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Antioxidant and antimicrobial potentials of mycelial extracts of *Hohenbuehelia myxotricha* grown in different liquid culture media

TETIANA KRUPODOROVA [1], VICTOR BARSHTEYN [1]*, MUSTAFA SEVINDIK [2]

[1] Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

[2] Department of Food Processing, Bahçe Vocational School, Osmaniye Korkut Ata University, Osmaniye, Turkey

Abstract

In addition to their nutritional properties, mushrooms have emerged as a health supplement because of their medicinal potential. Many studies have shown that mushrooms exhibit important biological activities. Here, the antioxidant and antimicrobial activities of *Hohenbuehelia myxotricha* (Lév.) Singer mycelia cultivated on Sabouraud dextrose broth (SDB) and glucose peptone yeast (GPY) medium were studied. The total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) of ethanolic extracts of mycelia were measured using Rel Assay kits. The antioxidant and oxidant potentials of *H. myxotricha* mycelial extracts were determined for the first time in the present study. The highest TAS, TOS, and OSI values of *H. myxotricha* were 5.416 ± 0.150 mmol/l, 1.320 ± 0.156 μmol/l, and 0.024 ± 0.003, respectively. Ethanolic mycelial extracts of *H. myxotricha* showed antimicrobial activities at concentrations from 25 to 200 μg/ml against all the studied bacteria (*Acinetobacter baumannii, Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Pseudomonas aeruginosa*) and fungi (*Candida albicans*, *C. glabrata,* and *Issatchenkia orientalis*) tested by the agar dilution method. The antifungal activity of the extract was more significant than its antibacterial activity. The antioxidant, oxidant, and antimicrobial potentials of *H. myxotricha* mycelia varied depending on the culture media used. GPY medium was more suitable for the synthesis of antibiotic compounds against *E. coli*, while SDB medium was more appropriate for producing metabolites with antioxidant and antifungal properties. Based on the results, ethanolic extract of *H. myxotricha* mycelia showed a significant pharmacological potential and could be used as a natural antioxidative and antimicrobial source for health benefit.

Key words: antioxidant, oxidant, antimicrobial, ethanolic extracts, mycelium, *Hohenbuehelia myxotricha*

Introduction

Reactive oxygen species (ROS) are generated during mitochondrial oxidative metabolism and during a cellular response to xenobiotics, cytokines, and bacterial invasion (Ray et al., 2012). Low levels of ROS are beneficial to cells, but when they reach high levels, they cause serious harm, for example, damage to nucleic acids, oxidization of proteins, and lipid peroxidation (Sánchez, 2017; Mhamdi and Van Breusegem, 2018). Endogenous antioxidants serve to reduce the effect of ROS, but a deficiency of endogenous antioxidants in organisms can lead to severe disorders such as cardiovascular diseases, diabetes, cancer, Alzheimer's disease, Parkinson's disease, and neural complications (Wei et al., 2008; Bal et al., 2017). In addition, because ROS can damage DNA, proteins, and other macromolecules, the changes accumulate over time and can cause aging of the organism (Sánchez, 2017). Hence, the presence of antioxidants in the diet plays an important role in preventing various diseases. Presently, natural products with medicinal properties (e.g., biologically active compounds from *Allium calocephalum* Wendelbo, galantamine from the plant family Amaryllidaceae, psychotrine from *Cephaelis acuminata* H. Karst., the alkaloid cap-

