ANTIMICROBIAL PROFILE OF EXTRACTS FROM THE FUNGUS SPECIES *LEPISTA PERSONATA*

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ABSTRACT

Nowadays, mushrooms are widely studied due to the various bioactive substances they produce. They define their antibacterial, antifungal, antioxidant, antitumor, cytostatic and anti-inflammatory properties which are beneficial for human health. This study aimed to determine the antimicrobial activity of different (hot and cold ethanol/ dichloromethane/ hexane) extracts from the fungus species Lepista personata against two test microorganisms: Escherichia coli NBIMCC K12 407 and Bacillus subtilis NBIMCC 3562. The antimicrobial assays were performed applying the classical disc diffusion method. The sensitivity of test microorganisms was determined according to the zone of inhibition which appeared around the discs after 24 h of incubation at 30°C - 37°C. All extracts were dissolved in 200 mM dimethyl sulfoxide, which was used as a control sample. Results showed that E. coli NBIMCC K12 407 is susceptible to dimethyl sulfoxide whereas B. subtilis NBIMCC 3562 showed no growth inhibition. All mushroom extracts also showed no inhibitory activity against B. subtilis NBIMCC 3562. At the same time, only the dichloromethane extract obtained after hot extraction inhibited the growth of E. coli NBIMCC K12 407.

Keywords: Lepista personata extracts, antimicrobial activity, Escherichia coli, Bacillus subtilis.

INTRODUCTION

Mushrooms were widely used in the traditional medicine of many countries around the world and became great resources for modern clinical and pharmacological research. Although the biological potential of many plants is already revealed, still in nature there are many other sources of substances with potential medicinal applications that are not discovered or well-studied. The mushroom kingdom is one of these hidden reserves of the environment which still has not been fully investigated [1].

Recently, mushrooms are investigated as possible source of substances with antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, cholesterol-lowering, antiviral, antibacterial, antiparasitic, antifungal, detoxicative, hepatoprotective, antidiabetic, antiobesity, neuroprotective, neuroregenerative, etc., activity. Also, substances derived from medicinal mushrooms can be used as painkillers and analgesics [2, 3]. Many novel biologically active compounds have been reported as a result of research on medicinal mushrooms [4].

Lepista personata (Synonyms*: Lepista saeva, Clitocybe saeva, Tricholoma amethystinum*, *Tricholoma personatum*, *Tricholoma personatum f. minor*, *Tricholoma personatum var. anserina*, *Tricholoma personatum var. saevum*, *Tricholoma saevum*, *Rhodopaxillus personatus*, *Rhodopaxillus saevus*, *Agaricus anserinus*, *Agaricus personatus β saevus)* is an edible mushroom,

saprotrophic, growing in gardens, parks, roadsides and deciduous woods [5].

Mercan and co-authors tested the antimicrobial activity of *Lepista nuda* extract *in vitro* by using the agar-well diffusion method with model microorganisms. It was observed that *Lepista nuda* extract did not demonstrate antibacterial activity against *Pseudomonas aeruginosa, Morganella morganii, Klebsiella pneumoniae, Proteus vulgaris* and *Bacillus subtilis*. On the other hand, the *L. nuda* extract showed antibacterial activity against *Micrococcus luteus, Micrococcus flavus, Staphylococcus aureus* ATCC 12598 and ATCC 25923, *Bacillus cereus* RSKK 863, *Yersinia enterocolitica* RSKK 1501, *Salmonella enteritidis, Escherichia coli*. The highest activity of *Lepista nuda* extract was observed against *Micrococcus luteus* and *Micrococcus flavus.* [6]. The extracts obtained from wild mushrooms *Fistulina hepatica, Lepista nuda, Leucopaxillus giganteus, Mycena rosea*, and *Russula delica* were originally reported as antimicrobials against in vitro biofilm formation by multiresistant clinical isolates of Gram-negative bacteria (*Acinetobacter baumannii, E. coli, Proteus mirabilis,* and *P. aeruginosa*) to solve multidrug resistance problems in public healthcare [7].

The sporophores of *L. nuda* contained high levels of vitamin B. Dulger at al. reported that the 60 % methanolic extract of *Lepista nuda* exhibited antimicrobial activity. The obtained results by using disc diffusion methods showed that four of the tested bacteria (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 11230, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538) were sensitive to the methanolic extract. *Klebsiella pneumoniae* and *Salmonella typhimurium* and the fungus *Candida albicans* and *Rhodotorula rubra* were resistant to the action of the extract [8]. Mushroom extracts could be an alternative as antimicrobials against pathogenic microorganisms resistant to conventional treatments. The microdilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). MIC results showed that *Lepista nuda* extract exhibited a bactericide effect upon *Pasteurella multocida* at 5 mg mL^{-1} and inhibited *Proteus mirabilis* at 20 mg mL^{-1} [9].

