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Assessment of antioxidant and anticancer attributes of crude extracts from edible mushrooms *Lycoperdon molle* and *Apioperdon pyriforme*

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ABSTRACT: The study analyzed extracts from two mushroom species, *Lycoperdon molle* and *Apioperdon pyriforme*, for their phenolic and flavonoid compound contents, antioxidant capacities, enzyme activities, and cytotoxic effects against human colorectal and breast cancer cells. LM extract was found to contain higher levels of phenolic compounds, including catechin, gallic acid, and myricetin. While AP extract had a higher concentration of gallic acid. Both extracts exhibited antioxidant activity, with LM showing slightly higher DPPH radical scavenging rates. However, their activities were weaker compared to standard antioxidants like gallic acid and quercetin. Enzyme assays revealed that both extracts inhibited CAT activity, activated GPx activity, and induced SOD activity. LM extract also inhibited GST activity, while AP extract showed activation at a certain concentration. In terms of cytotoxicity against cancer cells, LM extract displayed greater efficacy than AP, with lower IC_{50} values for both HT-29 colon cancer cells and MCF-7 breast cancer cells. Overall, the study highlights the potential differential bioactive properties of LM and AP extracts, with LM demonstrating stronger antioxidant and anti-cancer effects.

KEYWORDS: Antioxidant; anticancer; bioactive compounds; *Lycoperdon mole*; *Apioperdon pyriforme*

1. INTRODUCTION

Due to their significant nutritional and medicinal values, mushrooms can be considered functional foods. The biological activity of mushrooms has been confirmed by extensive studies and ongoing research. In the recent past, various compounds isolated from mushrooms have been widely observed in pharmaceutical applications. These mushroom components, including polysaccharides, lectins, polysaccharide-protein complexes, and various secondary metabolites, have been proven to possess important functions such as immunomodulatory, anticancer, and antioxidant effects [1-2].

Mushrooms also contain many secondary metabolites, including phenolic compounds, terpenes, and steroids. These secondary compounds isolated from mushrooms have been associated with a wide range of pharmacological effects, primarily antioxidant activity, as well as anticancer, anti-inflammatory, and immunomodulatory activities [3]. The antioxidants found in mushrooms are primarily phenolic compounds. Phenolic acids are divided into two main groups based on their chemical structures. Those in the first group contain hydroxybenzoic acid in their structures, with gallic acid being one of the important members of this group. Members of the second group, on the other hand, contain the hydroxycinnamic acid group in their structures, with caffeic acid being one of the most important examples in this group. Although phenolic compounds are non-nutritive compounds, they have various specific effects in biological systems, primarily antioxidant defense mechanisms [4]. Phenolic compounds demonstrate their antioxidant activities through chain-breaking reactions. The overall antioxidant activity of phenolic compounds depends on the involvement of phenolic hydrogen in radical reactions and the stability and chemical structure of the natural antioxidant formed during this reaction [5-7].

Lycoperdon molle Pers., a gastroid genus belonging to the family Lycoperdaceae within the order Agaricales (Basidiomycota), encompasses around 50 widely distributed species [8]. Commonly known as the

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smooth puffball, *L. molle* Pers. thrives in woodland areas with neutral to calcareous soil, characterized by globose to pyriform basidiocarps with pseudostipe and verrucose spores [9-10]. *Apioperdon pyriforme* (Schaeff.) Vizzini, a monotypic species of the genus *Apioperdon* (Kreisel & D. Krüger) Vizzini, was transferred from the genus *Lycoperdon* in 2017 due to notable phylogenetic and morphological distinctions. Commonly referred to as the pear-shaped puffball. *A. pyriforme* is a lignicolous species predominantly found in forests, gardens, or parks, particularly on decayed woods. Its key features include smooth spores, white subgleba, and non-poroid capillitium [9,11].

This study delves into the phenolic compositions and biological activities of etanolic extracts from *L. molle* (LM) and *A. pyriforme* (AP) mushrooms. The phenolic composition of ethanolic extracts was determined using ultra-performance liquid chromatography (UPLC). To the best of our knowledge, the effects of ethanolic extracts on antioxidant defense, drug metabolism enzymes, and anticancer properties have not been previously documented.

