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Research Paper

Antifungal and Demelanizing Activity of *Ganoderma lucidum* on Some Pathogenic Fungi and Electron Microscopic Study of Fungal Damage

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ABSTRACT

Introduction: In the last two decades, fungal diseases have increased for various reasons, including weakened immune systems, overuse of antibiotics, antifungals, and corticosteroids, as well as increased longevity. *Ganoderma lucidum* has a variety of biologically active compounds with medicinal functions. The present study aimed to investigate the antimicrobial and demelanizing activities of *G. lucidum* on some pathogenic fungi and to assess the damage caused to the fungi by scanning electron microscopy (SEM).

Materials & Methods: In this study, after preparing methanolic and chloroform extracts of *G. lucidum*, the antifungal activities of the methanolic and chloroform extracts were separately evaluated on *Sporothrix schenckii*, *Alternaria alternata*, *Mucor*, *Penicillium*, and *Trichophyton violaceum* based on the percentage of growth inhibition. Also, the inhibition of melanin production by *G. lucidum* was investigated in these fungi. Furthermore, the damage caused by the extracts to these fungi was studied by SEM.

Results: According to the results, the mean percentage of growth inhibition of the methanolic extract was 67.25±1.02, 100±0, 27.92±3.00, 100±0, and 100±0 for *S. schenckii*, *A. alternata*, *Mucor*, *Penicillium*, and *T. violaceum*, respectively, and that of the chloroform extract was 83.82±14.16, 100±0, 47.92±3.61, 100±0, and zero respectively. *G. lucidum* inhibited the production of melanin in *Penicillium*. Based on SEM observations, changes in morphology, production of pores, shrinkage of the surface of the mycelium and conidia, and a decrease in the number of conidia were observed.

Conclusion: According to the results, *G. lucidum* is a valuable mushroom with an antifungal effect against fungal pathogens and might be used to treat these diseases. However, more research is needed to investigate the possible side effects of consuming it.

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1. Introduction

Fungal pathogens are very challenging because they are eukaryotes and have evolutionary relationships with their human hosts [1]. The emergence of drug resistance is an evolutionary issue, a ubiquitous process based on natural selection for organisms that have an improved ability to survive and reproduce in the presence of drugs. This is not only a great threat to human health but also has significant economic consequences. In this regard, fungi are of interest for their medicinal value as well as their infectivity. Many medicinal mushrooms have a long history in traditional medicine, contributing to a healthy diet due to the presence of rich sources of vitamins, minerals, and proteins. More than 100 medicinal properties are attributed to fungi. Among these, we can mention antioxidant, anticancer, anti-diabetic, anti-allergic, anti-cholesterol, antimicrobial, and detoxification activities, strengthening the immune system, and protecting the liver, heart, and blood vessels [2]. Currently, almost 270 species of mushrooms with different therapeutic properties are used in the traditional medicine of many countries, such as Japan, China, Korea, and Russia. Among these, we can refer to *Ganoderma lucidum*, *Lentinula edodes*, and *Inonotus obliquus* as some examples [3, 4]. *G. lucidum* belongs to the phylum Basidiomycota, the order Aphyllophorales, the family Ganodermataceae, and the genus *Ganoderma*. This fungus is found as a saprophyte or facultative parasite of plants and can be found in nature mainly on live and dead wood of broadleaf trees, growing under high humidity and low-light conditions [5, 6]. *G. lucidum* has bioactive compounds, including polysaccharides, triterpenoids, proteins, and sterols with therapeutic properties, such as anti-cancer, antimicrobial, antiviral, antioxidant, and anti-diabetic activities. These active ingredients are responsible for protecting the cardiovascular system, whitening the skin, and strengthening the immune system, among others [3-5].

