мкг/мл для *H. erinaceus* 2530, *H. coralloides* 2332, *H. cirrhatum* 2393. Загалом, вища антибіотична активність зафіксована проти грампозитивних мікроорганізмів (*S. aureus*), незначна – проти грамнегативних (*Pseudomonas aeruginosa*). Отримані дані можуть використовуватися в подальших лабораторних напрацюваннях з ідентифікації та кількісного визначення нових антибіотиків, що синтезуються грибами роду *Hericium* sp.

Ключові слова: антибіотики; антимікотики; поверхневе культивування; глибинне культивування; мінімальна інгібуюча концентрація