* Corresponding author: Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, Kyiv, Ukraine; e-mail: barmash14@gmail.com

saicin from the genus *Capsicum*, and many others) are receiving increasing interest, as some synthetic drugs have been reported to have carcinogenic properties (Mohammed et al., 2019; Alrasheid et al., 2021). Therefore, the search for effective and nontoxic natural products is increasing, and it has been proven that antioxidants are present in all biological systems (Lee et al., 2004; Donglu et al., 2016). In addition to being a nutritious food, mushrooms have high medicinal properties as they contain important bioactive compounds. Mushrooms are rich in proteins (19–35% of dry weight), carbohydrates (50–65% of dry weight), fat (2–6% of dry weight), vitamins (thiamin, riboflavin, niacin, biotin, and ascorbic acid), and minerals (Ca, K, Mg, Na, P, Cu, Fe, Mn, Cd, Pb, and Se) (Rathore et al., 2017; Copetti, 2019; Vetter, 2019). Various mushrooms play an important role in the treatment of various diseases, including viral, bacterial, and fungal infections (Chang and Buswell, 1996; Khatua et al., 2013; Muszyńska et al., 2018). The mushroom kingdom contains many compounds with antioxidant properties, which include ergothioneine (a compound occurring in relatively few organisms) and glutathione (an insufficiently explored compound) (Kalaras et al., 2017; Quintero-Cabello et al., 2021).

Among the mushroom species, *Hohenbuehelia* sp. is currently of particular interest as it shows promising radioprotective (Li et al., 2015), antioxidant, and antimicrobial activities (Bala et al., 2011; Li et al., 2017). *H. myxotricha* is a rare representative of *Hohenbuehelia* species (Angeli and Scandurra, 2012). *H. myxotricha* is a fungus from Basidiomycota division, Agaricomycetes class, Agaricales order, and Pleurotaceae family. It was first described by French physician and mycologist Joseph-Henri Léveillé in the 19th century, and its current name was given by German-born mycologist Rolf Singer in 1951. There are very limited studies on this fungus, and its extracellular enzymatic activity, alternative substrates for the cultivation of its mycelia, and antibacterial activity of mycelia and culture liquid have been investigated only in the past few years (Krupodorova et al., 2014; Krupodorova and Barshteyn, 2015; Krupodorova et al., 2016). *H. myxotricha* was reported as a species that produces extracellular enzymes (Krupodorova et al., 2014); in their study, the authors reported for the first time the production of amylase, lipase, and urease by *H. myxotricha*. High biological efficiency of *H. myxotricha* mycelia cultivation on a soybean cake was also established (Krupodorova and Barshteyn, 2015). The antibacterial activities of *H. myxotricha* were observed against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* (Krupodorova et al., 2016).

The present study aimed to evaluate the total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and antimicrobial activities of *H. myxotricha* (Lév.) Singer mycelia cultivated in different liquid media.

Materials and methods

Fungal strain

H. myxotricha (synonyms: *Acanthocystis myxotricha*, *Agaricus myxotrichus*, *Calathinus myxotrichus*, *Dendrosarcus myxotrichus*, and *Pleurotus myxotrichus*) strain 1599 was kindly provided by the Mushroom Culture Collection (IBK) of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (Bisko et al., 2020). Stock cultures were maintained on beer wort agar slants at 4 °C.

Culture media and growth conditions

H. myxotricha was grown in Petri dishes (90 mm diameter) on glucose peptone yeast (GPY) agar medium pH 6.0 with the following composition (g/l): 25.0 glucose, 3.0 yeast extract, 2.0 peptone, 1.0 K_2HPO_4, 1.0 KH_2PO_4, 0.25 $MgSO_4 \cdot 7H_2O$, and 20.0 agar. The GPY liquid culture medium without agar and Sabouraud dextrose broth (HiMedia, India) pH 5.6 with the following composition (g/l): 20.0 dextrose and 10.0 peptone, were sterilized by autoclaving for 15 min at 121 °C. Flasks (250 ml) with 100 ml liquid culture medium were inoculated with three mycelial plugs of 8 mm diameter cut from the Petri dishes by using a sterile borer at the stage of actively growing mycelia. Mycelia were grown under static conditions (without agitation and in dark) in flasks for 14 days at 26 °C. The mycelium was then separated from the medium by filtration through a Whatman filter paper No. 4 (Whatman plc, UK) and washed with distilled water. The mycelium was dried at 60 °C (Snol-58/350, UMEGA, Republic of Lithuania) until it reached a constant weight.