In 2020, Rostami Nejad et al., by investigating the antimicrobial effect of *G. lucidum* mushroom extract on *Candida albicans* isolated from lung samples using two methods, disk diffusion and microdilution, showed that *G. lucidum* extract exhibited antifungal effects against *C. albicans* and could be used as an alternative to antifungal drugs in the treatment of this type of infection [7]. In 2023, Mousavi et al. reported that *G. lucidum* methanolic extract (GLME) has attracted much attention due to its exceptional antimicrobial and anticancer properties, which can be tuned by carefully controlling the content and concentration of the initial extraction. In this study, they described in detail its antimicrobial performance against gram-positive and gram-negative bacterial strains (*Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*). The results showed that the antibacterial activity of this product against *E. coli* was higher than that of streptomycin, with the diameter of the zone of inhibition being 44±0.09 mm and 30±0.11 mm, respectively [8].

The present study aimed to investigate the antimicrobial and demelanizing activity of *G. lucidum* on some pathogenic fungi and to assess the damage caused to the fungi by scanning electron microscopy (SEM).

2. Materials and Methods

G. lucidum was purchased from Islamic Azad University, Najafabad Branch. After sterilization with 70% ethanol and sterile water, the desired mushroom was dried, powdered, and prepared for extraction [9].

2.1. Extraction

Ten grams of *G. lucidum* mushroom powder were separately added to 50 mL of methanol and chloroform (99% Merck) and kept at -20 °C for seven days. Then, 150 mL of methanol and chloroform were added to the solution. The resulting solution was placed in an ultrasonic water bath at a temperature of 50 °C with a frequency of 59 kHz for 20 minutes. Next, the mushroom solution containing methanol or chloroform was placed on a shaker at 130 rpm for 48 hours. Finally, the desired extracts were filtered through No. 4 filter paper and placed in an oven to dry for 24 hours at a temperature of 45 °C [10].

2.2. Preparation of extract solution

Eight mg of the methanol extract was mixed with 12 mL of sterile water, and 6 mg of the chloroform extract was mixed with 12 mL of water to prepare the methanol and chloroform extract solutions, respectively. Dimethyl-sulfoxide (5%) and Tween 80 (20 µL) were used to dissolve the extract. The concentrations prepared for the extracts were 0.67 mg/mL and 0.5 mg/mL, respectively [10].

2.3. Tests to determine the sensitivity of fungi to extracts

For determining the growth inhibition percentage (GIP) of molds, first, 2 mL of each of the methanol and chloroform extracts were specifically mixed with 13 mL of Sabouraud dextrose agar (SDA) culture medium in

Petri dishes. After the media solidified, *Sporothrix schneckii*, *Alternaria alternata*, *Mucor*, *Penicillium*, and *Trichophyton violaceum* were directly inoculated in the center of each plate. As a control, inoculation was performed on SDA medium without the desired extracts for each fungus. The control and treatment plates were placed in an incubator at 25˚C. Then, the diameter of the control and treatment colonies was measured once every 24 hours for 5 days. All test steps were repeated three times for each of the extracts [11].

2.4. Preparation of samples of molds to examine the damage caused to the fungi by SEM

In the first step, the fungal colony was cut using a sterile scalpel and placed in 2.5% glutaraldehyde at 4 ˚C for 1 hour for initial fixation. In the second step, it was washed with 0.1 M sodium cacodylate buffer, and for secondary fixation, it was placed in 1% osmium tetroxide solution at room temperature for 1 hour and washed again with 0.1 M sodium cacodylate buffer.

Finally, the last step was performed by passing the sample through 25%, 50%, 70%, 90%, and 100% of ethanol, each for 10 minutes as a dehydration agent, and then drying the samples at ambient temperature.

After preparation, the sample was attached to a sample holder using double-sided carbon conductive tape and then coated with gold by the sputtering method, and imaging was done using a scanning electron microscope (SEM) [12]. Damage investigation was accomplished by SEM for each of the fungi *S. schneckii*, *A. alternata*, *Mucor*, *Penicillium*, and *T. violaceum*.