2. RESULTS AND DISCUSSION

In this research, we aimed to explore the biological potential of extracts obtained from LM and AP, two edible mushroom species, focusing on their chemical composition, antioxidant properties, and potential anticancer effects. Our study seeks to elucidate the health-promoting characteristics of these extracts and their possible applications in medicine and nutrition. The LM and AP extracts contained 8.2447 ± 0.29 mg and 4.6734 ± 0.13 mg of phenolic compounds per 100 g of dried sample (GAE/100 g), respectively. For flavonoid contents, LM showed 1.6648 ± 0.03 mg and AP exhibited 2.076 ± 0.08 mg of flavonoid compounds per 100 g of dried sample (QE/100 g). LM extract was found to be richer in phenolic compounds, while AP extract had a higher flavonoid content.

UPLC analysis of the phenolic composition revealed the presence of 5 out of 14 tested phenolic acids. The LM extract showed high levels of catechin, gallic acid, and myricetin, whereas the AP extract contained significant amounts of gallic acid and moderate levels of caffeic acid, vanillic acid, and myricetin. This finding highlights the distinct diversity of compounds between the two species within the same order, as detailed in Table 1.

Table 1. UPLC assessment of the phenolic compounds (mg/100 mL)

Compound	LM	AP
Apigenin	-	-
Catechin	8.03	-
Chlorogenic acid	-	-
Ferulic acid	-	-
Ellagic acid	-	-
Myricetin	4.06	0.94
Caffeic acid	-	0.58
Kaempferol	-	-
Luteolin	-	-
Quercetin	-	-
Gallic acid	11.36	8.95
Vanillic acid	-	0.82
Trans-cinnamic acid	-	-
p-coumaric acid	-	-

Our analysis of the chemical compounds in mushroom extracts revealed a wide range of bioactive compounds. Utilizing UPLC, we identified several phenolic compounds such as caffeic acid, catechin, gallic acid, myricetin, and vanillic acid (see Table 1), all of which are known for their beneficial health properties [21]. Furthermore, the UPLC results indicated high concentrations of gallic acid in both extracts. Gallic acid, a plant-derived phenolic compound, is notable for its nutraceutical importance and its antioxidant, anti-inflammatory, and anticancer effects [22].

Barros et al. [23] documented that the methanolic extract of LM had a total phenolic content of 11.48 mg/g extract, with notable amounts of p-hydroxybenzoic acid and vanillic acid. In a previous study of AP's nutritional composition in India, Altaf et al. [24] identified it as containing 11.5 g/100g protein, 8.78 mg/g

total phenolics, 0.44 mg/g total flavonoids, and 1.23 mg/g ascorbic acid. However, a detailed UPLC analysis of the AP mushroom has not yet been reported in the literature.

Understanding the chemical composition of these extracts is vital for elucidating their biological activities. Identifying specific compounds in these extracts helps clarify their potential mechanisms of action and targeted applications in medicine and functional foods.

One of the main objectives of this study was to assess the antioxidant activity of LM and AP mushroom extracts. The DPPH assay was used to measure the antioxidant capacity of the LM and AP extracts. Results showed that LM and AP extracts had scavenging rates of 77% and 72% respectively, at the highest concentration tested (Figure 1). The IC_{50} values were calculated as 0.6696 ± 0.0025 mg/mL for LM and 0.8663 ± 0.0086 mg/mL for AP extracts. For comparison, the positive controls, gallic acid, and quercetin, demonstrated lower IC_{50} values (gallic acid: 0.015 ± 0.007 mg/mL; quercetin: 0.017 ± 0.0012 mg/mL). Although LM exhibited a higher scavenging activity than AP, both extracts displayed weaker DPPH radical scavenging activity compared to the standard solutions of gallic acid and quercetin.