Extract preparation

Five grams of samples were taken from each of the mycelia samples obtained from liquid GPY or SDB medium and pulverized. The samples were then extracted

with 30 ml of ethanol (EtOH) with a heated magnetic stirrer for approximately 24 h. Subsequently, the obtained extracts were sieved through a filter paper and concentrated at 40 °C in a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator) until the solvent was completely evaporated.

Antimicrobial activity tests

Antimicrobial activity tests of EtOH extracts of mycelia samples were performed using the agar dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The minimum inhibitory concentrations (MIC) were determined in the tests. MIC values are the lowest extract concentrations that inhibit the growth of microorganisms (CLSI 2012; EUCAST 2014; EUCAST 2015). *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* (MRSA) ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC 19606 were used as bacterial strains and precultured in Muller Hinton Broth. *Candida albicans* ATCC 10231, *C. glabrata* ATCC 90030, and *Issatchenkia orientalis* (formerly *C. krusei*) ATCC 34135 were used as fungal strains and precultured in RPMI 1640 broth medium. The turbidity of the test bacterial and fungal suspensions was adjusted according to McFarland 0.5 scale to obtain a standard inoculum. The extracts were adjusted with distilled water to the concentrations of 12.5–800 μg/ml. The solvent (as control) used for the extraction was also tested for antimicrobial activity. Fluzole (Fluconazole; Nobel Ilac Sanayii Ve Ticaret A.S., Turkey), Ampholip (Amphotericin B; Bharat Serums and Vaccines, India), Amikacin (Provet Veterinary Products, Turkey), Alfasid (Ampicillin; Avis Ilac A.S., Turkey), and Flaprox (Ciprofloxacin; World Medicine Ilac San. Ve Tic. A.S., Turkey) were used as reference drugs (Bauer et al., 1966; Hindler et al., 1992; Matuschek et al., 2014).

TAS and TOS determination

TAS and TOS of the mycelial samples were determined using Rel Assay TAS kits (Erel, 2004) according to the manufacturer's protocol. Trolox was used as a standard in antioxidant tests. Hydrogen peroxide was used as a standard in oxidant tests. OSI (arbitrary unit: AU) was determined according to the following formula (Erel, 2005):

$$\text{OSI (AU)} = \frac{\text{TOS [μmol } H_2O_2 \text{ Equiv./l]}}{\text{TAS} \times 10 \text{ [mmol Trolox Equiv./l]}}$$

Statistical analysis

Statistical analyses were carried out with 5 replicates. The results are expressed as mean ± standard deviation (SD). SPSS software package, version 22.0 (SPSS, Inc. IL, USA) was sed for statistical analyses. A P value ≤ 0.05 was considered to be statistically significant.

Result and discussion

Antimicrobial activity of H. myxotricha mycelial extract

Microbial infections have been quite harmful for human health from past to present. Antibiotics are drugs used in the treatment of diseases caused by many different microorganisms (Tanhaeian et al., 2020). Presently, the effectiveness of the use of synthetic antibiotics is gradually decreasing due to the emergence and spread of multidrug-resistant bacteria (Fair and Tor, 2014; Dunai et al., 2019). In addition, the use of synthetic antibiotics may have side effects such as allergic reaction, vomiting, nausea, diarrhea, bloating, indigestion, abdominal pain, and fungal infections (Anderson, 2019). The discovery of new antimicrobial products has therefore become necessary, as microorganisms are increasingly acquire resistance to antibiotics (Klaus et al., 2020). The establishment of the presence of antibacterial activity in a poorly studied fungus *H. myxotricha* shows the potential of this species. Growing the fungus under controlled conditions allows, to a certain extent, to regulate the biosynthesis of its metabolites. In particular, the optimization of the culture medium can enhance secondary metabolite production. A previous study on the antimicrobial activity of different extracts of *Lentinus tigrinus* demonstrated that ethanolic extracts generally exhibited higher levels of activity against test microorganisms than other extracts (Sevindik, 2018a). It was also found that an ethanolic extract of *Stereum hirsutum* had higher antimicrobial and antioxidant activity than its methanolic extract (Sevindik et al., 2021a). Ethanol is a very suitable solvent for the extraction of polar and some nonpolar metabolites. It is also a preferred solvent because it is nontoxic to human and animal cells, unlike methanol. In the present study, the antimicrobial activities of ethanolic extracts of *H. myxotricha* mycelia produced in different culture media were determined. The obtained findings are shown in Table 1.

