

Investigation of The Antioxidant Activity And Total Phenolic Substance of *Fomes fomentarius* And *Ganoderma applanatum* Mushrooms Showing Therapeutic Properties

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Abstract: *Fomes fomentarius* (Ffo) and *Ganoderma applanatum* (Gap), which are mushroom species used in traditional medicine in Far East Asia, are widely found in our country and attract great attention with their antioxidant properties. In this study, the antioxidant activities of these mushrooms grown in our country were determined spectrophotometrically using by the DPPH method. The results were given as percent inhibition and also the IC₅₀ values of the fungal samples were calculated using calibration equation for DPPH. IC₅₀ values for *G. applanatum* and *F. fomentarius* were found to be 0.515 and 0.463 mg/mL, respectively. Additionally, total phenolic substances were analyzed by the Folin-Ciocalteu Method. Phenol content in methanolic extracts expressed in gallic acid equivalents (GAE) was found to be 8,447 and 10,300 mg/L for *G. applanatum* and *F. fomentarius*, respectively. In this study, both types of mushrooms were found valuable because they are rich in phenolic contents and antioxidant properties.

Keywords: *Fomes fomentarius* (Ffo), *Ganoderma applanatum* (Gap), Total phenolic substances, DPPH

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1. INTRODUCTION

Even though very few of the 2000 mushroom species belonging to the Basidiomycetes group of macrofungi can be considered food, they are one of the preferred ingredients of world cuisine with their unique tastes. In recent years, many studies have elucidated the medicinal properties of some useful wild edible mushroom species, and they can also be commercialized with their nutritional properties (Chang and Miles et al., 2008; Erbiai et al., 2021). Mushrooms show immunomodulatory, antitumor and antioxidant properties because they contain proteins, polysaccharides, terpenes, terpenoids and important antioxidants, including phenolic acids, glutathione and ergothioneine (Kalaras et al., 2017; Kozarski et al., 2015; Suarez-Arroyo et al., 2017; Li et al., 2019; Gonzalez-Palma et al., 2016). Recent research has shown that adding mushrooms to the daily diet increases the intake of important nutrients such as vitamin D, fiber and potassium (Fulgoni and Agarwal, 2021). Additionally, some mushroom species are known to have therapeutic effects on

disorders such as neurodegenerative and cardiovascular diseases, cancer, inflammation and diabetes (Umeno et al., 2017; Rahman and Abdullah, 2015).

Fomes fomentarius, belonging to the family Polyporaceae, order Polyporales, is a large, inedible, woody fungus that causes rot in some deciduous tree species. This mushroom has been used since ancient times as a pain reliever and to treat rheumatism, hemorrhoids, bladder disorders, esophagus, stomach and uterine cancers (Umeno et al., 2017). Some studies have revealed that *F. fomentarius* exhibits antimicrobial properties against many viruses and bacteria that cause diseases in humans (Lee et al., 2004). In addition, it has been determined that *F. fomentarius*, like other fungi, contains valuable bioactive compounds with potential antioxidant, antitumor, immunomodulatory and anti-inflammatory activities, which are the subject of chemical and biological studies (Grienke et al., 2014; Kolundzic et al., 2016; Bojin et al., 2020). Additionally, *Ganoderma applanatum*, which is in the order Polyporales

and the Ganodermataceae family, is another type of fungus that causes wood rot in various trees. Species of the *Ganoderma* genus are used in traditional medicine in Asia, especially in China and Japan. As a result of the studies, extracts obtained from *Ganoderma* species have been shown to be beneficial against diseases that may be caused by various microorganisms such as bacteria and fungi in humans (Wasser, 2011). *G. applanatum*, like *F. fomentarius*, has been accepted as a valuable group of organisms due to its wide range of biological activities such as antioxidant, antitumor, immunomodulatory, anti-hepatotoxic, antibacterial and antiviral effects (Oviasogie et al., 2015).

The aim of this study is determined, evaluate and comparing these features with each other the antioxidant properties and total phenolic properties of *G. applanatum* and *F. fomentarius* species mushrooms collected from Kastamonu Province of the Western Black Sea Region. The data obtained for this purpose will be used to show the economic value of this class of mushroom species, which are not very valuable in the region, and to show some of their therapeutic properties.

