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Functional properties of *Ganoderma lucidum* extract: antimicrobial and antioxidant activities

Mahdis Jamshidi TEHRANIAN[1], Mohammad JOUKI[2*], Mohammad Javad SHAKOURI[2], Sara JAFARIAN[1]

Abstract

This research was conducted to investigate the functional properties of *Ganoderma lucidum* extract. The antimicrobial activity was evaluated on some bacteria causing food spoilage like *Staphylococcus aureus* and *Escherichia coli*. Antioxidant activity was investigated by examining the effect of the extract on DPPH and ABTS free radicals, determining the amount of total phenolic compounds and flavonoids. Based on the results, the antimicrobial and antioxidant activities of the extract were increased by increasing the concentration. *Staphylococcus aureus* showed the highest sensitivity to Ganoderma extract with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 50 and 100 µg/mL, respectively. The total phenols amount was equal to 383.727 milligrams of gallic acid equivalent per gram (mg GAE/g) of the extract and the total flavonoid was equal to 28.047 milligrams of quercetin equivalents per gram (mg QE/g) of extract. The results of antioxidant activity tests showed that the concentration of 400 µg/mL had the highest scavenging activity of 85.9 and 90.12% for DPPH and ABTS free radicals, respectively. Therefore, considering the potential antioxidant activity and the rich amounts of phenolic compounds in Ganoderma extract, it can be used in the pharmaceutical and food industries instead of synthetic antioxidants and other chemical preservatives to delay lipid peroxidation and prevent the growth of food pathogens.

Keywords: *Ganoderma lucidum*; natural antimicrobial; antioxidant activity; total phenolic compounds.

Practical Application: One of the most important reasons for reducing the quality and taste of high-fat meat and dairy products is the formation of lipid oxidation compounds, which can increase economic losses due to the low quality of the product. Ganoderma extract as a natural functional compound has antioxidant and antimicrobial effects, which can be used in the form of a natural additive compound to enrich and control lipid oxidation in food products. Therefore, the use of this natural preservative can lead to better preservation of food products sensitive to oxidation and microbial spoilage.

1 INTRODUCTION

Microbiological health of food has long been a source of important concerns of consumers, producers, and control organizations. Microorganisms contaminating food can cause spoilage, decreased shelf life, and loss of organoleptic properties of food and can even lead to disease (Jouki & Khazaei, 2010; Sarabi-Jamab et al., 2020). *Ganoderma lucidum* Karst mushroom belongs to the Basidiomycota branch, *Aphyllophorales* order, *Ganodermataceae* (Polyporaceae) family and Ganoderma genus (Chang et al., 2002). This species has a bean-shaped basidiocarp with a base usually on the subsidiary section. The upper surface of the basidiocarp has concentric circles and can be seen in orange, red, purple, black-brown colors with a white or yellow to red-brown border. This species usually grows at the base of trees or in their lower cavities and is found as a saprophyte or a parasite of plants (Chang et al., 2002).

G. lucidum species have been reported from different regions of the world such as France, England, Canada, North America, Taiwan, China, Korea, and Japan (Hong & Jung, 2004), and seven species have been reported from Iran (Fakoor et al., 2007). The oldest reports related to Ganoderma species in Iran are from 1969-1971 (Keypour et al., 2013). According to the climatic conditions of the country and the presence of humid to hot and dry temperate climates, the distribution of these species in Iran is different. In temperate and humid areas (the edge of the Caspian Sea), the existence of species of this genus is a natural thing. This species is found in areas such as Tonkabon, Gilan, Gorgan, Mazandaran forests, and Ramsar (Keypour et al., 2010).

The growth of bacteria and fungi in food causes spoilage and reduces the quality of food products (Esazadeh Razelighi et al., 2016). The healthiness of food from microorganisms that cause spoilage, infection, and poisoning is the concern of many researchers and producers, food standards, and regulatory organizations. Despite the different methods of food preservation, diseases caused by infection and food poisoning still have high statistics in the world. On the other hand, the desire of consumers of food products for natural foods without chemical preservatives due to knowing the side effects of these compounds has encouraged researchers in the field of food safety and health to find antimicrobial agents of plant origin (Noshad & Sahraiyan,

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[1]*Islamic Azad University, Noor Branch, Department of Food Science and Technology, Noor, Iran.*
[2]*Islamic Azad University, North Tehran Branch, Department of Food Science and Technology, Tehran, Iran.*
*Corresponding author: m.jouki@iau-tnb.ac.ir

2021). Chemical drugs with all beneficial efficacy have many adverse side effects, and perhaps few pure substances have no adverse effects (Nostro et al., 2000).