The aim of this work is to investigate the antimicrobial profile of different extracts from the fungus species *Lepista personata*.

EXPERIMENTAL

Materials

Extracts obtaining

10 g of crude mushroom biomass was placed in a round flask and 50 mL of solvent was added. For cold extracts biomass was stirred for 6 h on a magnetic stirrer. Further, biomass was removed by filtration and the solvent was evaporated under a vacuum to dry solid. For hot extracts biomass with solvent was refluxed for 4 h. Further, the reaction mixture was chilled to room temperature, biomass was removed by filtration and the solvent was evaporated under vacuum to dry solid.

Microorganisms, Media and Culture Conditions

The bacterial strains *B. subtilis* NBIMCC 3562 and *E. coli* NBIMCC K12 407 were selected as test microorganisms and were obtained from the National bank for Industrial microorganisms and cell cultures (NBIMCC, Bulgaria). Exponential cultures (OD_{610} nm = 1.9) of both strains were obtained in Nutrient broth (NB)/Luria-Bertani (LB) broth, after cultivation in a shaker-incubator ES-20/60 (Biosan, Riga, Latvia, 120 rpm) at 30/37°C for 24 h, respectively.

In vitro antimicrobial activity assay

The antimicrobial assays were performed in triplicate by applying the classical disc diffusion method [10]. Commercially available discs were impregnated with 6 µL of each (hot and cold ethanol/dichloromethane/hexane) extract and placed on the surface of agar plates previously inoculated with the bacterial cultures of the two test strains. Their sensitivity was determined according to the zone of inhibition which appeared around the discs after 24 h of incubation at 30-37°C. All extracts were dissolved in 200 mM dimethyl sulfoxide (DMSO), which was used as a control sample. The *in vitro* method, applied in the analysis, follows the scheme of work in Fig. 1.

RESULTS AND DISCUSSION

Since the 1970s microbial resistance has become a serious threat to humanity all over the world. Inappropriate and irrational use of antimicrobial drugs provided favorable conditions for resistant microorganisms to emerge, spread and persist which

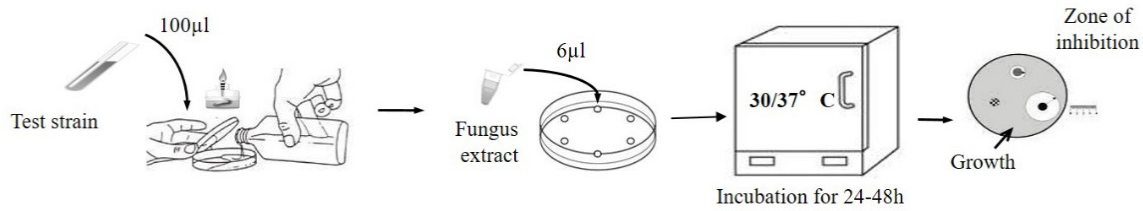

Fig. 1. Scheme of work of the *in vitro* method used for determination of the antimicrobial activity of the tested fungus extracts.

in return led to increased morbidity and mortality and enhanced stay in hospitals and cost of treatments [11, 12]. This led the pharmaceutical industry to focus the efforts on the development of new antimicrobial agents for the treatment of resistant infections.Natural products from fungi are considered an important source of novel antimicrobial compounds as they are long known for their antibacterial, antifungal, antioxidant, antitumor, cytostatic and anti-inflammatory activities [13]. For this purpose, the antimicrobial effect of several extracts from the fungus species *Lepista personata* was investigated.

Extracts were obtained both with hot or cold treatment with ethanol, dichloromethane or hexane and further were dissolved in 200 mM DMSO."Then sterile discs were impregnated with 6 µL of each extract and placed on the surface of agar plates previously inoculated with the corresponding test culture and incubated for 24 h at 30°C - 37°C. In the present study, the classical disc diffusion method was used to determine the antimicrobial activity of the fungus extracts against Gram-positive strain *B. subtilis* NBIMCC 3562 and Gram-negative strain *E. coli* NBIMCC K12 407. The obtained results are presented in Table 1 and Fig. 2.

Results showed that *E. coli* NBIMCC K12 407 is susceptible to 200 mM DMSO whereas *B. subtilis* NBIMCC 3562 showed no growth inhibition (Fig. 2). Our results are in disagreement with the general idea that Gram-negative bacteria are slightly more tolerant to hydrophobic chemicals than Gram-positive bacteria [14]. According to Dyrda et al., much higher concentrations (> 4.8 %) of solvents are needed to inhibit the growth of species like *Escherichia coli.* This is due to the natural tolerance mechanisms that some of the bacteria have developed [14].

