Research Article

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Antioxidant and antimicrobial potential of *Ganoderma lucidum* and *Trametes versicolor*

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Abstract

Objectives: The majority of research programs had been focused on extract from the fruiting body. However, fungal mycelium obtained by submerged cultivation also has higher pharmacological potential. The aim of this study was investigation of the antioxidant and antimicrobial potential of *Ganoderma lucidum* and *Trametes versicolor* fungal mycelium biomass obtained by submerged cultivation and liquid fluid.

Methods: The antioxidant activity was evaluated by comparing the oxidation kinetics of the reduced form of 2,6-dichlorophenolindophenol with atmospheric oxygen in the presence/absence of biological samples. A criterion for evaluating for assessing the antioxidant activity was the values of the inhibition constant of the oxidation reaction.

Antimicrobial activity of the fungal extracts was performed by agar disc diffusion method.

Results: A comparative analysis of antioxidant potential of *G. lucidum* and *T. versicolor* mycelium biomass and liquid fluid was conducted for the first time. It was found that antioxidant activity of fungal biomass depends on the cultivation time. The most active were the fractions obtained on the 21st day of fungal cultivation. Fungal extracts were more effective against Gram-positive bacteria compared to Gram-negative bacteria, micromycete and yeasts.

Conclusions: The results showed that submerged cultivation of mushrooms has significant industrial potential.

Keywords: antimicrobial activity; antioxidant activity; basidiomycetes; *Ganoderma lucidum*; *Trametes versicolor*.

Introduction

Extensive research on *Basidiomycetes* fungi has markedly increased, mainly due to their potential use in a variety of biotechnological applications, which are particularly important for the regulation of physiological functions in the human organism [1–3]. *Basidiomycetes* fungi represent a source of new polysaccharides with antitumor and immunostimulating properties. Medical mushrooms such as *Ganoderma lucidum* (Reishi), *Trametes versicolor* (Turkey tail), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga) and many others contain biologically active substances with pharmacological effect and have been collected and used for hundreds of years in Korea, China, Japan, and eastern Russia. Among world's largest manufacturers and exporters of different medicinal mushroom preparations are, Zhejiang Wanfeng Medicines Group Co. Ltd, China; Mycology Research Laboratory Ltd, UK; NAMMEX – North American Medicinal Mushroom Extracts, USA; Fungi Perfecti LLC, USA; Dimond Organics, USA; Concord, Australia and others.

Ikekawa et al. published one of the first scientific reports on antitumor activities of essences obtained from

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fruiting bodies of mushrooms belonging to the family *Polyporaceae* (*Aphyllphoromycetideae*) and a few other families, manifested as host-mediated activity against grafted cancer in animals [4–7]. After that the first three major drugs were developed from medicinal mushrooms. Three mushroom polysaccharides such as Lentinan (from *L. edodes*), Schizophyllan (from *Schizophyllum commune*), and Krestin (from *Coriolus versicolor*) are currently available to the pharmaceutical industry [8–10]. For almost 40 years, medicinal mushrooms have been intensively investigated for medicinal effects in *in vivo* and *in vitro*, and many new antitumor and immunomodulating polysaccharides have been identified and put into practical use [11, 12].

Fungal-delivered polysaccharides especially from edible and medicinal species belonging to *Basidiomycetes*, possesses potential immuno-modulatory, antitumor, hypoglycemic, antibacterial, anti-HIV, antiviral effects. These polysaccharides are of different chemical composition, with most belonging to the group of beta-glucans. Differences in activity can be correlated with solubility in water, size of the molecules, branching rate and form. Polysaccharides have been well investigated in the fruit body, mycelia and produced extracellular in the culture medium in *Agaricus blazei*. Polysaccharides from fruit bodies represented glucans with different types of glucose unit connections or heteroglucans; culture mycelia contained glucomannans, and mannan-protein complexes were produced in a culture medium under submerged cultivation [13]. Additionally, *Basidiomycetes* mushrooms contain other compounds that inhibit blood aggregation, and reduce cholesterol and sugar levels [14, 15].