Extracts obtained from medicinal plants have bioactive compounds and can be applied as a source of natural antimicrobial substances against a wide range of pathogenic microorganisms. In this regard, the resistance of bacteria to antibiotics is increasing, which made people think of replacing effective antimicrobial agents with fewer side effects instead of antimicrobial agents with fewer effects and more unwanted side effects. Therefore, extracts of medicinal plants have been used to overcome many microorganisms, and these antimicrobial effects have been proven against bacteria and fungi (Rezaei et al., 2013). Therefore, on this basis, this research approached the antibacterial and antioxidant activities of Ganoderma mushroom.

2 MATERIALS AND METHODS

2.1 Ganoderma mushroom extract

To extract Ganoderma mushroom essence, the dried parts were first turned into powder using an electric grinder, and then 50 g of the powder was mixed with ethanol solvent (80%v/v) into a decanter using the percolation method. After 1 h, the decanter valve was opened and the solution containing the extract and ethanol comes out drop by drop. Then, the obtained extract was filtered with filter paper and the ethanol solvent in the extract was removed with a rotary evaporator.

2.2 Preparation of bacterial and fungal suspension

The lyophilized Staphylococcus aureus (PTCC 1431) and Escherichia coli (PTCC 1769) were purchased from the Iranian Scientific and Industrial Research Organization. The vials were opened under sterile conditions under a laminar hood and then spread onto relevant sterile culture medium and incubated at 37°C for 24 h.

2.3 Antibacterial activity of extract

Different concentrations of 6.25, 12.5, 25, 50, 100, 200, and 400 µl/mL of the extract were prepared by dilution with sterile distilled water and the antibacterial activity was investigated for each microorganism by disk diffusion method. A suspension with a concentration of 1.5×10^8 of each microorganism was prepared in physiological serum and inoculated on Mueller Hinton Agar (MHA) culture medium. Sterile blank disks impregnated with different extract treatments were used on the culture medium to investigate the non-growth aura of bacteria (inhibitory zone). The plates, along with the control — which includes a plate containing antibiotic discs for bacteria as a positive control and a sterile blank disc containing sterile distilled water as a negative control —, were kept at 37±2°C for 24 h until there was no growth (Fakoor et al., 2007).

2.4. Minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC) of Ganoderma extract, the tube dilution method was used with a series of sterile test tubes containing 1 mL of Mueller Hinton Broth culture medium by adding 1 mL of the first treatment of Ganoderma extract to the first tube. Next, 1 mL of the contents of the first tube was added to 1 mL of the culture medium of the second tube, and thus, 1.2 times more dilutions in the tubes were prepared from the extract. Subsequently, 1 mL of the contents of the last tube was discarded. A total of 9 test tubes were used; two test tubes — one positive control (absence of extract, which becomes cloudy with the addition of bacteria and their growth) and one negative control (a mixture of culture medium and plant extract) — and 7 tubes for different treatments of extracts. The impact of the extract on the growth of Staphylococcus aureus and Escherichia coli was compared with the turbidity of the control tube. In the next step, 20 µl of the suspension of microbes was added to all the tubes and incubated at 37°C for 24 h and, after incubation, the growth and turbidity created in the tubes compared to the turbidity created in the control tube or the positive control were verified. The amount of effective substance in the last tube before the tube in which turbidity was observed was as the MIC of Ganoderma extract on the studied bacteria (Mohajerfar et al., 2013).

2.5 Minimum bactericidal concentration

To determine the minimum lethal concentration, 10 µl of MIC tube and other non-turbid tubes were cultured in MHA culture medium and incubated at 37±2°C for 24 h, and the lowest concentration at which no growth was observed was considered as minimum bactericidal concentration (MBC) (Mohajerfar et al., 2013).

2.6 Total phenolic compounds

The amount of total phenolic compounds was measured by Folin–Ciocalteu reagent method (Ordonez et al., 2006). In this method, 0.5 mL of extract with a concentration of 50 µg/mL was removed, then 2.5 mL of Folin–Ciocalteu reagent was added. After 5 min, 2 mL of sodium carbonate solution was added, and the solution was allowed to rest. Two hours later, the absorbance of the samples was measured by an ultraviolet spectrophotometer at a wavelength of 760 nm against a blank. To draw the gallic acid (GA) standard curve, GA standard solutions with concentrations of 25, 50, 100, 200, and 400 µg/mL were prepared. Subsequently, the average absorption was placed in the equation of the line obtained from drawing the standard curve of GA and the result was reported as the total phenolic compounds of the extract based on the equivalent amount of "mg GA/g of extract".