Table 1. Minimum inhibitory concentration (μg/ml) values of *H. myxotricha* mycelial extract and commercial drugs

Media/Antibiotics	A	B	C	D	E	F	G	H	I
Mycelial extract, grown on SDB	50	50	100	200	200	100	25	25	25
Mycelial extract, grown on GPY	50	50	100	100	200	100	50	25	50
Ampicillin	1.56	3.12	1.56	3.12	3.12	–	–	–	–
Amikacin	–	–	–	1.56	3.12	–	–	–	–
Ciprofloxacin	1.56	3.12	1.56	1.56	3.12	–	–	–	–
Fluconazole	–	–	–	–	–	–	3.12	3.12	–
Amphotericin B	–	–	–	–	–	–	3.12	3.12	3.12

A – *S. aureus*, B – MRSA, C – *E. faecalis*, D – *E. coli*, E – *P. aeruginosa*, F – *A. baumannii*, G – *C. albicans*, H – *C. glabrata*, I – *I. orientalis*, (–) – not established

A negative control of the solvent (ethanol) was also tested to ensure that any activity on the microorganisms is not caused by the solvent. The solvent did not show any inhibitory activity against the tested pathogenic bacteria and fungi. The effect of the influence of commercial antibiotics was also determined. The antimicrobial activity of the ethanolic extract of *H. myxotricha* mycelia was significantly lower than that of commercial antibiotics. This was, however, an expected result because the crude extract was analyzed, while the tested antibiotics contain purified isolated substances.

