



Edible Mushroom Extracts: Evaluating Phenolic Content, Antioxidant Capacity, and Anticancer Effects

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Abstract: Edible mushrooms have also been recognized as valuable sources of bioactive compounds with potential therapeutic benefits. Although several edible mushrooms have demonstrated anticancer effects, limited studies have investigated their impact on colorectal cancer cells. Furthermore, it is of interest to further examine the relationship between the total phenolic content, antioxidant activity, and anticancer potential of edible mushroom extracts. Therefore, this study aimed to evaluate the anticancer effects of ethanolic extracts from eight edible mushrooms sourced from Wang Nam Khiao Farm on HT-29 cells and to investigate the relationship between their total phenolic content, antioxidant capacity, and anticancer activity. Total phenolic content was the highest in *Lentinus edodes*. The strongest antioxidant property was observed in *Pleurotus ostreatus*. Based on anticancer results, the mushrooms were classified into three groups: vigorous activity (*P. ostreatus*, *Auricularia auricular-judae*, *Pleurotus djamor*, and *Pleurotus sajor-caju*), moderate activity (*Tremella fuciformis* and *Volvariella volvacea*), and low activity (*Flammulina velutipes* and *L. edodes*). A positive relationship was generally observed between total phenolic content, antioxidant activity, and anticancer efficacy, suggesting that phenolic compounds may play a role in mediating anticancer effects. Interestingly, *L. edodes* was an exception, exhibiting elevated total phenolic content and antioxidant capacity, yet demonstrating low anticancer activity, indicating that additional bioactive compounds or mechanisms may contribute to its anticancer effects. These findings highlight the promising role of edible mushrooms as potential sources of natural antioxidants and anticancer agents for the prevention of colon cancer. Further chemical characterization and mechanistic studies are required to elucidate the compounds responsible for the biological activities.

Keywords: Edible mushroom; phenolic compound; antioxidant activity; anticancer activity

1. Introduction

Mushrooms can be divided into three main groups: medicinal mushrooms, such as *Cordyceps militaris*, *Ganoderma lucidum*, and *Schizophyllum commune*; edible mushrooms, including *Auricularia auricular-judae*, *Tremella fuciformis*, *Pleurotus sajor-caju*, and *Volvariella volvacea*; and poisonous mushrooms, such as *Amanita phalloides* and *Cortinarius rubellus* [1–2]. However, numerous edible mushrooms have long been appreciated not only as nutritious food but also as valuable sources of bioactive compounds with potential health

benefits [3,4]. Numerous studies have demonstrated that mushrooms possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, primarily due to the presence of secondary metabolites such as polysaccharides, terpenoids, and phenolic compounds [5–6]. However, the amount of these active ingredients in each mushroom varies according to the species, environmental conditions, cultivation techniques, and the stage of growth at the time of harvest [7–12]. Phenolic compounds, in particular, have been extensively studied for their antioxidant activity, which involves eliminating reactive oxygen species and preserving cellular integrity against oxidative stress [13–16]. The total phenolic content is often correlated with antioxidant capacity in natural products, including those of mushroom extracts [16–18]. Since oxidative stress plays a crucial role in carcinogenesis, antioxidants derived from edible mushrooms are considered promising agents for cancer prevention and therapy [19].

Several edible mushrooms have demonstrated the ability to kill human cancer cells, particularly breast cancer cells, through interfering with cancer cell growth, for example, by stopping the cell cycle and triggering apoptosis [20–30]. Few studies have examined the impact of mushroom extracts on colorectal carcinoma cells, and none have investigated the relationship between the total phenolic content, antioxidant ability, and the anticancer effect of edible mushroom extracts [31–34]. Our study includes *A. auricular-judae*, which is a widely consumed edible mushroom; however, research on its specific biological activities, such as anticancer effects, is significantly less extensive than that of other edible mushroom species. Hence, this research aims to evaluate the potential of various edible mushroom extracts to inhibit the proliferation of colon cancer cells while also investigating the relationship between the total phenolic content, antioxidant capacities, and anticancer efficiency exhibited by these extracts. It was hypothesized that edible mushroom extracts could inhibit the proliferation of colon cancer cells by exhibiting a positive relationship with total phenolic content and antioxidant ability. The findings from this study could lead to molecular-level studies of the cancer cell inhibition mechanisms of mushroom extracts, potentially resulting in the development of healthy food products from edible mushrooms in the future.