2.5. Investigating the activity of *G. lucidum* on the inhibition of melanin production by molds

To determine the depigmentation of molds by methanolic and chloroform extracts of *G. lucidum* mushroom, 2 mL of the extracts were separately mixed with 13 mL of SDA medium in a plate. After cooling the medium, *S. schneckii*, *A. alternata*, *Mucor*, *Penicillium*, and *T. violaceum* were separately inoculated in the center of each plate. As a control, each fungus was cultured separately on SDA medium without the *G. lucidum* extracts. The control and treatment plates were placed at room temperature. Then, the fungi were compared with the control in terms of pigment production, and depigmentation was observed under a light microscope [10, 13].

2.6. Data analysis

The Mean±SD values were determined based on the results of the Mann-Whitney and Friedman tests comparing the percentage of growth inhibition between the two methanolic and chloroform extracts.

3. Results

3.1. Investigating the antimicrobial effect of *G. lucidum* mushroom on pathogenic fungi

3.1.1. Inhibition of *Mucor*

According to the Mann-Whitney test, comparing the GIP between the methanolic and chloroform extracts, it was found that on all the first (P=0.046), second (P=0.050), third (P=0.046), fourth (P=0.050), and fifth (P=0.046) days, the GIP of the chloroform extract was significantly higher than that of the methanolic extract (Figure 1).

3.1.2. Inhibition of *Penicillium*

Based on the Mann-Whitney test, it was discovered that, in comparison with the methanolic extract, the GIP of the chloroform extract was significantly lower on the second (P=0.043), third (P=0.046), fourth (P=0.043), and fifth (P=0.050) days. On the first day, there was no significant difference between the GIPs of the two extracts (P=1.00) (Figure 1).

3.1.3. Inhibition of *A. alternata*

According to the Mann-Whitney test, comparing the percentage of growth inhibition between the two extracts, it was found that on the second (P=0.046), third (P=0.050), fourth (P=0.050), and fifth (P=0.050), the GIP of the chloroform extract was significantly lower than that of the methanolic extract. On the first day, no significant difference was observed between the GIP of the two extracts (P=1.00) (Figure 1).

3.1.4. Inhibition of *S. schenckii*

According to the Mann-Whitney test, comparing the percentage of growth inhibition between the methanolic and chloroform extracts, it was found that in the first (P=0.046), second (P=0.050), third (P=0.050), fourth (P=0.046), and fifth (P=0.050), the GIP of the chloroform extract was significantly higher than that of the methanolic extract (Figure 1).

3.1.5. Inhibition of *T. violaceum*

According to the analysis of the results by the Friedman test, there was a significant difference in the GIP of the methanolic extract between the first and fifth days (P=0.007). The Wilcoxon post hoc test was performed with Bonferroni adjustment for pairwise comparisons between the five days of measurement. The results are demonstrated in Figure 1. A noticeable inhibition effect was found for this fungus with the chloroform extract (Figure 1).

3.2. Electron microscope images of fungi treated with methanolic and chloroform extracts of *G. lucidum*

When fungi are exposed to *G. lucidum* extracts, the mycelium becomes wrinkled or crushed. The hyphae collapse, with holes created on their surface. This indicates the destruction of the cell membrane and the subsequent leakage of the cytoplasmic contents. The SEM images clearly illustrate the aforementioned damage. These are shown in Figures 2 and 3 for the *Mucor*, *Penicillium*, *A. alternata*, *S. schneckii*, and *T. violaceum*.

3.3. Investigating the ability of *G. lucidum* to inhibit melanin production of molds

Morphological changes in the melanization of *Penicillium* and its depigmentation by the methanolic extract are evident in Figure 4. Morphological changes in the conidiophores revealed that the tested fungus was melanized. To better detect the depigmentation activity, microscopic slides were prepared from the control and the methanol extract. Also, *Sporothrix*, *Alternaria*, *Mucor*, and *T. violaceum* were also tested, but no depigmentation was observed (Figure 4).