2. MATERIAL AND METHOD

2.1. Preparation of mushroom samples

Taxonomy was determined of fresh *G. applanatum* collected from Taşköprü district of Kastamonu province and fresh *F. fomentarius* type mushrooms collected from Küre District was carried out at from Kastamonu University Faculty of Forestry Engineering. Deionized water was used in every stage of this study obtained from the Milli-Q system (18.2 MX/cm³, Human Power I Plus, Korea) Deionized water obtained from the Milli-Q system (18.2 The collected mushrooms were cut into small portions and dried under oven at 25°C for 48 hours Mushroom extracts were prepared according to standard protocol with minor modifications (Lee et al., 2004).

First, 2.5 g the dried and powdered mushroom was measured and added 20 ml methanol solution (80%) on sample. The extract mixture, which was kept at room temperature for 3 hours, was filtered through a filter crucible. 5 mL of methanol (80%) was added to the filtrate and then filtered again. The homogenate was centrifuged at 5000 rpm and 7500 rpm for 10 min (18°C), respectively, and the resulting supernatant was used for measurement.

2.2 Determination of Antioxidant Activity

DPPH radical scavenging method was used for antioxidant activity determinations. This method is based on monitoring the maximum absorbance of the purple solution of the DPPH (1,1-diphenyl-2-picryl hydrazyl) radical measured at 517 nm with decreasing color change (Bozdogan et al., 2018). In this study, absorbances were measured by spectrophotometer (SHIMADZU UVM-1240 UV-VIS, Shimadzu Corp., Kyoto, Japan). Antioxidant activities of two mushroom species were determined using the DPPH radical scavenging method and the results were calculated as percentage inhibition (%). For this, 30 µM concentration DPPH solution was used. Absorbance changes were measured in the concentration range of 1.66-6.66 mg/mL for each mushroom extract versus blank. DPPH radical damping percentage was calculated by the following formula:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Here, A_0 is the control absorbance for the blank and A_1 is the absorbance obtained for the fungal samples in solution. Additionally, IC_{50} (mg/mL) calculations were made, indicating the amount of antioxidant required to reduce the initial DPPH concentration by 50% (Frankel and Meyer, 2000).

2.3. Determination of Total Phenolic Substance

Total phenolic components of mushroom extracts were determined using Folin-Ciocalteu reagent as standard. Total phenolic components extracted from mushrooms by methanolic extraction methods, were measured at 760 nm. To implement this method, 4.5 mL of deionized water and 0.1 mL of Folin-Ciocalteu reagent were used. After 3 minutes, 0.3 mL Na₂CO₃ (2%) solution and 0.1 mL extract solution were added to the solution and stirred (Chandler and Dodds, 1983; Slinkard and Singleton, 1977).

In our total phenolic analysis, first the phenolic substances against the concentration of gallic acid were graphed. Using this calibration chart, the total phenolic concentrations of *G. applanatum* and *F. fomentarius* mushrooms were calculated as gallic acid equivalent. The equation found for gallic acid, which was used as a standard, was used to calculate the concentration of phenolic compounds:

$$\text{Absorption} = 0.0264 \text{ gallic acid (mg)} + 0.0462 \quad (R^2 = 0.989)$$

2.4. Statistical Analysis

Standard deviation data among antioxidant concentration of mushrooms were calculated using descriptive statistical analysis with SPSS software for Windows version 13 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

Antioxidant activities of mushroom extracts prepared at different concentrations were determined. Accordingly, it was determined that the inhibition values of both mushroom samples increased with concentration (Figure 1). Inhibition values in the concentration range of 0.20-0.80 mg/mL were varied from 20.35-84.59% for *G. applanatum* and 23.88-82.18% for *F. fomentarius*. At all concentrations, *F. fomentarius* showed higher % inhibition than *G. applanatum* except at the highest concentration.

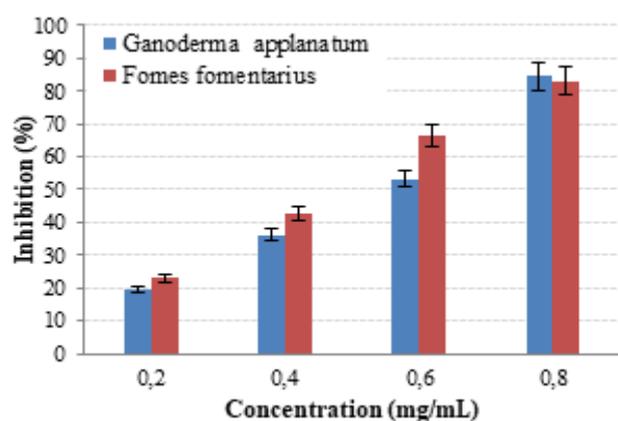


Figure 1. Inhibition (%) graph for *Ganoderma* and cultivated mushrooms at concentrations of 20-80 mg/mL. The calculated results are given as mean \pm SEM (standard error of the mean).