The MIC values of mycelia samples of *H. myxotricha* varied from 25 to 200 μg/ml depending to the tested pathogens. Our results indicate that the mycelial extract of *H. myxotricha* contained effective antimicrobial compounds with a broad spectrum of activity against the tested pathogens, both gram-positive and gram-negative [(gram-positive: *S. aureus* (MIC 50 μg/ml), MRSA (MIC 50 μg/ml), *E. faecalis* (MIC 100 μg/ml); gram-negative: *E. coli* (MIC 100–200 μg/ml), *P. aeruginosa* (MIC 200 μg/ml), *A. baumannii* (MIC 100 μg/ml)] and fungus: *C. albicans* (MIC 25–50 μg/ml), *C. glabrata* (MIC 25 μg/ml), *I. orientalis* (MIC 25–50 μg/ml). To the best of our knowledge, the present study is the first report to demonstrate the antimicrobial effect of *H. myxotricha* against *E. faecalis*, *P. aeruginosa*, *A. baumannii*, and *C. glabrata*. The gram-negative bacteria *A. baumannii*, *E. coli*, and *P. aeruginosa* with an MIC value between 100 and 200 μg/ml were less sensitive than the gram-positive bacteria *S. aureus* and MRSA with an MIC value of 50 μg/ml (Table 1). This tendency can be explained by the structural intricacy of gram-negative bacteria as their cell walls are less permeable (Walsh, 2003). The antifungal activity of the ethanolic extract of *H. myxotricha* mycelia (MIC value ranging from 25 to 50 μg/ml) was stronger than its antibacterial activity (MIC value ranging from 50 to 200 μg/ml). This tendency was also observed for extracts in a similar experiment with the same pathogens (*S. aureus*, MRSA, *E. faecalis*, *E. coli*, *P. aeruginosa*, *A. baumannii*, *C. albicans*, *C. glabrata,* and *C. krusei*) for the extracts of *Cerrena unicolor* fruiting bodies (Sevindik, 2018b). In the present study, the cultivation medium had no effect on the antibacterial activity of the extract, except for *E. coli*; however, the extract of *H. myxotricha* mycelia grown in SDB medium (MIC 25 μg/ml for *C. albicans*, *C. glabrata*, and *I. orientalis*) showed more effective antifungal activity than the extract of the strain grown in GPY medium (MIC 50 μg/ml for *C. albicans* and *I. orientalis,* and MIC 25 μg/ml for *C. glabrata*). Thus, it was observed that the antimicrobial activities of the same mushroom strain cultivated in different culture media can change depending on the medium used. This observation is in line with other studies that reported on the effect of carbohydrate composition of the cultivation medium for manifestation of the antimicrobial activity of *Pleurotus ostreatus* (Barakat and Sadik, 2014), *Cantharellus cibarius* (Popova, 2015), *Lentinula edodes*, and *Fomitopsis betulina* (Krupodorova et al., 2019). It should be noted that the tested mycelial extract inhibited the growth of *S. aureus* and MRSA at the same MIC value of 50 μg/ml. Many studies on the significant antimicrobial potential of the extracts of mushrooms (Basidiomycetes, Ascomycetes) have been appropriately summarized in different reviews (Alves et al., 2012; Ranadive et al., 2013; Sharma et al., 2014; Shen et al., 2017; Rosenberger et al., 2018; Román

et al., 2020). In previous studies, by using the disc diffusion method, the mycelia of *H. myxotricha* obtained after growth in GPY medium was shown to exert some effect against *Bacillus subtilis* ATCC 6633 (zone of inhibition (ZOI): 13.3 ± 0.7 mm) and after growth in amaranth flour medium against *Escherichia coli* 06, (ZOI: 10.0 ± 0.0 mm), *Staphylococcus aureus* 209 (ZOI: 15.0 ± ± 1.0 mm), and *B. subtilis* (ZOI: 19.0 ± 1.0 mm) (Krupodorova et al., 2016). The antibacterial activity against *S. aureus* and *E. coli* has also been noted for water extract (75–100% inhibition) and ethanolic extract (25–100% inhibition) of fruiting bodies of *Hohenbuehelia* sp. (Bala et al., 2011). Some activity (hazy/very hazy inhibition zone) against *S. aureus* was recorded for the methanolic extracts of *Hohenbuehelia mastrucata* (Fr.) Singer culture liquids (Suay et al. 2000). It is important to note that the effective concentrations of *H. myxotricha* mycelial extract against all the tested pathogens were lower than those of *L. tigrinus* (Sevindik, 2018a), *Ganoderma lucidum* (Bal, 2019), and *Lactifluus rugatus* (Sevindik, 2020) mushrooms. Previous studies have shown that the different extracts of *L. tigrinus* caprophores were effective against *S. aureus* (MIC values: 200 μg/ml for ethanol extract, 200 μg/ml for methanol extract, and 800 μg/ml for dichloromethane extract), MRSA (MIC values: 200 μg/ml for ethanol extract, 400 μg/ml for methanol extract, and 800 μg/ml for dichloromethane extract), *E. faecalis* (MIC values: 200 μg/ml for ethanol extract, 200 μg/ml for methanol extract, and 800 μg/ml for dichloromethane extract), *E. coli* and *P. aeruginosa* (MIC value: 880 μg/ml for ethanol extract), *C. albicans* (MIC values: 400 μg/ml for ethanol extract, 800 μg/ml for methanol extract, and 800 μg/ml for dichloromethane extract), *C. glabrata* (MIC values: 100 μg/ml for ethanol extract, 200 μg/ml for methanol extract, and 400 μg/ml for dichloromethane extract), and *C. krusei* (MIC values: 200 μg/ml for ethanol extract, 200 μg/ml for methanol extract, and 400 μg/ml for dichloromethane extract) (Sevindik, 2018a). The methanolic extract of *G. lucidum* fruiting bodies also possessed antimicrobial activity against *S. aureus* and MRSA (both MIC 200 μg/ml), *C. albicans and C. krusei* (both MIC 50 μg/ml), and *C. glabrata* (MIC 100 μg/ml) (Bal, 2019). The extracts of *L. rugatus* fruiting bodies exhibited activities against *S. aureus* and MRSA (MIC values: 100 μg/ml for methanol extract and 200 μg/ml for dichloromethane extract), *E. faecalis* (MIC values: 100 μg/ml for methanol extract and 400 μg/ml for dichloromethane extract), *E. coli* (MIC values: 200 μg/ml methanol extract, 400 μg/ml for dichloromethane extract), *P. aeruginosa* and *A. baumannii* (MIC values: 50 μg/ml for methanol extract and 200 μg/ml for dichloromethane extract), and *C. albicans*, *C. glabrata*, and *C. krusei* (MIC value: 1000 μg/ml for methanol and dichloromethane extracts) (Sevindik, 2020). The results of our studies clearly showed that the ethanolic extract of *H. myxotricha* mycelia contains potential antimicrobial compounds.