4. Discussion

Today, many antifungal drugs are used to treat pathogenic fungal infections, but they have side effects. *G. lucidum* is used as an effective medicine to treat various diseases and infections in China and other Asian countries. Studies have shown that this mushroom contains various chemical compounds, including polysaccharides, triterpenoids, alkaloids, and ergosterol [14, 15]. The results of this research revealed that the methanolic and chloroform extracts of *G. lucidum* exhibited an inhibitory effect on diverse species of mold fungi.

In this study, a comparison was made between methanolic and chloroform extracts over five consecutive days at 25 ˚C, and the results showed that the maximum inhibitory effect for *Mucor* and *Sporothrix* was observed with the chloroform extract at different times. In the cases of *Penicillium*, *Alternaria*, and *T. violaceum*, most of the inhibitory effect was observed with the methanolic extract.

Specifically, the maximum GIP of *G. lucidum* mushroom extracts on *Mucor* during the first five days was 27.92±3 for the methanolic extract, and the minimum

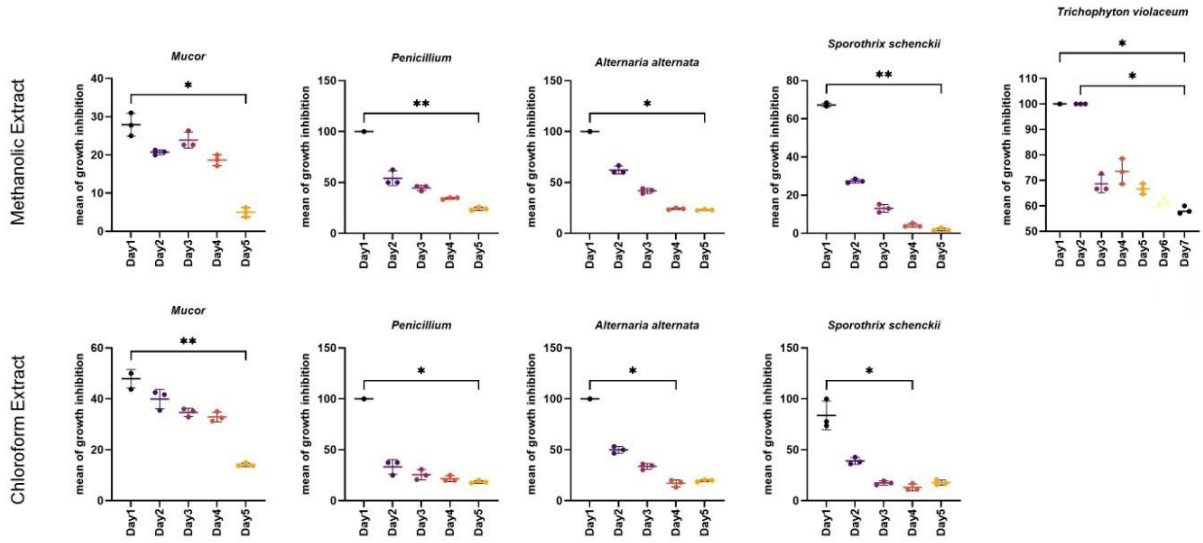

Figure 1. Mean±SD GIP of *Mucor*, *Penicillium*, *A. alternata*, *S. schneckii*, and *T. violaceum* by the methanolic and chloroform extracts of *G. lucidum* from the first to the fifth day

Note: Significant differences are marked with an asterisk (*).

