



Antioxidant and Antimicrobial Activity of a Medicinal Mushroom, *Ganoderma lucidum*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study aimed to evaluate the antioxidant and antimicrobial activity, along with the determination of major fatty acids by GC-MS and the content of total phenolic compounds of *Ganoderma lucidum* naturally grown in Türkiye. The antioxidant activity was determined by widely used DPPH radical scavenging method. *G. lucidum* methanol extract exhibited a 90.81% scavenging activity of DPPH. The content of total phenolic compounds was determined as 235.19 µg GAE/mL by Folin-Ciocalteu's phenol reagent method. The major fatty acids of *G. lucidum* were characterized by GC-MS. 2,6-di(t-butyl)-4-hydroxybenzoic acid (RT: 30.021 min), hexadecanoic acid (RT: 36.221 min), 9,12-octadecadienoic acid (RT: 39.949 min), 9-octadecenoic acid (RT: 40.076 min) and octadecanoic acid (RT: 40.601 min) were found to be the predominant fatty acid compounds. The methanol and acetone extracts of *G. lucidum* were also tested against pathogenic microorganisms via agar disc diffusion method and exhibited various levels of inhibition. The highest antimicrobial activity was detected as 17.67±0.47 mm against *Enterococcus faecalis* ATCC 29212 strain by acetone extract.

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1. INTRODUCTION

Bioactive compounds can be obtained from the fruiting bodies, mycelium, and spores of medicinal mushrooms [1]. Known by many names in China (Lingzhi), Japan (Reishi), and Korea (Mannentake), *Ganoderma lucidum* is one of the principal medicinal mushrooms and has been used since ancient times [2]. The traditional medical use of mushrooms, particularly *G. lucidum*, which dates back more than 4,000 years, has garnered the interest of numerous researchers, particularly those from Far Eastern nations [3]. The traditional use of *G. lucidum* as medicine includes treating bronchial cough, cancer, coronary heart disease, hypertension, anorexia, sleeplessness, dizziness, chronic hepatitis, hypercholesterolemia, mushroom poisoning, and hypertension [4,5].

Large, dark-textured, glossy-surfaced *G. lucidum* is a fungus that typically grows on the hardwood of trees, however, it can also grow on the stumps or roots of large deciduous hosts [6]. With a complex spore wall consisting of an outermost primary layer, an interior secondary pigmented layer, and darkly stained interwall pillars, *G. lucidum* possesses the general traits of the Ganodermataceae family. The elliptical, truncated apex of basidiospores is complemented by a relatively small number of slender interwall pillars and a smooth wall. Its pileus, or basidiocarp, is stipitate and covered in a coating of dark red laccate material. The medium-length, clavate, thick walled pilocystidia have tapering shafts. There are six different colors of *G. lucidum* based on fruiting bodies that are blue, purple, yellow, white, red, and black [7-9]. The background tissue exhibits concentric growth zones and ranges in hue from cinnamon-buff to pink-buff.

Anticancer, antibacterial, and antioxidant capabilities of *G. lucidum* have been demonstrated in numerous research to provide health benefits. By using a variety of solvents, including methanol, chloroform, acetone, or water, several studies have extracted metabolites from *G. lucidum* [10,11]. Nucleotides, glycoproteins, phenols, steroids, terpenoids, and polysaccharides are examples of secondary metabolites found in *G. lucidum* extracts [12]. A major portion of *G. lucidum*'s immunomodulatory, antioxidant, antitumor, and antibacterial qualities stem from its polysaccharide

composition [13,14]. Its antitumor, anti-inflammatory, antioxidant, antihepatitis, antimalarial, hypoglycemic, antimicrobial, and antiinflammatory properties are all attributed to its triterpene concentration [15,16]. Many pathogenic bacteria have been tested to determine the antibiotic activity of *G. lucidum* extracts [11]. While prior research on *G. lucidum* has demonstrated many health benefits, there is currently limited scientific data to back up these assertions.

This study aims to determine the content of total phenolic compounds, antioxidant and antimicrobial activity and to characterize the major fatty acids by gas chromatography-mass spectrometry (GC-MS) of *Ganoderma lucidum* extracts.

2. MATERIALS AND METHODS

2.1 Fungal Material

The fungal sample used in this study was obtained from Yalova province, Türkiye, in 2021. *G. lucidum* sample was identified due to its morphological characteristics according to the current literature [17-20]. The fungal sample was then air-dried, pulverized, and kept in a zipper bag in laboratory conditions away from direct sunlight at room temperature (RT), until use.