Antioxidant and oxidant status of the mycelial extract of H. myxotricha

Mushrooms are known to be an important source of antioxidants (Lu et al., 2018; Nkadimeng et al., 2020; Sevindik et al., 2020). In our present study, the TAS, TOS, and OSI values of mycelia samples of *H. myxotricha* grown in SDB and GPY media were determined. The obtained values are given in Table 2.

To the best of our knowledge, the TAS, TOS, and OSI values of mycelial extracts of *H. myxotricha* have been determined for the first time. The effect of the nutritional media on the antioxidant and oxidant status of *H. myxotricha* was assessed, and it was found that the mycelium produced in SDB medium had a higher TAS value of 5.416 ± 0.150 mmol/l. The ethanolic extract of the mycelium grown in GPY medium also showed higher TOS (2.623 ± 0.157 μmol/l) and OSI (0.058 ± 0.004) values. Differences in the antioxidant activity of the cultivated fungus *Pleurotus citrinopileatus*, depending on the content of the substrate used for growth, was also reported previously (Gürgen et al., 2020). The technique used in this work for assessing the antioxidant and oxidative potential has been successfully tested for fruiting bodies of different species of Basidiomycetes belonging to various ecological and systematic groups (Akgül et al., 2016; Sevindik et al., 2016; Akgül et al., 2017; Bal et al., 2017, 2019a; Sevindik, 2018a; Sevindik et al., 2018a,b,c; Sevindik, 2019, 2019a; Bal, 2019; Gürgen et al., 2020; Krupodorova and Sevindik, 2020; Mushtaq et al., 2020; Sevindik, 2020; Sevindik et al., 2020, 2020b). It was found that the TAS values of the mycelia extracts of *H. myxotricha* grown in SDB (TAS 5.416 mmol/l) and GPY (TAS 4.549 mmol/l) media were higher than those of wild-type *Macrolepiota procera* (2.805 mmol/l) (Akgül et al., 2016), *Ompholatus olearius* (2.827 mmol/l) (Sevindik et al., 2016), *Auricularia auricula* (1.010 mmol/l), *Tra-*

Table 2. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) values of *H. myxotricha* mycelial extract

Media	TAS [mmol/l]	TOS [μmol/l]	OSI
Mycelial extract, grown on SDB	5.416 ± 0.150	1.320 ± 0.156	0.024 ± 0.003
Mycelial extract, grown on GPY	4.549 ± 0.136	2.623 ± 0.157	0.058 ± 0.004