2. Materials and Methods

2.1 Preparation of edible mushroom extracts

Eight edible mushrooms (*Auricularia auricular-judae*, *Flammulina velutipes*, *Lentinus edodes*, *Pleurotus djamor*, *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Tremella fuciformis*, and *Volvariella volvacea*) were purchased from Wang Nam Khiao Farm, an organic mushroom farm in Nakhon Ratchasima Province, Thailand. The mushroom identities were confirmed morphologically by referring to the literature [35]. The mushrooms were cleaned by rinsing them with clean water and then air-dried until they were slightly moist. They were subsequently chopped into small pieces and then dried at 60°C for 24 hours in a hot-air oven to a constant weight. Then, the samples were ground into a fine powder using a blender. To prepare the extracts, 30 g of each sample was added to 150 mL of a 1:4 (v/v) water and 95% ethanol. The mixtures were then incubated in a shaker for 4 h at 80°C and 150 rpm. The supernatant was separated by centrifugation at 5,000 rpm for 15 min. The solvent was then evaporated using a rotary evaporator heated to 60°C, as modified from Sawangwan et al. [36]. The obtained crude extract was stored at -20°C until further investigation.

2.2 Determination of total phenolic content

The total phenolic content of eight edible mushroom extracts was determined using the Folin–Ciocalteu colorimetric method adapted from Seephonkai et al. [37]. Briefly, 10 µL of the extract was mixed with 50 µL of Folin–Ciocalteu reagent (Sigma-Aldrich, Saint Louis, USA) and 65 µL of distilled water in a 96-well plate. After incubation at room temperature for 8 minutes, 125 µL of 7.5% (w/v) sodium carbonate was added to the mixture. The reaction was allowed to proceed in the dark at room temperature for 2 h. The absorbance was measured at 765 nm using a microplate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA). Gallic acid was used as a standard to construct a standard curve.

2.3 Determination of antioxidant activity

The antioxidant activity of the mushroom extracts was determined using the DPPH radical scavenging assay, modified from the method of Loypimai et al. [38]. Briefly, 50 μ L of mushroom extract (0–5 mg/mL) was combined with 150 μ L of 0.3 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Saint Louis, USA) solution prepared in methanol in a 96-well microplate. For half an hour, the reaction mixture was kept in the dark at room temperature. The absorbance was then measured at 517 nm using a microplate reader. The percentage of DPPH radical inhibition was calculated using the following equation.

$$\% \text{ DPPH radical inhibition} = (\text{Mean OD}_{\text{blank}} - \text{Mean OD}_{\text{sample}}) / \text{Mean OD}_{\text{blank}} \times 100$$

A graph was plotted to show the relationship between the percentage of DPPH radical inhibition and the concentrations of mushroom extract. The effective concentration required to scavenge 50% of DPPH radicals (EC_{50}) was calculated from the linear regression equation obtained from the dose–response curve. The EC_{50} value reflects the concentration of the antioxidant required to decrease the initial DPPH concentration by 50%.

2.4 Cell culture

The HT-29 human colon cancer cell line (HT-29 cells) was purchased from iCell Bioscience Inc. (China). Cells were propagated and maintained in DMEM enriched with 10% FBS and 1% (v/v) antibiotic-antimycotic solution (all from Gibco, Thermo Fisher Scientific Inc., USA). The incubator provided a humidified atmosphere of 5% CO_2 and a temperature of 37°C.

2.5 Evaluation of anticancer activity

The anticancer activity of edible mushroom extracts against HT-29 cells was assessed by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT Sigma-Aldrich, Saint Louis, USA) assay. The experiment included untreated HT-29 cells as a control and culture medium as a blank control. HT-29 cells were seeded in triplicate into 96-well plates at a density of 5×10^4 cells/well and incubated for 24 hours to allow for cell adhesion. Subsequently, these cells were treated with edible mushroom extracts for 48 h. The extracts were prepared across six concentrations (5 to 0.156 mg/mL) using a two-fold serial dilution method. After treatment, the MTT solution was added to the wells, and the plates were incubated for 4 hours. Subsequently, the solution was removed, and 100% DMSO was added to dissolve the formazan crystals. Cell cytotoxicity was assessed by measuring absorbance at 570 nm with a microplate reader. The percentage of viable cells was then calculated based on the following equation.

$$\text{Cell viability (\%)} = \text{Mean OD}_{\text{sample}} / \text{Mean OD}_{\text{blank}} \times 100$$

Cell viability was calculated as a percentage relative to the untreated control. The half-maximal inhibitory concentration (IC_{50}) was then determined using a nonlinear regression equation.