2.7 Total flavonoids

The amount of total flavonoid compounds in the extracts was determined according to the method of Chang et al. (2002), by using aluminum chloride reagent. Briefly, first, 0.5 mL of extract with a concentration of 100 µg/mL was taken, then 1.5 mL of methanol and 100 µl of aluminum chloride, 100 µl of 1 M potassium acetate and 3 mL of water was added. After 30 minutes, the absorbance at a wavelength of 415 nm compared to the blank was measured using a dual visible-ultraviolet spectrophotometer.

Quercetin was considered as a standard with concentrations of 15.62, 31.25, 62.5, 125, 250 µg/mL for drawing the calibration curve. A blank solution was also prepared in the same way without extract. The amount of flavonoids was reported based on the amount equivalent to "mg quercetin /g of extract".

2.8 DPPH free-radical scavenging activity

The extract with a concentration of 800 µg/mL was used for dilution and stocks with concentrations of 6.25, 12.5, 25, 50, 100, 200, and 400 µg/mL were prepared. Afterward, 1 mL of each stock was transferred to a new tube and 1 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was added to each. The control solution contained 1 ml of methanol and 1 ml of DPPH reagent. A control was included for each extract. Then it was kept in darkness for 30 min and the absorbance of the samples was read at a wavelength of 517 nm at different concentrations of different extracts (Jouki et al., 2021). The scavenging activity of DPPH free radicals was calculated according to the following Equation 1:

$$\text{Scavenging activity (\%)} = \frac{\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{DPPH}}} \times 100 \quad (1)$$

2.9 Antioxidant capacity using ABTS radicals

The scavenging activity of the Ganoderma extract on 2,2-azino-bis-(3-eyhlbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was measured according to the method of Modi et al. (2014). ABTS cationic solution was prepared by mixing 1 mL of potassium dichromate (100 mM) and 25 mL of ABTS (10 mM) and kept overnight in the dark at 25°C. 0.05 µl of ABTS radical cationic solution was added to 2 mL of the Ganoderma extract at different concentrations and the mixtures were left undisturbed for 20 min and then absorbance was measured at 734 nm. BHT was used as a reference standard.

2.10 Statistical analysis

Experimental data were statistically analyzed using SPSS statistical software (version 23.0; SPSS, Inc., IL). To compare the difference between means, Duncan's multiple range test was used and $P<0.05$, and correlation coefficient analysis was performed between different parameters.

3 RESULTS AND DISCUSSION

3.1 Antibacterial activity of the extract

The results of antibacterial activity test of Ganoderma extract on *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method (DDM) in Figure 1 show that the extract has more antibacterial effects on gram-positive *Staphylococcus aureus* compared to gram-negative *Escherichia coli*, which at a concentration of 400 mg/mL with an average diameter of inhibitory zone of 19.5 mm had the most antimicrobial effects on *Staphylococcus aureus*.

The results of this research showed that the hydroalcoholic extract of Ganoderma mushroom had significant antimicrobial effects on *Staphylococcus aureus* and *Escherichia coli* bacteria; moreover, there were significant effects on gram-positive *Staphylococcus aureus* compared to *Escherichia coli*, so that the treatments of 400 and 6.25 µg/mL in *Staphylococcus aureus* with the average diameter of the non-growth halo of 19.5 and 7.5 mm and in *Escherichia coli* with the average diameter 14.5 and 6.33 mm diameter of lack of growth, respectively, had the maximum and minimum diameter of lack of growth. In this regard, in a study conducted by Yoon et al. (1994) on the antibacterial effect of Ganoderma mushroom aqueous extract, it was shown that this extract can inhibit the growth of 15 Gram-positive and Gram-negative bacteria.

The results of MIC and MBC of different treatments of the extract in Table 1 show that the lowest MIC and MBC were on gram-positive bacteria *Staphylococcus aureus*.

In accordance with the results of this research, Jonathan and Awotona (2010) showed inhibitory effects of three Ganoderma extracts on some pathogenic bacteria. Results showed that all the screened fungi (*G. austral, G. applanatum*, and *G.*

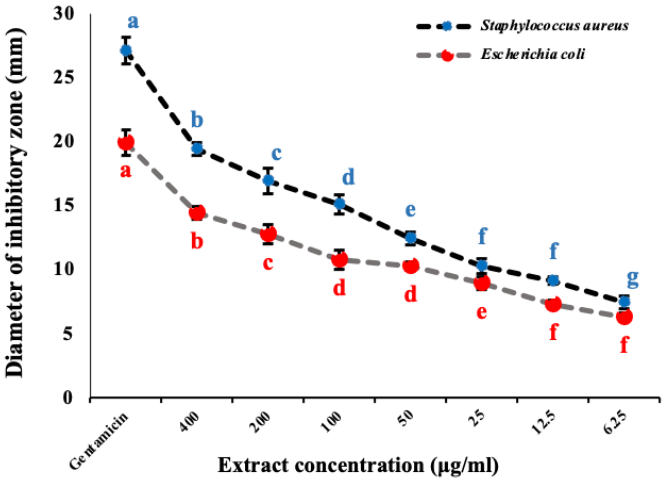

Figure 1. Average diameter of inhibitory zone (mm) of *Staphylococcus aureus* and *Escherichia coli* bacteria under the influence of different concentrations of *Ganoderma* extract.