Values are presented as mean ± SD

metes versicolor (0.820 mmol/l) (Akgül et al., 2017), *Daedalea quercina* (0.312 mmol/l), *Inonotus hispidus* (2.922 mmol/l), *Fomes fomentarius* (3.270 mmol/l), *Fuscoporia torulosa* (4.033 mmol/l), *Trametes gibbosa* (0.590 mmol/l), *Trichaptum biforme* (0.802 mmol/l) (Bal et al., 2017), *Gyrodon lividus* (2.077 mmol/l) (Bal 2018), *L. tigrinus* (1.748 mmol/l) (Sevindik, 2018a), *Cyclocybe cylindracea* (4.325 mmol/l) (Sevindik et al., 2018a), *Laetiporus sulphureus* (1.748 mmol/l) (Sevindik et al., 2018c), *Cerioporus varius* (2.312 mmol/l) (Sevindik, 2019), *Pholiota limonella* (2.378 and 2.263 mmol/l) (Sevindik et al., 2018b), *Lepista nuda* (3.102 mmol/l) (Bal et al., 2019a), *Infundibulicybe geotropa* (1.854 mmol/l) (Sevindik et al., 2020), *Octaviania asterosperma* (3.410 mmol/l) (Sevindik et al., 2020b), *L. rugatus* (3.237 mmol/l) (Sevindik, 2020), *Suillus granulatus* (3.143 mmol/l) (Mushtaq et al., 2020), *Ramaria stricta* (4.223 mmol/l) (Krupodorova and Sevindik, 2020), and *Pleurotus citrinopileatus* cultivated on different substrates (2.428–3.125 mmol/l) (Gürgen et al., 2020), but lower than that of *Cerrena unicolor* (6.706 mmol/l) (Sevindik, 2018b). The TAS value of 5.416 mmol/l for the mycelial extracts of *H. myxotricha* grown in SDB medium was quite close to that of *Cantharellus cibarius* (5.268 mmol/l) (Sevindik, 2019a) and *G. lucidum* (5.509 mmol/l) (Bal, 2019). On the basis of these results, it can concluded that for *H. myxotricha*, SDB medium is more appropriate than GPY medium for producing metabolites with antioxidant properties. The effect of carbohydrate sources on the antioxidant activity of basidiomycetes has also been documented (Barros et al., 2008; Barakat and Sadik, 2014; Zhang et al., 2015; Bai et al., 2020). The comparison of our results with the data from other similar studies proved that *H. myxotricha* contained significant antioxidant compounds. The TAS value is an indicator of the total antioxidant compounds produced by the mushroom (Gürgen et al., 2020). The differences among the TAS values for the abovementioned fungus species may be attributed to the potential of these species to produce different antioxidant compounds and the differences in the environments in which they grow. It was also found that the ethanolic extract of *H. myxotricha* mycelia grown in SDB and GPY media had significantly lower TOS (1.320 μmol/l) and OSI (0.024) values than those for previously studied fungi species such as *M. procera* (TOS: 6.596 and 10.349 μmol/l, OSI: 0.235 and 0.367) (Akgül et al., 2016), *O. olearius* (TOS: 14.210 μmol/l, OSI: 0.503) (Sevindik et al., 2017), *A. auricula* (TOS: 23.910 μmol/l, OSI: 2.367), *T. versicolor* (TOS: 17.760 μmol/l, OSI: 2.166) (Akgül et al., 2017), *D. quercina* (TOS: 6.868 μmol/l, OSI: 2.201), *I. hispidus* (TOS: 6.534 μmol/l, OSI: 0.224), *F. fomentarius* (TOS: 2.601 μmol/l, OSI: 0.080), *F. torulosa* (TOS: 2.969 μmol/l, OSI: 0.074), *T. gibbosa* (TOS: 3.522 μmol/l, OSI: 0.597), *T. biforme* (TOS: 4.356 μmol/l, OSI: 0.543) (Bal et al., 2017), *C. unicolor* (TOS: 19.308 μmol/l, OSI: 0.288) (Sevindik, 2018b), *G. lividus* (TOS: 13.465 μmol/l, OSI: 0.651) (Bal, 2018), *L. tigrinus* (TOS: 19.294 μmol/l, OSI: 1.106) (Sevindik, 2018a), *C. cylindracea* (TOS: 21.109 μmol/l, OSI: 0.488) (Sevindik et al., 2018a), *P. limonella* (TOS: 4.742 and 33.022 μmol/l, OSI: 0.199 and 1.459) (Sevindik et al., 2018b), *L. sulphureus* (TOS: 19.294 μmol/l, OSI: 1.106) (Sevindik et al., 2018c), *C. varius* (TOS: 14.358 μmol/l, OSI: 0.627) (Sevindik, 2019), *C. cibarius* (TOS: 6.380 μmol/l, OSI: 0.121) (Sevindik, 2019a), *G. lucidum* (TOS: 10.177 μmol/l, OSI: 0.185) (Bal, 2019), *L. nuda* (TOS: 36.920 μmol/l, OSI: 1.190) (Bal et al., 2019a), *I. geotropa* (TOS: 30.385 μmol/l, OSI: 1.639) (Sevindik et al., 2020), *O. asterosperma* (TOS: 7.548 μmol/l, OSI: 0.221) (Sevindik et al., 2021b), *L. rugatus* (TOS: 8.178 μmol/l, OSI: 0.254) (Sevindik, 2020), *R. stricta* (TOS: 8.201 μmol/l, OSI: 0.194) (Krupodorova and Sevindik, 2020), and *S. granulatus* (TOS: 18.933 μmol/l, OSI: 0.603) (Mushtaq et al., 2020). The TOS value is an indicator of the total oxidant compounds produced in the