2.2 Preparation of the Extracts

One g of powdered *G. lucidum* sample was mixed with 40 mL solvent (methanol and acetone separately), homogenized with a WiseTis HG-15D homogenizer (Daihan Scientific, China) at 8000 rpm for 5 min, and incubated overnight at RT and in an ultrasonic bath (Sonorex, Bandelin, Germany) at 40°C for 30 min. The samples were filtered through Macharey Nagel (Germany) filter papers, and the solvents were removed by a rotary evaporator (HeiVap Value, Heidolph, Germany) until dryness. The residues were re-suspended with enough solvent to obtain 200 mg/mL extracts. These extracts were used to perform antioxidant and antimicrobial assays.

2.3 Total Phenolic Compounds and Antioxidant Activity

The content of total phenolic compounds of *G. lucidum* methanol extract was determined by

spectrophotometric Folin-Ciocalteu's phenol reagent method [21]. Since the phenol reagent method is also referred to as Gallic Acid Equivalence (GAE), a standard curve was established with gallic acid to evaluate the amount of total phenolic compounds in the extract. A 100 µL of methanol extract was added to 1 mL phenol reagent, shaken, and incubated for 5 min at room temperature. Then 1 mL of 7.5% Na₂CO₃ solution was added to the mixture and incubated in the dark for another 90 min at room temperature. The absorbance of the blue color formed in the test tubes was measured by a spectrophotometer (MultiSkan GO, Thermo) at 765 nm and results expressed as µg GAE/mL extract.

The antioxidant activity of *G. lucidum* methanol extract was determined by performing a DPPH radical scavenging assay [22]. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a dark-colored powder that consists of free radicals. A 5-step dilution of the extract was prepared with methanol. One hundred µL of extract from each dilution was added to 2.9 mL 0.1 mM DPPH solution prepared with methanol, shaken vigorously, and incubated at RT in the dark for 15 min. The absorbance of the samples and the blank DPPH solution were measured by a spectrophotometer at 517 nm. The DPPH radical scavenging activity of the *G. lucidum* methanol extract was evaluated by the formula below;

$$\% SA = \frac{ADPPH - ASample}{ADPPH} \times 100$$

where %SA is % DPPH scavenging activity of the extract, ADPPH is the absorbance of 0.1 mM DPPH solution and ASample is the absorbance of the sample.

2.4 GC-MS Analysis

The major fatty acids of *G. lucidum* were determined by GC-MS following the extraction performed with hexane. Briefly; 1 g of sample was mixed with 30 mL n-hexane and homogenized at 8000 rpm for 5 min. The homogenate was incubated at RT for 6 hours and in an ultrasonic bath at 30 °C for 30 min. Then the sample was filtered through Macharey Nagel filter paper and the solvent was removed by a rotary evaporator until dryness. The residue was resuspended with 5 mL n-hexane. Esterification and phase separation of the fatty acids were achieved by applying 2M KOH in methanol and 1 N HCl solutions. The upper layer

after the phase separation was dried with anhydrous Na₂SO₄ and passed through a 0.45 µm nylon filter before injection to GC-MS.

The major fatty acids of the *G. lucidum* hexane extract were determined by a Shimadzu QP2010 GC-MS Ultra system equipped with a Restek Rxi-5MS column (30 m * 0.25 mmID * 0.25 µm df). The injection port temperature was set to 240 °C. One µL of sample was injected to the system in split mode with a ratio of 1:40 by AOC2.0i autosampler. The carrier gas was Helium with a constant flow rate of 1.1 mL/min. The initial oven temperature was 40 °C for 1 min, then heated up to 160 °C at a rate of 5 °C/min and held for 3 min, and finally heated up to 250 °C at a rate of 5 °C/min and held for 11 min. The interface and the ion source temperatures were 270 and 200 °C, respectively. The total analysis time was 57 min. All spectra were acquired in electron impact (EI) mode and the mass range was 40-650 m/z in full scan mode. Wiley mass spectra library (W9N11) and Flavour and Fragrance Natural and Synthetic Compounds library (FFNSC 1.2) were used for the identification of the major components detected by MS.

2.5 Antimicrobial Activity

The disc diffusion method was performed for the determination of *G. lucidum* methanol and acetone extracts against test microorganisms. *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Salmonella kentucky*, *Staphylococcus aureus* ATCC 25923, *Salmonella infantis*, *Salmonella typhimurium* SL1344, *Staphylococcus epidermidis* DSMZ 20044, *Pseudomonas aeruginosa* DSMZ 50071, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231 were grown in Luria-Bertani (LB) broth medium at 37 °C for 24 hours. The turbidity of the microorganism strains was in accordance with the 0.5 McFarland standard.