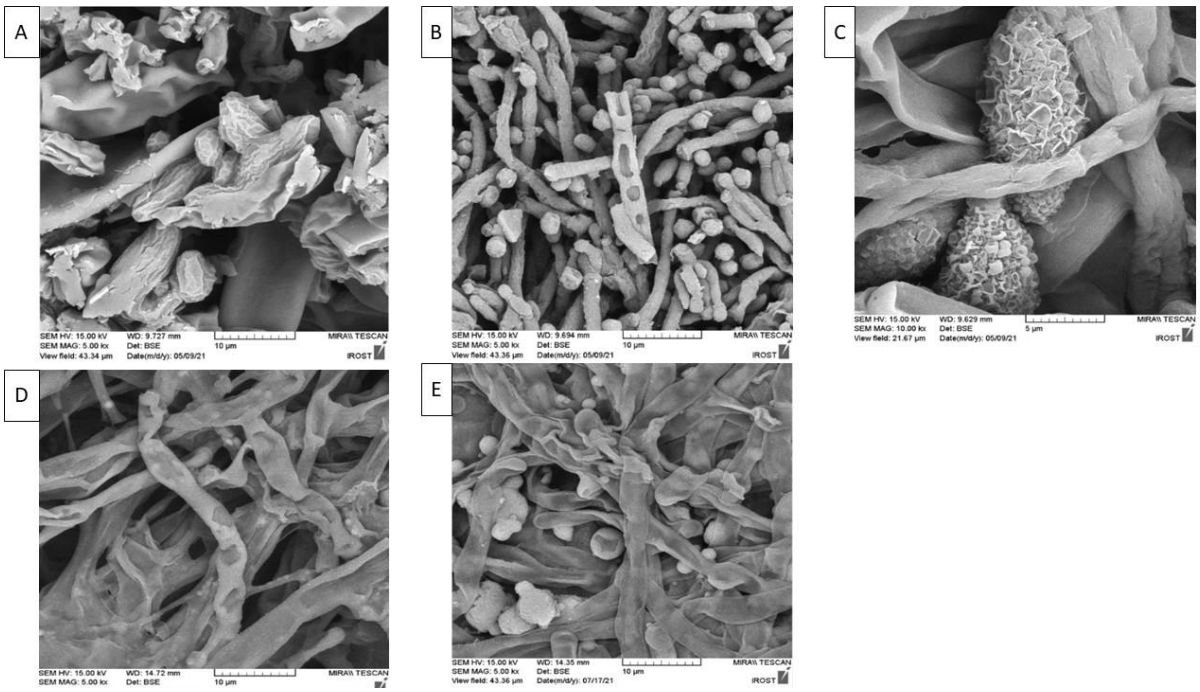

Figure 2. SEM images of the structural damage of *Mucor* (a), *Penicillium* (b), *A. alternata* (c), *S. schneckii* (d), and *T. violaceum* (e) caused by the methanolic extracts of *G. lucidum*

GIP on the fifth day was 1.25±5. For the chloroform extract, the maximum GIP on the first day was 47.92±3.61, and the minimum inhibition percentage on the fifth day was 14.17±0.72. The comparison of the GIPs for *Penicillium* during the same period revealed that the highest inhibitory effect for both extracts was on the first day with 100% inhibition, and the lowest effect on the fifth day was 27.27±1.7 for the methanolic extract and 27.27±1.7 for the chloroform extract.

Comparing different days for both extracts, the results showed that the methanolic extract had a higher percentage of inhibition than the chloroform extract. The results of the GIP for *A. alternata* were reported as 100% inhibition on the first day for both extracts, while on the second day, the maximum inhibition was 62.22±3.85 for the methanolic extract. The results showed that the methanolic extract had the greatest inhibitory effect on this fungus. The investigation carried out on *S. schneckii* revealed that the best inhibitory effect was observed with the chloroform extract, with a GIP of 82.82±14.16 on the first day and 17.8±2.43 on the fifth day, while for the methanolic extract, these values were 67.25±1.02 on the first day and 20.3±0.79 on the fifth day. Also, while no fungal growth was observed in the chloroform extract, the methanolic extract showed high efficacy against this fungus. Based on the results obtained in this research, it is probable that some morphological changes occur in the cell wall of the fungi due to the *G. lucidum* metabolites that finally prevent their growth.