Table 1. Different concentrations of *Ganoderma* mushroom extract in determining the minimum inhibitory concentration and the minimum bactericidal concentration of studied microorganisms.

Microorganisms	Ganoderma mushroom extract treatments (µg/ml)							MIC	MBC
	6.25	12.5	25	50	100	200	400		
S. aureus	+	+	+	-	-	-	-	50	100
E. coli	+	+	+	+	+	-	-	200	400

+ Microorganism growth; - Lack of microorganism growth.

lucidum,) demonstrated various degrees of antibacterial activity. For the ethanolic extract, the highest antibacterial activity (18.3 mm) was demonstrated by *G. lucidum* purified extract against *Bacillus cereus*. The crude extract of the same fungus produced 11.0 mm zone of inhibition for *B. cereus*. They also report that the widest inhibitory zone (20.3 mm) was obtained with the crude methanolic extract of *G. lucidium* against Proteus mirabilis while the highest antifungal activity (24.3 mm) was seen in the ethanolic extracts of *G. lucidium* against *Aspergillus niger*. The lowest zone of inhibition (2.3 mm) was demonstrated with the aqueous extract of *G. australe* against *Escherichia coli* and 2.7 mm with the purified extract of *G. australe* against *Penicillum oxalium*. The MIC for the ethanol extract ranges between 1.7 and 5.0 mg/mL for bacteria and between 2.0 and 6.0 mg/mL for fungi.

In a study, the antimicrobial activity of the extract was tested against Gram-positive and Gram-negative bacteria. Among fifteen bacterial species tested, the antimicrobial activity was observed against "Micrococcus luteus" (Marzhoseyni et al., 2023). Then, the effects of antimicrobial compounds were compared with four types of antibiotics (cefazolin, ampicillin, chloramphenicol, and oxytetracycline). Results showed that *Ganoderma* enhanced antibiotic effects when administered with cefazolin against *Klebsiella oxytoca* and *Bacillus subtilis* (Liu et al., 2023; Shi et al., 2023). Sheena et al. (2003) reported that the methanol extract of *G. lucidum* showed significant antibacterial activity against *E. coli*, *Salmonella species*, and *Bacillus subtilis*.

Phytochemical analysis of the extract showed the presence of lipid derivatives including sterols and triterpenoid acids (Keypour et al., 2008). In addition, Keypour et al. (2010) reported that the aqueous extract of *Ganoderma lucidum* has antibacterial properties against the Gram-negative *Pseudomonas aeruginosa* at a concentration of 500 mg/mL. In the present study, Ganoderma extract at concentrations of 100 and 200 µg/mL had lethal effects against *Staphylococcus aureus* and *Escherichia coli*, respectively.

3.2 Antioxidant activity of Ganoderma *extract*

The results of total phenolic compounds and flavonoids in *Ganoderma* extract are shown in Table 2. In this study, the average total phenols and total flavonoids obtained from 100 mg/ml of the extract were 383.727±4.848 (mg GAE/g dw) and 28.047±0.181 (mg QE/g dw), respectively. Özbek et al. (2020) studied the antioxidant compounds and phenolic of Pistachio (*Pistacia vera* L.) and the results obtained indicated relationships between the tested parameters, *i.e.* ethanol concentration and extraction yield. The maximum yield was obtained with 50% ethanol (32.9 g of dry extract/100 g of dry matter). The total phenolic content was found in the range of 21.3–39.3 mg/g extract as GA equivalent, also reported, the antioxidant activity (AA) was determined using three different tests. The best antioxidant activity with the lowest half-maximal inhibitory concentration (IC50) value (0.70 mg/mL) was obtained for 40% ethanol, and the lowest antioxidant activity was obtained for 100% ethanol with the highest IC50 value of 2.73 mg/mL.