2.6 High-performance liquid chromatography (HPLC) analysis of phenolic compounds

Quantification of gallic acid, tannic acid, and caffeic acid was analyzed using an HPLC system (HP 1100, Hewlett-Packard, Germany) with a UV-Vis detector and a reverse-phase C18 column (4.6 \times 250 mm, 5 μ m) at 30 °C. The mobile phase consisted of 5% acetic acid (A) and acetonitrile (B), delivered isocratically at a flow rate of 1.0 mL/min. Gallic and tannic acids were detected at 280 nm, while caffeic acid was detected at 320 nm [39].

2.7 Statistical analysis

The data, reported as mean \pm standard error of the mean (SEM), from three independent experiments were statistically analyzed using SPSS for Windows, version 21 (SPSS, Chicago, IL, USA). A completely randomized design (CRD) was employed. Normally distributed data were subjected to one-way analysis of variance (ANOVA), with statistical significance set at $p < 0.05$.

3. Results and Discussion

Eight edible mushrooms (*A. auricular-judae*, *F. velutipes*, *L. edodes*, *P. djamor*, *P. ostreatus*, *P. sajor-caju*, *T. fuciformis*, and *V. volvacea*) were extracted using ethanol and investigated for their total phenolic content, antioxidant activity, anticancer effect, and some phenolic compound content.

3.1 Total phenolic content of edible mushroom extracts

Phenolic compounds, a significant class of secondary metabolites, are crucial for antioxidant activity and are known to help prevent cancer. Their antioxidant potential is primarily due to their ability to scavenge free radicals, reduce transition metal ions, and inhibit lipid peroxidation [14,40]. To examine the total phenolic content of eight edible mushroom extracts, a Folin-Ciocalteu reagent was used in this study. As shown in Figure 1, *L. edodes* exhibited the highest concentration of total phenolic content at 1.53 mg/mL, followed by *V. volvacea* at 1.27 mg/mL, *P. sajor-caju* at 1.24 mg/mL, *P. ostreatus* at 1.17 mg/mL, *P. djamor* at 1.15 mg/mL, and *A. auricular-judae* at 1.08 mg/mL. *T. fuciformis* and *F. velutipes* exhibited low levels of total phenolic content at 0.43 mg/mL and 0.56 mg/mL, respectively.

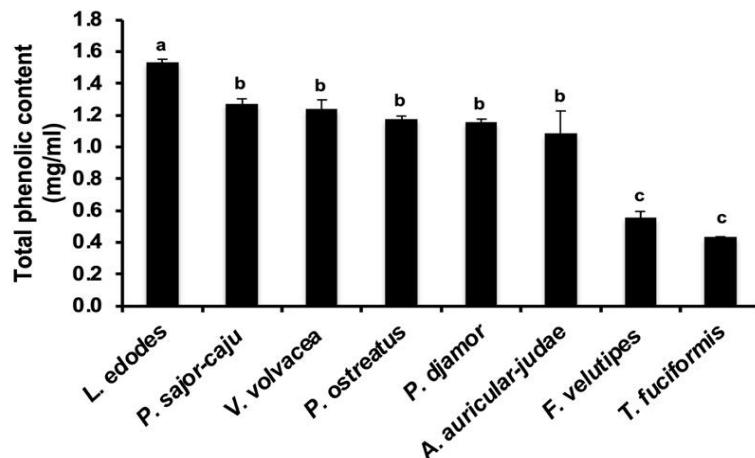


Figure 1. Total phenolic content of eight edible mushroom extracts using Folin-Ciocalteu reagent. Values presented are mean \pm SEM derived from three independent experiments. Bars with different letters (a, b, c) indicate statistically significant differences from each other ($P < 0.05$).

Cheung et al. [14] compared the total phenolic content of two edible mushrooms (*L. edodes* and *V. volvacea*), which were extracted by four extractants (petroleum ether, ethyl acetate, methanol, and distilled water). They found that both *L. edodes* and *V. volvacea* had similar maximum total phenolic content when using ethyl acetate (0.10 mg GAEs/mg of extract for *L. edodes* and 0.09 mg GAEs/mg of extract for *V. volvacea*) and water (0.08 mg GAEs/mg of extract for *L. edodes* and 0.10 mg GAEs/mg of extract for *V. volvacea*) as an extractant, respectively. Their results were consistent with our results, which showed comparable total phenolic content in *L. edodes* and *V. volvacea*.

Boonsong et al. [13] investigated the total phenolic content of five edible mushrooms in Thailand (*L. edodes*, *V. volvacea*, *P. eous*, *P. sajor-caju*, and *A. auricular-judae*), which were extracted using three different solvents (ethanol, diethyl ether, and distilled water). The highest total phenolic content was found in the condition using distilled water as an extractant. In addition, *L. edodes* had the highest total phenolic content in all extractants, followed by *V. volvacea*, *P. eous*, *P. sajor-caju*, and *A. auricular-judae*, respectively. Their results corresponded with our findings, which indicated that the highest total phenolic content was observed in *L. edodes*, and the ranking of total phenolic content in other mushrooms was similar.