All tested mushroom extracts (ethanol, hexane and dichloromethane) also showed no inhibitory activity against *B. subtilis* NBIMCC 3562. These results are in agreement with the results reported by Mercan et al. in 2006 [6]. They also demonstrated the lack of antibacterial activity of an ethanol extract of *Lepista nuda* against *B. subtilis* ATCC 6633 [8].

At the same time, only the dichloromethane extract, obtained after hot extraction, inhibited slightly the growth of *E. coli* NBIMCC K12 407. All the other

Table 1. Antimicrobial activity of fungus extracts against *E. coli* NBIMCC K12 407 and *B. subtilis* NBIMCC 3562.

Samples	Zones of inhibition [mm] against	
	E. coli NBIMCC K12 407	*B. subtilis* NBIMCC 3562
Control (200 mM DMSO)	10 ± 0.20	nd
Hot ethanol extract	9 ± 0.18	nd
Cold ethanol extract	10 ± 0.20	nd
Hot hexane extract	8 ± 0.17	nd
Cold hexane extract	8 ± 0.17	nd
Hot dichloromethane extract	10.5 ± 0.20	nd
Cold dichloromethane extract	9.5 ± 0.19	nd

**Descriptive statistics expressed as mean ± standard deviation from three replicates; **nd - not detected*

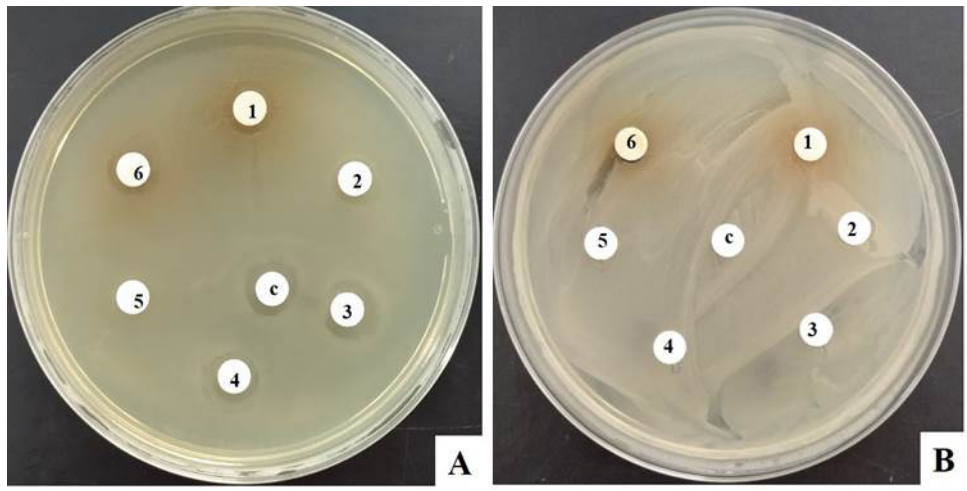

Fig. 2. Antibacterial activity measured by the agar diffusion method against *E. coli* NBIMCC K12 407 (A) and *B. subtilis* NBIMCC 3562 (B) used as test microorganisms for fungus extracts: (A) - C - control, 1 - hot ethanol extract, 2 - cold hexane extract, 3 - hot CH_2Cl_2 extract, 4 - cold CH_2Cl_2 extract, 5 - hot hexane extract, 6 - cold ethanol extract; (B) - C - control, 1 - cold ethanol extract, 2 - cold hexane extract, 3 - cold CH_2Cl_2 extract, 4 - hot CH_2Cl_2 extract, 5 - hot hexane extract, 6 - hot ethanol extract.

mushroom extracts showed smaller inhibition zones when compared with the control sample (containing 6 µL 200 mM DMSO) (Table 1). This means that dichloromethane has a higher ability to extract the secondary metabolites responsible for these activities than the other two solvents. The dichloromethane extract of the mushroom species *Termitomyces striatus* was also found to inhibit the growth of *E. coli* at concentrations of 6.25, 12.5, 25, 50, 100 and 200 mg mL^{-1} [15].

CONCLUSIONS

The dissolution of the mushroom extracts in DMSO defines to a greater extent their antimicrobial activity towards *E. coli* NBIMCC K12 407. Among extracts, only the dichloromethane extract, obtained after hot extraction, showed slightly higher inhibitory activity against *E. coli* NBIMCC K12 407. However, *B. subtilis* NBIMCC 3562 shows resistance to DMSO as well as to all the mushroom extracts.

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