So far, more than 100 types of polysaccharides have been isolated from *G. lucidum* (Moradali et al., 2007). The polysaccharide present in *G. lucidum* mushroom and other mushrooms strengthens the immune system, while having antioxidant, antibacterial, antiviral, and protection properties against radiation. Chitin and chitosan polysaccharides found in the cell wall of all fungi have medicinal properties (Badalyan et al., 2007). Studies conducted on polysaccharides have shown that some β-Dglucans isolated from fungi exist in right-handed three-dimensional spirals and have biological activity (Yang et al., 2007).

The results of antioxidant effects of *Ganoderma* mushroom extract showed that this functional extract has significant antioxidant activities, so at the highest concentration (400 µg/mL) this extract has 85.9% scavenging effects on DPPH free radicals. The results of the analysis of flavonoid compounds and total phenol were calculated as 28.047 and 383.727 mg/g of dry weight of the extract, respectively. Figure 2 shows the percentage inhibition of ABTS radicals by Ganoderma extract. The results clearly show that the highest percentage of inhibitory activity among the concentrations was related to the concentration of 400 µg/mL. As Ali showed, among commonly used solvents, methanol is the best solvent for dissolving phenols, flavonoids, triterpenoids, and glycosides. Therefore, the high level of scavenger activity of the methanolic extract of Ganoderma is proven. In this study, a positive correlation was observed between scavenging activities on DPPH and ABTS free radicals.

Kamra and Bhatt (2012) investigated the effects of using different solvents on the antioxidant properties of Ganoderma extract. They showed that the antioxidant properties are the higher in dichloromethane, aqueous extract, methanol, ethyl acetate, and hexane solvents, respectively. Preliminary phytochemical analysis of methanol and aqueous extracts revealed

Table 2. Average amount of total phenols and flavonoids in Ganoderma mushroom extract.

Ganoderma extract	Total phenols (mg GAE/g dw)	Total flavonoids (mg QE/g dw)
	383.727±4.848	28.047±0.181

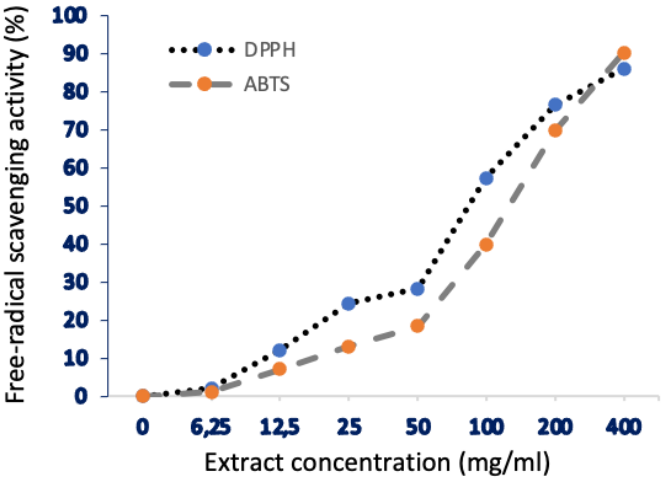

Figure 2. Scavenging activity (%) against DPPH and ABTS free radicals in different concentrations of extract.

the presence of phenolics, flavonoids, and ascorbic acid. Hence, the antioxidant activity observed *in vitro* may be due to these plant compounds, which need further investigation to isolate the purified compounds. Zhang et al. (2016), by investigating the key components on the antioxidant activity of polysaccharide extract from *Ganoderma atrum*, found that the antioxidant activities of polysaccharide from *Ganoderma atrum* (PSG) are attributed to phenolic and protein components and carbohydrate fraction is responsible for the immunomodulatory activity of PSG.

4 CONCLUSION

This study aimed to investigate the functional properties of *Ganoderma lucidum* extract. Results showed that the hydroalcoholic extract of Ganoderma mushroom has significant antimicrobial effects on *Staphylococcus aureus* and *Escherichia coli* bacteria. The extract has more antibacterial effects on Gram-positive *Staphylococcus aureus* compared to Gram-negative *Escherichia coli*, which had the most antimicrobial effects on *Staphylococcus aureus* at a concentration of 400 µg/mL with an average diameter of the inhibition zone of 19.5 mm. *Staphylococcus aureus* showed the highest sensitivity to Ganoderma mushroom extract with MIC and MBC of 50 and 100 µg/mL, respectively. The results of the antioxidant effects of Ganoderma extract showed that this functional extract has significant antioxidant activity. The amount of total phenol was equal to 383.727 mg GAE/g extract and total flavonoid was equal to 28.047 mg QE/g extract. The results of the DPPH free radical scavenging activity test showed that concentrations of 400 µg/mL had the highest scavenging activity with 85.9 and 90.12% for DPPH free-radicals, and ABTS free-radicals, respectively.

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