Helano et al. 2021 [10] showed that the antimicrobial activity of medicinal mushrooms can be due to compounds with high and low molecular weights. They also believe that the ganoderic terpene compounds have the highest antifungal activity. Furthermore, phenolic acids and their derivatives, such as hydroxybenzoic acid, have been identified in *G. lucidum*, and these compounds may exhibit antifungal activity against fungi such as *Aspergillus fumigatus*, *Aspergillus niger*, and etc. These compounds can affect the membrane of fungal cells and prevent the growth of fungi. In the study by Azadbakht et al. 2015 [16] methanolic solvents extracted compounds such as anthocyanins, saponins, tannins, flavonoids, phenols, polyphenols, lactones, triterpenoids, glycosides, and resins. Chloroform solvent can also extract bioactive compounds such as terpenoids, flavonoids, anthraquinones, glucosinolates, cannabinoids, steroids, and alkaloids from the plant. The extraction of such compounds with a special molecular structure that can penetrate the fungal cell can result in high antifungal activity against pathogenic fungi. It is possible that methanol and chloroform solvents efficiently extracted the antimicrobial compounds of *G. lucidum*.

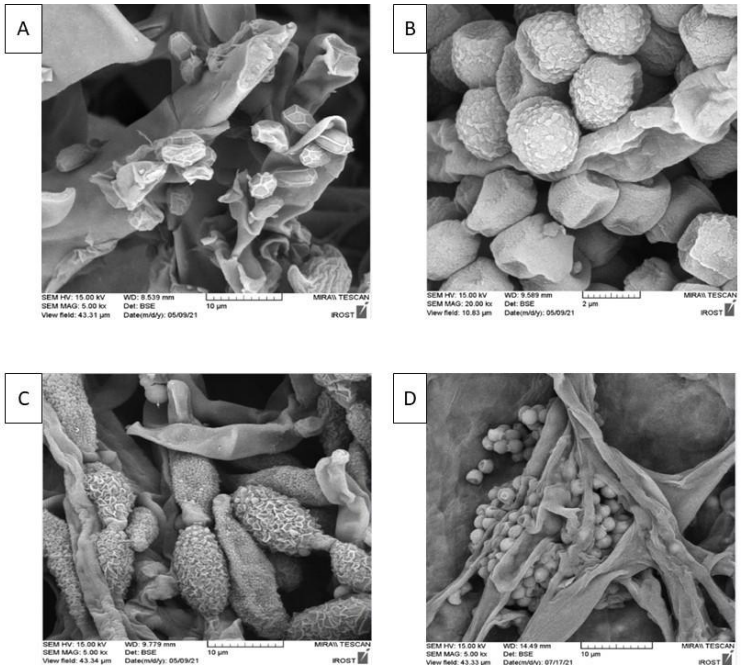

Figure 3. SEM images of the structural damage of *Mucor* (a), *Penicillium* (b), *A. alternata* (c), and *S. schneckii* (d) caused by the chloroform extracts of *G. lucidum*

The results of the present study showed that the chloroform extract of *G. lucidum* had a greater inhibitory effect on *Mucor*, *S. schneckii*, and *T. violaceum* than the methanol extract, while a lesser effect was observed on *Penicillium* and *A. alternata*. These findings are consistent with the results of Rostami Nejad et al. (2020), who found that *G. lucidum* extract showed significant anti-*C. albicans* activity and had a low MIC [7]. Also, the study by Mousavi et al. (2023) showed that the methanol extract of *G. lucidum* inhibits a wide range of bacteria and fungi, and the effect of organic extracts is stronger than that of aqueous extracts [8]. A review study titled "*G. lucidum*: A look at antimicrobial and antioxidant properties with secondary metabolite development" also