Besides, it has been reported that the mycelium and fruiting bodies of *Basidiomycota* fungi contain a large number of biologically active substances, such as polysaccharides, triterpenes, phytohormones [16–18]. They contain carbohydrates (60% of dry weight) [19–21]. Various classes of carbohydrates are represented by free and bound sugars, mono- and polysaccharides. All of them have immunomodulatory, antitumor, and antimicrobial properties. Chihara et al. isolated an antitumor polysaccharide from shiitake and named it lentinan (D-glucan) [22]. The molecular formula of lentinan is $(C_6H_{10}O_5)_n$.

Fungal-delivered polysaccharides especially from edible and medicinal species belonging to *Basidiomycetes*, possesses potential immuno-modulatory, antitumor, antioxidant, antibacterial, and antiviral effects. In spite of many researchers' efforts for the production of bioactive metabolites by mushrooms, the physiological and engineering aspects of submerged cultures (production kinetics, structural features, biological activity and biosynthesis control

methods of some bioactive compounds) are still far from being thoroughly studied.

G. lucidum, commonly referred to as Lingzhi in China, is a fungus which has been widely used through the centuries for the general promotion of health and longevity in Asian countries. It has been known to have numerous pharmacological effects including immunomodulating, antiinflammatory, anticancer, antidiabetic, antioxidative and radical-scavenging, and antiaging effects [23]. The potency of *G. lucidum* depends chiefly on its chemical constituents, namely the triterpenes and polysaccharides that make up the fruiting body, mycelium or spores. Most mushrooms are 90% water by weight. However, *G. lucidum* consists of 26–28% carbohydrate, 3–5% crude fat, 59% crude fibre, and 7–8% crude protein [24]. In addition, *G. lucidum* contains a wide variety of bioactive constituents such as terpenoids, steroids, phenols, glycoproteins, and polysaccharides. Numerous authors have shown that triterpenes and polysaccharides are the major physiologically active components of *G. lucidum* [25].

Along with *G. lucidum*, *T. versicolor* has a long history of medical use in Asia, dating back hundreds of years in traditional Asian medicine. Subsequent research led to identification of two closely related proteoglycan constituents of *T. versicolor* with anticancer activity: Krestin and polysaccharide peptide. High-molecular-weight polysaccharides or polysaccharide-protein complexes from mushrooms appear to enhance innate and cell-mediated immune responses, and exhibit antitumor activities in animals and humans. Polysaccharides of *G. lucidum* and *T. versicolor* have a number of biological activities, including antioxidant, antitumor and antimicrobial.

Traditionally, bioactive components have been extracted from fruiting bodies [26]. However, the production of medicinal mushrooms' fruiting bodies usually takes several months, and it is difficult to control the quality of the final product. By contrast, the growth of pure mushroom cultures in submerged conditions in a liquid culture media permits acceleration of the growth speed, resulting in high biomass yield in several days. The culture media in which mycelium grows is made of chemically pure and ecologically clean substances. Mycelial biomass production forms a future platform for fully standardized production of safe mushroom's based dietary supplements containing bioactive components. The present investigation deals with investigation of the antioxidant potential of *G. lucidum* and *T. versicolor* fungal mycelium biomass obtained by submerged cultivation and liquid fluid as well as their evaluation against some pathogenic bacteria, fungi and yeasts.

Materials and methods

Fungal strains

Ganoderma lucidum 1621 and *Trametes versicolor* 353 strains from the Mushroom culture collection of the M.G. Kholodny Institute of Botany (Kiev, Ukraine) were used in this study.

Media

- 1) GPD medium which has the following composition, g/L: glucose – 25; peptone – 3; yeast extract – 3; KH_2PO_4 – 1; K_2HPO_4 – 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.25; pH 5.5.
- 2) Synthetic medium A, g/L: glucose – 25; $(\text{NH}_4)_2\text{HPO}_4$ – 4; K_2HPO_4 – 1; KH_2PO_4 – 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.5; CaCl_2 – 0.1; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ – 0.02; FeSO_4 – 0.02; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ – 0.001; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.02; pH 5.5.

Fungal cultivation was carried out in two ways:

Stationary culture: Five disks (5 mm in diameter) cut from 7 days old culture and added to 500 mL Erlenmeyer flasks with 100 mL sterile liquid medium. The flasks were placed in a thermostat (darkness, $26 \pm 1^\circ\text{C}$).

Submerged culture: Inoculation was carried out with a suspension of mycelium, which was obtained by homogenizing of the fungal culture in a liquid medium (glucose-peptone or synthetic medium). This suspension (10 mL) added on 500 mL Erlenmeyer flasks with 100 mL sterile liquid medium. The flasks were placed in a rotary shaker (150 rpm, darkness, $26 \pm 1^\circ\text{C}$).