fungus, and it shows how much the endogenous oxidants in the fungus are suppressed by the endogenous antioxidants (Gürgen et al., 2020). The mycelia of *H. myxotricha* used in our study were grown in two different culture media. The analysis revealed that the TOS values of the ethanolic extract of *H. myxotricha* mycelia changed depending on the culture media used. The TOS value was two times lower in mycelia obtained in SDB medium (1.320 μmol/l) than that of mycelia grown in GPY medium (2.623 μmol/l). The study samples were also found to have very low TOS values (1.320 and 2.623 μmol/l) as compared to the above-mentioned mushroom species, which were collected from their natural environment. This difference might be due to the lesser environmental impact on *H. myxotricha* metabolite composition in the culture environment as compared to the natural habitat. In addition, the low level of oxidant compounds produced in the mushroom and the excess of endogenous antioxidants led to a stronger antioxidant defense system (Sharifi-Rad et al., 2020). Therefore, the OSI value was low. The obtained results showed that *H. myxotricha* has a significant antioxidant potential.

Conclusions

Presently, the antioxidant and antimicrobial properties of fungi are important research topics. The results of the present study show that *H. myxotricha* exhibits significant antioxidant and antimicrobial activities. The antioxidant, oxidant, and antimicrobial potentials of the ethanolic extract of *H. myxotricha* mycelia varied depending on the culture medium used. GPY medium stimulated the synthesis of a high amount of antibacterial compounds against *E. coli*. However, the antioxidant and antifungal properties of the ethanolic extract of *H. myxotricha* mycelia were better after growth in SDB medium. Thus, our results confirm the importance of finding an optimal cultivated medium that provides the best manifestation of antimicrobial and antioxidant activities. Further research is needed to isolate and identify the active compounds responsible for the antioxidant and antimicrobial activities that can provide new sources in pharmacological drug designs.

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Competing interests

The authors declare that they have no competing interests.

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