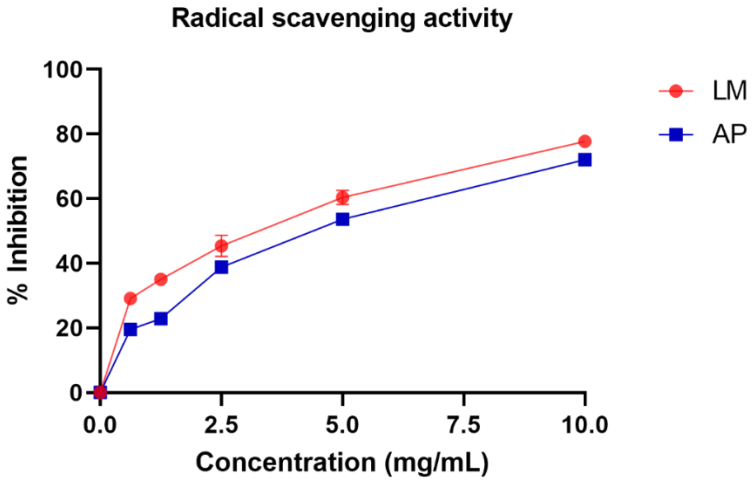

Figure 1. DPPH activity (IC_{50} values, mg/mL extract) of the extracts

Previous studies has demonstrated notable DPPH free radical scavenging activity in methanol, ethanol, and acetone extracts of LM, which corroborates our results [25-27]. These results align with earlier research highlighting the presence of various bioactive compounds, such as phenolic compounds and flavonoids, in mushrooms known for their antioxidant properties. The detected antioxidant activity is probably attributed to compounds like polysaccharides, β-glucans, and polyphenols, which are known for their capacity to scavenge free radicals and mitigate oxidative stress [28].

This indicates that these mushroom extracts have potential in reducing oxidative damage and may help in preventing chronic diseases associated with oxidative stress, such as cardiovascular diseases, diabetes and diabetic complications, cancer, chronic inflammatory diseases and neurodegenerative disorders [29].

We conducted experiments using various concentrations (ranging from 0.625 to 10 mg/mL) of mushroom extracts to evaluate their effects on CAT, GPx, GST, and SOD enzymes. The results revealed that LM and AP extracts inhibited CAT enzyme activity by 4% to 15% across all concentrations. Conversely, both LM and AP extracts activated GPx enzyme activity within the concentration range of 0.625–10 mg/mL, with peak activities observed at 5 mg/mL, reaching 21% and 28%, respectively. Additionally, LM extract inhibited GST activity by up to 8% across the tested concentrations, whereas AP extract demonstrated a 6% activation at 5 mg/mL. Furthermore, both LM and AP extracts induced SOD enzyme activity by up to 10% across all concentrations. A summary of all enzyme assay results is presented in Figure 2.