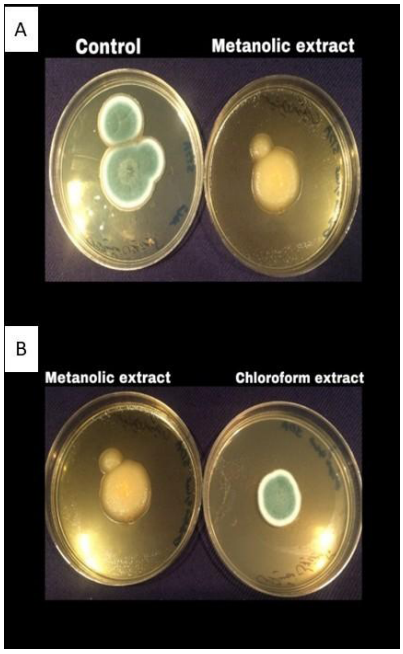

Figure 4. Morphological changes and melanization in *Penicillium* culture treated with the methanolic extract compared with *Penicillium* culture without extract (control) and the chloroform extract

stated that organic extracts and the ganodermin protein are able to inhibit the growth of fungal species, such as *Fusarium oxysporum*, *Trichoderma viride*, and *A. alternata* [17]. SEM images in the present study showed that the extracts caused hyphal disintegration and cell membrane perforation, which is consistent with the antifungal mechanisms reported in these references. Our findings on the effect of methanol on reducing melanin production in *Penicillium* are also noteworthy and are in line with previous reports on the demelanizing activity of *G. lucidum* glucan sulfate [13]. Also, the observation of significant daily differences in the growth inhibition rate indicates the time- and concentration-dependent effects of the extracts, which are consistent with the MIC results and the effect of boiled extracts in the studiy by Idu et al. (2025) [18]. Therefore, the results of this study not only confirm the broad antifungal effect of organic extracts of *G. lucidum*, but also provide preliminary evidence of the antimelanogenic activity of these extracts and highlight the importance of using natural compounds with a broad spectrum of activity in the treatment of fungal infections.

Therefore, it can be stated that the mold GIP results of methanolic and chloroform extracts compared to other extracts in our research are consistent with most of the previous studies, providing further evidence for the higher efficacy of *Ganoderma* mushroom extracts in inhibiting fungal growth.

The antioxidant, antibacterial, and DNA protection activity of the protein extracted from *G. lucidum* shows that the protein extracts of *G. lucidum* mycelium and fruiting bodies have antibacterial activity. Among the tested protein estracts, 25 µg of protein from the fruiting body extract and 115 µg of protein from the mycelial extract were obtained. It was found that *G. lucidum* fruiting body protein extract can inhibit all tested bacteria except *S. aureus*, while the protein extract from the *G. lucidum* mycelium can inhibit all tested bacteria except *P. aeruginosa* [19]. According to the studies conducted by researchers, *G. lucidum* G2 mushroom has proteins with antimicrobial properties against a wide range of gram-positive and gram-negative bacteria, especially *Staphylococcus epidermidis* [20].

Studies have shown that the pigment of fungi plays an important role in protecting them against immune cells. By producing pigments, fungi inhibit the toxic oxygen metabolites produced by the host's macrophages and neutrophils, thus avoiding the host's immune system. The production of pigment by the fungus contributes to pathogenicity inside the host [6]. Polysaccharides or sulfated glucans are usually found in marine organisms, but recently they have also been reported in fungi. Sulfated β-1,3-D-glucan extracted from *G. lucidum* mycelium shows antimicrobial activity against pathogenic bacteria and fungi. This structure was also non-cytotoxic to human cells. Sulfated glucan from *G. lucidum* is soluble and has significant therapeutic activity compared to non-sulfated *Ganoderma glucan*. As a result, *G. lucidum*, which contains such compounds, has different biological activities, including antifungal and depigmenting effects [13]. Genes responsible for melanin production in fungi, including alb1, polyketide synthase PKS, arp1, citalone reductase, arp2, hydroxynaphthalene reductase, abr1, abr2, laccase, and ayg1, are involved in melanin biosynthesis. As a result, compounds such as sulfated glucans may inhibit the expression or activity of genes involved in fungal melanin production [10]. Helano et al. 2021 [10] and Wan-Mohtar et al. 2017 [13] investigated the potential of *G. lucidum* methanol extract to induce the demelanization of *A. niger* over 72 hours. The color of *A. niger* changed from black to white. In the present research, morphological changes during the demelanization process showed that the number of *Penicillium* conidia following treatment with the methanol extract of *G. lucidum* decreased compared to the control. Also, the colony morphology changed from filamentous to mucoid, and the color of the colony changed from blue-green to colorless. Therefore, it is possible that the methanol extract of *G. lucidum*, containig compounds such as sulfated glucans, is directly involved in inhibiting or modifying polyketide synthase type 1. However, more studies should be conducted to investigate the mechanism of demelanization. Also, the depigmentation ability of other molds, such as *S. schneckii*, *A. alternata*, *Mucor*, and *T. violaceum* was investigated here, but no depigmentation was observed.