Antioxidant activity determination

Antioxidant activity (AOA) was defined by method described by Kapich [27]. Fungal mycelium was cultured for 7, 14, 21, and 28 days under stationary conditions (darkness, $26 \pm 1^\circ\text{C}$). 15 g of fungal biomass was mixed with 30 mL of deionised-distilled water in vortex for 60 min at 150 rpm. The homogenate was centrifuged at $5000 \times g$ for 10 min at 4°C and filtered through filter paper; clear supernatant was stored at -20°C for further analysis.

The antioxidant activity (K_i) was evaluated by comparing the oxidation kinetics of the reduced form of 2,6-dichlorophenolindophenol with atmospheric oxygen in the presence/absence of biological samples.

The calculation was made according to the formula:

$$K_i = \frac{(K_c - K_e)}{C}$$

where K_c and K_e are the constants of substrate oxidation in the control and experimental trial, respectively; C is the concentration of biological material in the cuvette.

Antimicrobial activity determination

The test microorganisms used in this study included Gram-positive and Gram-negative bacteria, yeasts and fungi provided by the Russian Collection of Microorganisms.

Mixture of ethyl acetate and 10% methanol fungal extract was prepared as a follow: *G. lucidum* 1621 and *T. versicolor* 353 were cultured on glucose-peptone medium for 14 and 21 days in submerged conditions. After that 20 g of fresh homogenized biomass was extracted with 10 mL of ethyl acetate by stirring at 120 rpm for 24 h at room temperature and left for 24 h at 4°C . The ethyl acetate fraction was evaporated on a rotary evaporator under vacuum and 1 mL of methanol (10%) was added. Obtained extract was stored at -20°C for further analysis.

The antimicrobial activity of the *G. lucidum* 1621 and *T. versicolor* 353 extracts was assayed by agar disc diffusion method. All bacteria species were grown on agar plates for 24 h at 37°C . All fungal species were grown on agar plates for 72 h at 26°C . A sterile 10 mm-diameter filter discs with *G. lucidum* 1621 and *T. versicolor* 353 extracts were placed on the bacteria/fungal plates. After that agar plates were incubated under suitable conditions depending upon the test microorganism (for 24 h at 37°C for bacteria and yeasts and for 96 h at 30°C for fungi). The antibacterial/antifungal activity was assayed by measuring the zone of growth inhibition surrounding the filter discs.

A sterile 10 mm-diameter filter discs with ethyl acetate and 10% methanol were placed on the bacterial/fungal plates as control.

Statistical analysis

All the analyses were performed in triplicate, and the results will be expressed as mean SD values of the three sets of observations. The results were expressed as mean $\pm$ standard deviation using Excel 2010.

Results

Antioxidant activity of *Ganoderma lucidum* 1621 and *Trametes versicolor* 353

Dynamics of the antioxidant activity of the culture fluid and fungal biomass of *T. versicolor* and *G. lucidum* presented in Table 1.

The obtained results showed that the antioxidant activity of the fungal mycelium and the culture liquid of *G. lucidum* 1621 and *T. versicolor* 353 vary depending on the cultivation time. With an increase of cultivation time, the antioxidant activity of the fungal biomass increased (the maximal value observed on the 14th day). The antioxidant activity of the culture fluid, in contrast, decreased.

The culture liquid of fungal strains had difference in antioxidant activity. Antioxidant activity of the of *T. versicolor* culture liquid vary from 0.6×10^{-3} to 1.4×10^{-3} L/(mL $\times$ min), and *G. lucidum* ranged from 0.4×10^{-3} to 0.9×10^{-3} L/(mL $\times$ min). The antioxidant activity of *T. versicolor* biomass ranged from 1.1×10^{-3} to 5.7×10^{-3} L/(mL $\times$ min), and *G. lucidum* from 0.8×10^{-3} to 2.5×10^{-3} L/(mL $\times$ min). The maximal antioxidant activities of *T. versicolor* and *G. lucidum* fungal biomass were 5.7×10^{-3} L/(mL $\times$ min) and 2.5×10^{-3} L/

Table 1: Antioxidant activity of the culture fluid and biomass of *G. lucidum* 1621 and *T. versicolor* 353.