A hundred µL of test microorganisms in broth media were inoculated on LB agar medium with a sterile Drigalski spatula. Sterile paper discs with a 6 mm diameter including 25 µL extracts prepared with methanol and acetone were placed on LB agar medium and incubated at 37 °C for 24 hours. The solvents and empty discs were used as negative control while the discs containing 30 µg Vancomycin and 10 µg of Gentamycin were used as positive control. The diameters of the inhibition zones around paper discs were measured following the incubation

period and expressed as mm to evaluate the antimicrobial activity. All experiments were conducted as triplicates.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Compounds and Antioxidant Activity

Phenolic compounds are one of the major classes of secondary metabolites comprised of at least one phenol group, along with terpenoids and alkaloids. *G. lucidum* extracts include phenolic acids, flavonols, flavons, and stilbenes which contribute to the antioxidant and anti-inflammatory effect of the mushroom [23]. Evaluation of the *G. lucidum* methanol extract in terms of total phenolic compounds was performed by Folin-Ciocalteu's phenol reagent method. According to Fig 1, the content of total phenolic compounds was determined as 235.19 µg GAE/mL extract. The studies on bioactive compounds and secondary metabolites of *G. lucidum* indicate that the content of phenolic compounds can vary between 2.54 and 7.38 mg GAE /g DW according to the extraction technique with a yield range of 3.64-8.55 % [24]. It was also stated that the phenolic content could be affected by the drying method. Dong et al. [25] indicated that the microwave drying of *G. lucidum* samples showed the highest phenolic compound content with 4.38 mg GAE/g DW when compared to the freeze-dried, air-dried, and oven-dried samples.

Fig. 1 also indicates the DPPH radical scavenging activity of the *G. lucidum* methanol extract. The maximum DPPH scavenging activity was determined as 90.81 % of 0.1 mM DPPH from the 200 mg/mL fungal extract, which was quite high when compared to other medicinal mushrooms. The DPPH scavenging activity determined in this study was also in accordance with other studies conducted with *Ganoderma* species [26,27]. Although the antioxidant activity of the medicinal mushroom extracts is generally associated with the presence of the phenolic compounds in the investigated samples [28], in the case of *Ganoderma* species triterpenoids and polysaccharides also take place [29,30]. It was considered that the difference occurred in the content of total phenolic compounds and the DPPH radical scavenging activity between this study and the literature might be due to the geographic position of the sampling areas, meteorological differences, the growth stage of the sample, extraction method and solvent.

3.2 Fatty Acid Composition

The major fatty acid composition of the *G. lucidum* sample was determined and characterized by the GC-MS technique by comparing the mass spectra with the Wiley mass spectra library and the literature. According to the results, the major fatty acid components were identified as 2,6-di(t-butyl)-4-hydroxybenzoic acid with a concentration of 5.24 % at a retention time (RT) of 30.021 min; hexadecanoic acid with a concentration of 21.09 % 36.221 min; 9,12-octadecadienoic acid with a concentration of 4.82 % at RT of 39.949 min; 9-octadecenoic acid with a concentration of 11.62 % at RT of 40.076 min and octadecanoic acid with a concentration of 13.91 % at RT of 40.601 min. Since *G. lucidum* is widely credited for its bioactive compounds such as polysaccharides, proteins, terpenoids, polyphenols, and peptidoglycans with antioxidant, anti-inflammatory, antibacterial, antiviral, immunomodulatory, and antitumoral activity [30], it is considered that fatty acid content may be another factor for *G. lucidum* to exhibit above-mentioned health beneficial effects.

3.3 Antimicrobial Activity

G. lucidum is referred to as "lingzhi, reishi, or the mushroom of immortality" concerning the bioactive compounds it possesses, such as phenolics, steroids, terpenoids, fatty acids, proteins, polysaccharides, and glycoproteins. In this study, methanol and acetone extracts of *G. lucidum* were investigated in terms of antimicrobial activity by disc diffusion method using 9 bacterial and one fungal pathogen strains.

Table 1 indicates the inhibition zones formed by acetone and methanol extracts of *G. lucidum* against test microorganisms. The largest inhibition zones were formed by acetone extract with diameters of 17.67±0.47 and 17.00±0.82 mm against *E. faecalis* and *P. aeruginosa*, respectively. The smallest zones formed by acetone extract were observed against *S. epidermidis* and *S. typhimurium* with a zone diameter of 11.00±0.82 mm. On the other hand, the largest inhibition zone formed by methanol extract was observed against *S. typhimurium* with a diameter of 14.33±0.47 mm while the smallest inhibition zone formed against *L. monocytogenes* with a diameter of 6.33±0.47 mm.