IC₅₀ and total phenolic substance values used to compare the antioxidant activities of both mushroom extracts are presented in Table 1. Accordingly, it was found to be 0.52 \pm 0.05 mg/mL and 0.46 \pm 0.04 mg/mL for *G. applanatum* and *F. fomentarius*, respectively. Additionally, total phenolic substance values in gallic acid equivalents (GAE) were found to be between 8.45 \pm 0.12 mgGA/L and 10.30 \pm 0.13 mg GA/L, respectively. *G. applanatum* and *F. fomentarius* showed very close total phenolic content to each other. Similarly, IC₅₀ values were found to be close to each other. *F. fomentarius* with higher total phenolic concentration showed higher activity, as expected. These results showed that there was a strong correlation between negatively correlated IC₅₀ values and total phenolic substance content, our results were shown similarity to previous studies (Gülçin et al., 2003).

Table 1. IC₅₀ values and total phenolic substances of *Ganoderma applanatum* (Gap) and *Fomes fomentarius* (Ffo) mushrooms

	Total phenolic substances (mgGA/L)	IC ₅₀ (mg/mL)
<i>Ganoderma applanatum</i> (Gap)	8.45 \pm 0.12	0.52 \pm 0.05
<i>Fomes fomentarius</i> (Ffo)	10.30 \pm 0.13	0.46 \pm 0.04

In the anticancer and antioxidant activity determination study conducted by Kolniak-Ostek et al. (2022) with *G. lucidum*, also known as the mushroom of immortality, the results showed that the characterization of compounds belonging to polyphenolic acids, flavonols, flavanols, flavones and stilbenes is composed of polyphenolic compounds (Kolniak-Ostek et al., 2022). *G. applanatum*, which is widely used in traditional Asian medicines, was previously optimized by liquid shake flask fermentation to investigate the media composition of the fungal strain and the antioxidant activity of exopolysaccharides. The results revealed that *G. applanatum* strain was able to scavenge exopolysaccharides, hydroxyl radicals, and superoxide anion radicals, which was positively associated with antioxidant activity (Zhong-Hua et al., 2015). A comparative study was carried out to elucidate the chemical properties between *G. applanatum* and *G. lucidum*, which belong to the

Basidiomycota group, and the results showed that the total phenol and flavonoid content, betulinic acid and also antioxidant activity of *G. applanatum* measured by DPPH radical scavenging and FRAP methods were higher than the other, but they showed that *G. lucidum* had higher total polysaccharide and protein content (Mohammadifar et al., 2020). *Fomes* mushroom, many biological activities have been studied in recent years, has been used for medicinal purposes for centuries thanks to its active compounds. *F. fomentarius* antioxidant enzyme activity, enzymatic activity, antimicrobial activity, antifungal activity are existed by extracts or isolated compounds from fermentation broth, mycelia and fruit bodies (Aoki et al., 1993; Chen et al., 2008).

As a result of the information compiled by Elkhateeb et al. (2020), they stated that *F. fomentarius* and *Polyporus squamosus* have medical potential and can be used as a natural source for pharmacological research with their antioxidant and antimicrobial properties (Elkhateeb et al., 2020). These studies, in line with our data, have shown that *G. applanatum* and *F. fomentarius* species have medicinal values thanks to their effective antioxidant properties, which are in good correlation with the rich total phenolic substance amount.

4. CONCLUSIONS

DPPH radical scavenging activity and total phenolic substance contents were determined and each of features compared *G. applanatum* and *F. fomentarius* species mushrooms. Results: Although the *F. fomentarius* type mushroom showed a higher antioxidant activity than the *G. applanatum*, it was found that both mushroom species were valuable in terms of both their total phenolic content and antioxidant activities. This study will provide a resource for the use of both mushroom species in alternative medicine industry applications for therapeutic purposes, thanks to their antioxidant properties.

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ETHICS COMMITTEE APPROVAL

N/A

PEER-REVIEW

Externally peer-reviewed.

AUTHOR CONTRIBUTIONS

Conceptualization: S.Ü., M.K., T.B.; Investigation: T.B.; Material and Methodology: M.K., T.B.; Supervision: T.B.; Visualization: M.K.; Writing-Original Draft: T.B.; Writing-review & Editing: S.Ü., M.K., T.B.; Other: All authors have read and agreed to the published version of manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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