The main function of the cell wall is to provide the shape and integrity of the cell, acting as an osmotic barrier. Hence, observation of changes in the cell structure of bacteria and fungi by SEM can help to clarify the mode of action of antimicrobial agents, including mushroom extracts. In addition, much attention has been paid to the use of natural antimicrobial compounds derived from natural sources such as plants, animals, bacteria, algae, and fungi to reduce the harm caused by microorganisms and improve the quality and shelf life of food [21]. According to the research of Turecka et al. (2018) [22], antifungal substances prevent the formation of an essential lipid in the cell membrane of the fungus called ergosterol and inhibit the synthesis of β-glucan or chitin in the fungal cell wall. They cause cellular abnormalities, cell aggregation, malformation, growth inhibition, and ultimately fungal cell death.

Some phenolic compounds, such as tannins, have a toxic effect on fungi when combined with fungal proteins. Also, among other phenolic compounds, such as phenolic acids, flavonoids, xanthones, and anthocyanins, some are lipophilic, and this property enables them to affect membrane components and inhibit the transport of substances across the membrane [23]. Also, the observations from scanning electron microscopy in the research of Muniyappan et al. (2023) showed that the extract of *G. lucidum* can stop the germination of *Colletotrichum gloeosporioides* and cause contraction of the mycelium and abnormalities in the spores of this fungus [24].

5. Conclusion

In this research, the effect of methanolic and chloroform extracts of *G. lucidum* on *S. schneckii*, *A. alternata*, *Mucor*, *Penicillium*, and *T. violaceum* molds was investigated by scanning electron microscope. Abnormal irregularities on the surface of conidia and mycelium were observed in the images. The results showed that the mycelia of the five fungi were shriveled, fragmented, and constricted, which indicates membrane damage and plasmolysis of the cellular contents. Also, the conidia of the mold fungi treated with the two mushroom extracts were deformed, and perforations in the conidia were observed. Regarding the morphological changes shown by the electron microscope and the investigation of the inhibitory effect of methanolic and chloroform extracts of *G. lucidum* on the destruction of the spore cell wall and hyphae of *S. schneckii*, *A. alternata*, *Mucor*, *Penicillium*, and *T. violaceum* in the present study, we found that the extracted compounds cause morphological changes in the fungal structures and significantly inhibit their growth. GLME changed the penicillium colony morphology from filamentous to mucoid and the color of the colony from blue-green to colorless.

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Compliance with ethical guidelines

There were no human subjects or animal experiments involved in this study.

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Authors' contributions

Conceptualization, study design, and supervision: Mahboobeh Madani; Data acquisition: Sayeh Jamadi Khiyabani; Analysis and data interpretation: Sayeh Jamadi Khiyabani and Seyed Masoud Hashemi Karouei; Statistical analysis: Seyed Masoud Hashemi Karouei; Writing the original draft: Mahboobeh Madani and Sayeh Jamadi Khiyabani; Review and editing: Pegah Shakib.

Conflict of interest

The authors declared no conflict of interest.

Data availability

All relevant data are included within the paper and its supporting information files.

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