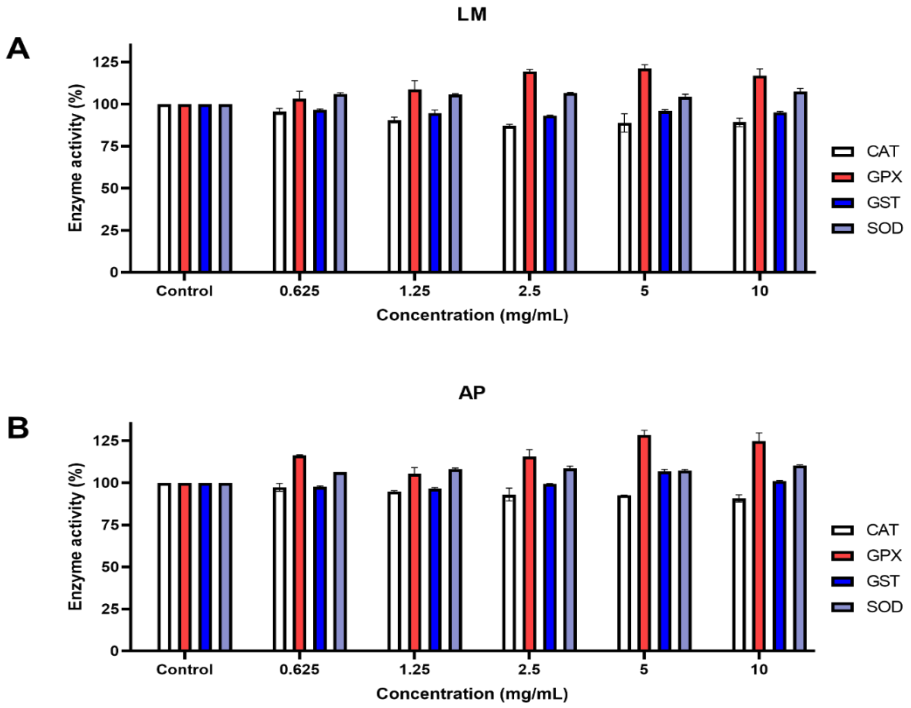

Figure 2. The effects of (A) LM and (B) AP extracts on enzymes (CAT, GPx, GST and SOD)

The catalase (CAT) enzyme catalyzes the conversion of hydrogen peroxide into water, thereby preventing its accumulation and subsequent cellular damage. Our findings indicate that both LM and AP extracts inhibited CAT enzyme activity. Glutathione peroxidase protects cells from oxidative damage by catalyzing the conversion of hydrogen peroxide (H_2O_2) and organic hydroperoxides into water and alcohols. Our results demonstrated that both extracts effectively stimulated GPx enzyme activity at all concentrations, suggesting that consuming these mushrooms as functional foods could enhance the antioxidant defense system. Glutathione S-transferase (GST) is crucial for detoxification, catalyzing the conjugation of reduced glutathione (GSH) to xenobiotic substrates. LM extract inhibited GST enzyme activity at all tested doses compared to the control, while AP extract activated GST enzyme activity. Thus, consumption of AP mushrooms may enhance detoxification-related enzyme activity, whereas the strong inhibitory effect of LM on GST enzyme activity could be beneficial in overcoming GST-mediated drug resistance. Superoxide dismutase converts superoxide anions (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen. This process protects cells from oxidative damage by managing oxidative stress and reducing the harmful effects of reactive oxygen species (ROS). This reaction manages oxidative stress in cells by decreasing the reactivity and toxicity of superoxide anions. Our study shows that both LM and AP extracts enhanced SOD enzyme activity at all concentrations, indicating that LM and AP are potent sources of antioxidant molecules and suggesting their potential as food supplements to strengthen aginst oxidative stress damage.

To evaluate the cytotoxic effects of LM and AP extracts, human colorectal (HT-29) and breast cancer (MCF-7) cell lines were exposed to concentrations ranging from 7.81 to 500 µg/mL. The inhibitory concentrations (IC_{50} values) of the extracts are detailed in Table 2.

Table 2. Cytotoxic activity (IC_{50} values, µg/mL samples)

		HT-29	MCF-7
LM	24 h	>500	482 ± 18
	48 h	>1000	494 ± 5
AP	24 h	>1000	>500
	48 h	>1000	>1000
Doxorubicin	24 h	246 ± 10	54 ± 4
	48 h	115 ± 10	29 ± 5

Both LM and AP extracts demonstrated relatively low cytotoxicity against HT-29 colon cancer cells. However, LM extract was more effective, reducing HT-29 cell viability by 36%. Additionally, LM extract exhibited greater potency against MCF-7 breast cancer cells, with IC_{50} values of 482 µg/mL at 24 hours and 494 µg/mL at 48 hours. In contrast, doxorubicin displayed IC_{50} values of 246 µg/mL (24 hours) and 115 µg/mL (48 hours) for HT-29 cells, and 54 µg/mL (24 hours) and 29 µg/mL (48 hours) for MCF-7 cells. Therefore, LM extract showed a more pronounced anti-cancer effect compared to AP, inhibiting 35% of HT-29 cells and 50% of MCF-7 cells (Figure 3).