Strain	Cultivation time, days	AOA culture fluid, 10^{-3} L/(mL $\times$ min)	AOA biomass, 10^{-3} L/(mL $\times$ min)
<i>T. versicolor</i> 353	7	1.4 ± 0.1	1.1 ± 0.1
	14	1.2 ± 0.1	5.7 ± 0.2
	21	0.7 ± 0.1	4.8 ± 0.2
	28	0.6 ± 0.1	3.2 ± 0.3
<i>G. lucidum</i> 1621	7	0.9 ± 0.1	0.8 ± 0.02
	14	0.7 ± 0.1	2.5 ± 0.1
	21	0.6 ± 0.1	1.8 ± 0.2
	28	0.4 ± 0.1	1.3 ± 0.1

Each value is the mean of three observations ($p < 0.05$).

(mL $\times$ min), respectively on the 14th day of fungal cultivation. Analysis of these data indicates that antioxidant activity of fungal biomass depends on the biological characteristics of the strains and the cultivation time.

Antimicrobial activity of *Ganoderma lucidum* 1621 and *Trametes versicolor* 353

Antimicrobial activity *G. lucidum* 1621 and *T. versicolor* 353 was tested against Gram-positive and Gram-negative bacteria, micromycete and yeasts. The fungal antibacterial properties may correlate with the synthesis of organic acids, triterpenoids or steroids during fungal growth [28]. It was found that fungal extracts were more effective against Gram-positive bacteria compared to Gram-negative bacteria like *Escherichia coli* or *Comamonas terrigena*. Discs with mixture of ethyl acetate and 10% of methanol did not show antibacterial or antifungal activity (Table 2).

The most active were the fractions obtained on the 21st day of fungal cultivation. The most sensitive to fungal extracts were *Rhodococcus rhodochrous* ATCC 13808, *Leuconostoc mesenteroides* VKPM B-4177, *Micrococcus luteus* NCTC 8340. Among Gram-positive bacteria, the least sensitive to *G. lucidum* 1621 extracts were *Bacillus mycoides* 537 and *Staphylococcus aureus* INA 00762. Only extract obtained from biomass of *T. versicolor* 353, cultured during 21 days had a little antimicrobial effect against these bacteria. The higher activity against the Gram-positive strains may be due to the fact that Gram-positive bacteria has less stable cell wall which is composed of phospholipids and proteins and is the major site of interaction with antimicrobial compounds [29].

The study of the antifungal activity showed that fungal extracts did not have any antimicrobial activity against *Aspergillus niger* INA 00760. It should be noted that such

Table 2: Antimicrobial activity of *G. lucidum* 1621 and *T. versicolor* 353.

Test culture	The diameter of the zone of inhibition, mm			
	<i>T. versicolor</i> 353		<i>G. lucidum</i> 1621	
	14 days	21 days	14 days	21 days
Gram-positive:				
<i>Bacillus subtilis</i> ATCC 6633	8	10	6	12
<i>Bacillus mycoides</i> 537	–	6	–	–
<i>Bacillus licheniformis</i> 72	4	10	5	9
<i>Rhodococcus rhodochrous</i> ATCC 13808	10	16	12	14
<i>Leuconostoc mesenteroides</i> VKPM B-4177	12	26	8	10
<i>Micrococcus luteus</i> NCTC 8340	14	18	12	16
<i>Staphylococcus aureus</i> FDA209P	12	15	7	9
<i>Staphylococcus aureus</i> INA 00761	7	12	5	7
<i>Staphylococcus aureus</i> INA 00762	–	6	–	–
Gram-negative:				
<i>Escherichia coli</i> ATCC 25922	5	7	6	8
<i>Comamonas terrigena</i> ATCC 8461	–	–	–	–
<i>Pseudomonas aeruginosa</i> ATCC 27853	4	5	3	4
Micromycetes:				
<i>Aspergillus niger</i> INA 00760	–	–	–	–
Yeast:				
<i>Saccharomyces cerevisiae</i> RIA 259	–	–	–	–
<i>Candida albicans</i> INA 00763	–	–	–	–

activity is extremely rare even among traditional producers of antibiotics – actinomycetes.