3.2 Antioxidant activity of edible mushroom extracts

Since the association between total phenolic content and antioxidant activity was reported in much research [7, 10–12, 41], the antioxidant activity of eight edible mushroom extracts was investigated in this study by DPPH radical scavenging assay. As shown in Figure 2, all mushroom extracts demonstrated a dose-dependent increase in antioxidant activity. When the EC₅₀ values of all mushroom extracts were compared, the data are shown in Figure 3 and Table 1. *P. ostreatus*, *L. edodes*, *A. auricular-judae*, *P. djamor*, *P. sajor-caju*, and *V. volvacea* exhibited high antioxidant activity, indicated by their low EC₅₀ values. *F. velutipes* displayed the lowest antioxidant activity, indicated by the highest EC₅₀ value, followed by *T. fuciformis*.

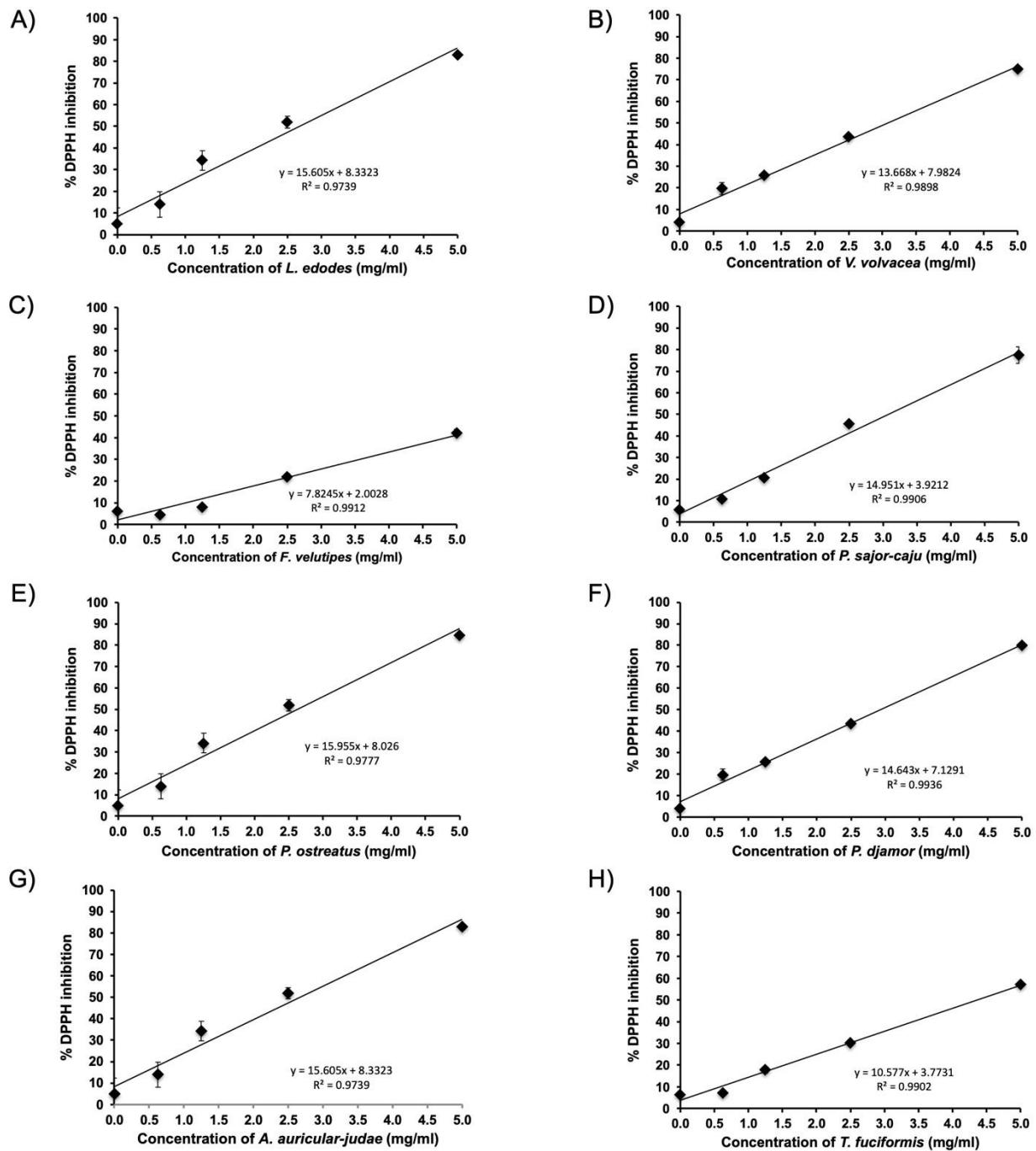


Figure 2. Percentage of DPPH inhibition of eight mushroom extracts by DPPH assay. *L. edodes* (A), *V. volvacea* (B), *F. velutipes* (C), *P. sajor-caju* (D), *P. ostreatus* (E), *P. djamor* (F), *A. auricular-judae* (G), and *T. fuciformis* (H). Values presented are mean \pm SE derived from three independent experiments.