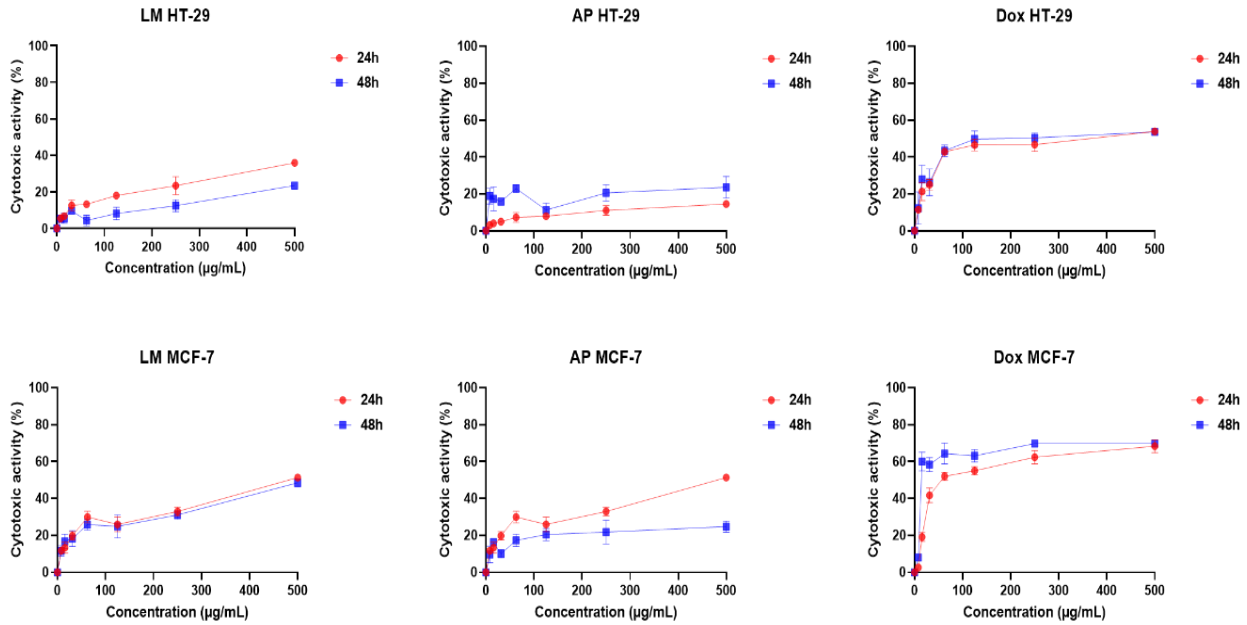

Figure 3. Cytotoxic effects (IC_{50} values, µg/mL samples)

A major discovery from our study was the examination of the anticancer potential of these mushroom extracts, representing the first investigation of its type. In vitro experiments revealed significant cytotoxic effects of both LM and AP extracts against colorectal and breast cancer cell lines (see Figure 3). Notably, LM extract exhibited stronger anti-cancer activity against both HT-29 and MCF-7 cells compared to AP.

In a separate study, Emsen et al. [30] demonstrated that methanol and water extracts from *L. molle* mushrooms showed no mutagenic effects on human lymphocytes at tested concentrations of 1-25 mg/L. The anticancer properties observed in the LM extract may be attributed to the abundance of secondary metabolites present in the extract. UPLC analysis revealed high concentrations of significant phenolic compounds such as gallic acid, catechin, and myricetin in the LM extract (see Table 1). These compounds have been thoroughly investigated for their antioxidant and anticancer effects. [31].

These results are consistent with earlier studies suggesting that bioactive compounds from mushrooms, such as polyphenols, polysaccharides, and lectins, have anticancer effects through mechanisms like regulating apoptosis, causing cell cycle arrest, and boosting immune responses [32-33,7].

3. CONCLUSION

As a conclusion, our study on the biological properties of extracts derived from *L. molle* Pers. and *A. pyriforme* (Schaeff.) Vizzini edible mushrooms underscore their potential as rich sources of antioxidants and bioactive substances. These discoveries emphasize the significance of including mushrooms in our diet to harness their potential health advantages. Continued exploration in this field offers hope for the creation of novel therapeutic approaches and functional food products. In this study, we have, to our knowledge, revealed for the first time the capacity of LM and AP extracts to modulate the activity of crucial antioxidant enzymes, alongside their potential as anticancer agents.