Discussion

Extensive research on *Basidiomycetes* fungi has markedly increased, mainly due to their potential use in a variety of biotechnological applications, which are particularly important for the regulation of physiological functions in the human organism. *Basidiomycetes* fungi represent a source of new polysaccharides with antitumor and immunostimulating properties.

Fungal-delivered polysaccharides especially from edible and medicinal species belonging to *Basidiomycetes*, possesses potential immuno-modulatory, antitumor, antioxidant, antibacterial, and antiviral effects. In spite of

many researchers' efforts for the production of bioactive metabolites by mushrooms, the physiological and engineering aspects of submerged cultures (production kinetics, structural features, biological activity and biosynthesis control methods of some bioactive compounds) are still far from being thoroughly studied. Thus, it is very important to focus on studying different types of edible mushroom extracts to find a source of physiologically beneficial and non-toxic medicines against the multidrug-resistant pathogens [30].

The antimicrobial properties of medicinal mushrooms have been studied by numerous authors. Some mushroom extracts were reported to have antimicrobial activity against Gram-positive compared to Gram-negative bacteria [31–34], which is in agreement with the results obtained in present study.

According to Kamra and Bhatt, a water extract of the *G. lucidum* fruit body has inhibitory effect against *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Enterococcus faecalis*, but *Listeria monocytogenes* was resistant to this extract. A moderate inhibitory effect of fungal extract was observed against *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Streptococcus mutans*, and the least effect against *Bacillus subtilis*. Organic solvent extracts of *G. lucidum* (hexane, dichloromethane, ethyl acetate and methanol) have inhibitory effect to *Bacillus cereus*, *Enterobacter aerogenes*, *S. aureus*, *E. coli*, and *P. aeruginosa* [35]. Gram-negative bacteria *E. coli*, *Shigella flexneri*, and *Proteus mirabilis* were resistant to the methanol extract of *T. versicolor* which is in agreement with our results obtained [32].

Heleno et al. reported that methanol extract of *G. lucidum* showed higher antibacterial activity against *S. aureus* and *B. cereus* than the antibiotics ampicillin and streptomycin, whereas *S. aureus* and *B. cereus* were the most susceptible bacteria [36]. In contrast, in the study conducted by Sheena et al. the tested strain *S. aureus* had a resistance to methanol extract of *G. lucidum*, but *E. coli*, *B. subtilis*, and *Salmonella typhimurium* were moderately sensitive to this extract [37]. Quereshi et al. tested the antimicrobial activity of various solvent extracts of *G. lucidum* fruit body against six species of bacteria (*E. coli*, *S. aureus*, *K. pneumoniae*, *B. subtilis*, *Salmonella typhi* and *P. aeruginosa*). It was found that acetone extract of *G. lucidum* had significant antibacterial activity against all studied strains [38]. Janeš et al. reported that methanol extracts of *T. versicolor* fruit body had little antibacterial effect against *P. aeruginosa*, but did not have inhibitory effect against *E. coli*, *E. faecalis*, and *S. aureus* [39]. Studies of various extracts from *T. versicolor* fruit body indicated no inhibitory effect against micromycetes [40].

The explanation for the different sensitivity of the fungal strains to Gram-negative and Gram-positive bacteria may be the fact that the peptidoglycan of Gram-negative bacteria is surrounded by a membrane that restricts diffusion through its lipopolysaccharide (LPS) covering. This LPS layer plays an important role in ensuring selective permeability [41]. On the other hand, Gram-positive bacteria lack an external membrane, but they are characterized by a thick hydrophilic porous structure that makes them more permeable. Consequently, Gram-positive bacteria are expected to be more sensitive to mushroom extracts [42, 43]. Further work is required to fully understand the mechanisms involved in the antimicrobial activity of mushroom extracts in order to search for new natural antimicrobial agents.

This issue is very important and requires further study. It should be noted, that numerous research was conducted using extracts obtained from fruiting bodies but in current research the fungal mycelium obtained by submerged cultivation was used for extract preparation. In this study a compared analysis of antioxidant potential of *G. lucidum* and *T. versicolor* mycelium biomass and culture fluid was conducted for the first time.

The results showed that submerged cultivation of mushrooms has significant industrial potential, but its success on a commercial scale depends on increasing product yields and development of novel production systems that address the problems associated with this technique of mushroom cultivation. The obtained results can be used to develop biotechnology for the production of biologically active substances of fungal origin with wide therapeutic and medicinal properties.

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