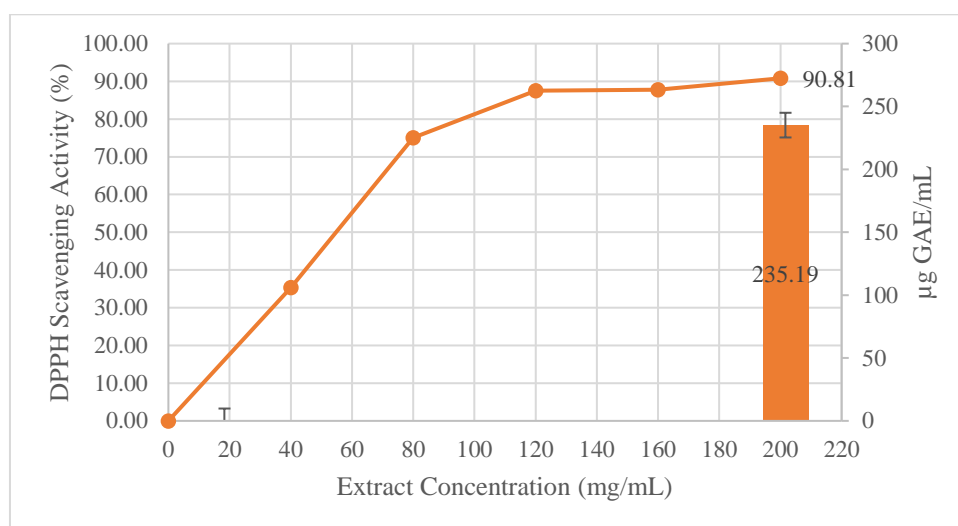


Fig. 1. DPPH scavenging activity and content of total phenolic compounds of *Ganoderma lucidum* methanolic extract

Table 1. Antimicrobial activity of *Ganoderma lucidum* acetone and methanol extracts against test microorganisms

	Inhibition Zone Diameter (mm)			
	Acetone	Methanol	VA	CN
<i>Escherichia coli</i> ATCC 25922	13.00±0.82	nd	12	20
<i>Staphylococcus aureus</i> ATCC 25923	12.00±0.82	nd	17	22
<i>Staphylococcus epidermidis</i> DSMZ 20044	11.00±0.82	12.33±0.47	7	14
<i>Pseudomonas aeruginosa</i> DSMZ 50071	17.00±0.82	13.67±0.47	nd	20
<i>Enterococcus faecalis</i> ATCC 29212	17.67±0.47	9.67±0.47	nd	nd
<i>Listeria monocytogenes</i> ATCC 7644	14.33±0.47	6.33±0.47	26	15
<i>Salmonella typhimurium</i> SL1344	11.00±0.82	14.33±0.47	8	21
<i>Salmonella infantis</i>	14.33±0.47	9.67±0.47	8	18
<i>Salmonella kentucky</i>	13.67±0.47	7.67±0.47	7	10
<i>Candida albicans</i> ATCC 10231	nd	8.67±0.47	nd	nd

*VA: Vancomycin (30 µg/disc), CN: Gentamycin (10 µg/disc), nd: not detected

Various levels of inhibition were detected against tested pathogen microorganisms in studies conducted with *Ganoderma* species extracted by numerous solvents and methods. Bioactive compounds such as terpenes, polysaccharides, lectins [31], ganomycin and triterpenoids [32], and also peptides [33] extracted from *G. lucidum* were reported to exhibit strong antibacterial activity against a wide range of pathogen microorganisms including *E. coli*, *S. aureus*, *B. subtilis*, *L. monocytogenes*, *S. typhimurium*. Moreover, crude extracts obtained by various solvents (ethanol, acetone, chloroform, hexane...etc.) also show inhibition against pathogens [34-37]. The inhibition zones determined in this study varied between moderate and high values depending on the extraction solvent and the test microorganism

when compared to control antibiotic discs. Overall inhibition effect of *G. lucidum* acetone extract was higher against test microorganisms than methanol extract, considering that the polarity of the solvent may alter the effectiveness of the extract by extracting more active compounds from the sample.

4. CONCLUSION

G. lucidum, which has many medicinal uses, is one of the most popular mushroom species as a natural source of bioactive compounds. This study aimed to contribute the antimicrobial and antioxidant properties along with the major fatty acid components and the content of total phenolic compounds of *G. lucidum* mushroom naturally grown in Türkiye. According to the

results obtained from this study, the authors recommend additional research into the antimicrobial activity of *G. lucidum*, which may be considered a valuable candidate as a natural source for highly needed novel antimicrobial agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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