4. MATERIALS AND METHODS

4.1 Sample Collection and Identification

The mushroom specimens were collected from two distinct locations: *L. molle* (LM) samples were obtained from Belgrad Forest in Istanbul, while *A. pyriforme* (AP) samples were sourced from the Beşikdüzü region of Trabzon. The identification of these specimens was performed by Dr. Ilgaz Akata. The samples were then cataloged with the reference codes LM-2015 and AP-2015.

4.2 Extraction and Phenolic Compound Analysis

For the ethanol extraction, the mushroom samples were initially pulverized into a fine powder with the aid of liquid nitrogen. Ethanol was then employed to extract the compounds from these powders using a previously optimized extraction protocol [12]. The total phenolic and flavonoid compound of the samples were measured using the Folin-Ciocalteu and aluminum chloride colorimetric assays. Results were quantified as milligrams of gallic acid equivalents (GAE) and quercetin equivalents (QE) per gram of extract, respectively [13].

4.3 Ultra-Performance Liquid Chromatography (UPLC) Analysis

The phenolic and flavonoid components in the mushroom extracts were examined using a UPLC system that included a pump, degassing unit, autosampler, and photodiode array detector (PDA). The separation process utilized a reverse-phase C18 column with a gradient flow method, with detection wavelengths set at 285, 320, and 360 nm. The mobile phase A was composed of water with formic acid (19:1), and mobile phase B was methanol, with a flow rate of 0.9 ml/min over 85 minutes. Pure standards of 14 phenolic compounds, as shown in Table 1, were employed as positive controls [13].

4.4 Antioxidant Activity Assessment

In this study, the antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method to evaluate the antioxidant activities of phenolic compounds in the prepared extracts. In this method, the change in absorbance of DPPH treated with different sample concentrations was measured. A graph was plotted corresponding to the measured absorbance values against the concentrations, and the sample amount required to reduce the DPPH concentration by half was determined in µg/mL using the equation y=ax+b, and expressed as the IC_{50} value [14] .

4.5 Antioxidant Enzyme Assays

The activities of antioxidant enzymes (CAT, GST, GPx, and SOD) were assessed using cytosol extracted from bovine liver, following previously established protocols [15]. CAT activity was measured by the formation of red quinoneimine dye from hydrogen peroxide breakdown at 520 nm [16]. GST activity was determined by assessing the thioether formation with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm [17]. GPx activity was measured by monitoring the decrease in reduced NADPH at 340 nm in the presence of cumyl hydroperoxide, as described by Paglia and Valentine [18]. SOD activity was evaluated following the method of Geller and Winge [19].

4.6 Cell Culture

To investigate the anticancer effects of mushroom extracts, colon (HT-29) and breast cancer (MCF-7) cell lines were used. The cells were grown in DMEM and RPMI-1640 (Invitrogen GIBCO) cell culture media containing 10% fetal bovine serum (FBS), 20 mM L-glutamine, and 1% penicillin under conditions of 37°C and 5% CO_2, and were passaged at specific densities.

4.7 MTT Assay

The cytotoxic effects of the mushroom extracts were evaluated using the MTT assay [20]. Cells ($1x10^4$) were seeded into 96-well tissue culture plates and incubated for 18 hours. They were then treated with various concentrations of the extracts, along with vehicle (DMSO) and positive control (Doxorubicin). Cytotoxicity was quantified by calculating the IC_{50} values from the dose-response inhibition curves.

4.8 Statistical Analysis

Each experiment was performed at least three times independently, and the data are expressed as the mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA and Student's t-test, with a *p*-value of < 0.05 indicating statistical significance.

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Author contributions: Concept – N.D., O.O., O.Y.; Design – N.D.; O.O., O.Y.; Supervision – O.Y.; Resources – N.D., O.O.; Materials – N.D.; Data Collection and/or Processing – N.D., O.O.; Analysis and/or Interpretation – N.D., O.O.; Literature Search – N.D., O.O.; Writing – N.D., O.O.; Critical Reviews – N.D.

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