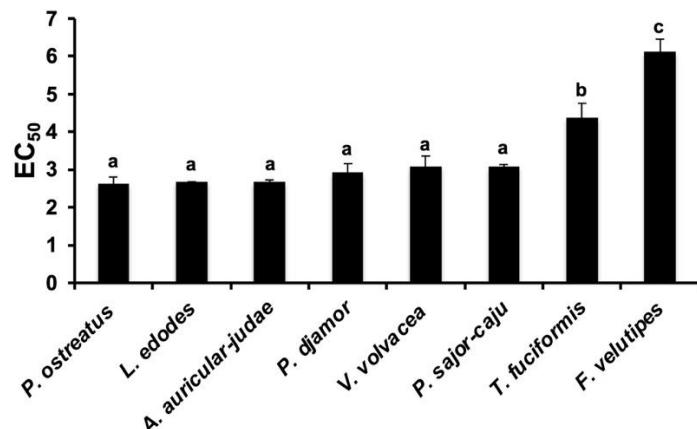


Figure 3. Half maximal effective concentration (EC₅₀) of eight mushroom extracts. These data were obtained from Figure 2, which shows the concentration of each mushroom extract required for 50% DPPH inhibition. Values presented are mean \pm SEM derived from three independent experiments. Bars with different letters (a, b, c) indicate statistically significant differences from each other ($P < 0.05$).

Table 1. Comparison of IC₅₀, EC₅₀, and total phenolic content of eight mushroom extracts.

| Mushroom extracted | IC ₅₀ (mg/mL) | EC ₅₀ (mg/mL) | Total phenolic content (mg/mL) |
|---------------------------|-----------------------------|-----------------------------|-----------------------------------|
| <i>A. auricular-judae</i> | 0.15 ± 0.03^a | 2.67 ± 0.05^a | 1.08 ± 0.12^b |
| <i>F. velutipes</i> | 0.94 ± 0.07^c | 6.13 ± 0.38^c | 0.56 ± 0.06^c |
| <i>L. edodes</i> | 1.04 ± 0.08^c | 2.67 ± 0.01^a | 1.53 ± 0.05^a |
| <i>P. djamor</i> | 0.20 ± 0.02^a | 2.93 ± 0.22^a | 1.15 ± 0.06^b |
| <i>P. ostreatus</i> | 0.11 ± 0.01^a | 2.63 ± 0.18^a | 1.17 ± 0.12^b |
| <i>P. sajor-caju</i> | 0.26 ± 0.02^a | 3.07 ± 0.06^a | 1.24 ± 0.11^b |
| <i>T. fuciformis</i> | 0.49 ± 0.06^b | 4.37 ± 0.33^b | 0.43 ± 0.04^c |
| <i>V. volvacea</i> | 0.53 ± 0.03^b | 3.08 ± 0.29^a | 1.27 ± 0.04^b |

Data were expressed as mean \pm SEM.

Means with different letters (a, b, c) within a column of each group are statistically significant differences from each other ($P < 0.05$).

Our findings align with those of Hussein et al. [42], who reported that *A. auricular-judae* extracted using methanol demonstrated the highest antioxidant activity (EC₅₀ = 0.08 mg/mL) among seven wild edible mushrooms (*Polyporus tenuiculus*, *L. sajor-caju* W, *L. squarrosulus*, *Macrolepiota procera*, *Panus conchatus*, *A. auricular-judae*, and *L. sajor-caju* D). Oke & Aslim [43] also displayed that *A. auricular-judae* extracted using distilled water (EC₅₀ = 0.309 mg/mL) exhibited greater antioxidant activity compared to *P. eryngii* (EC₅₀ = 0.545 mg/mL). Ikay Koca & Gençcelep [44] conducted a study on the antioxidant activity of 24 wild edible mushrooms, including *P. ostreatus*, which is also utilized in our experiment. The EC₅₀ values of the same mushroom reported by various research groups exhibit inconsistency. The observed variation may be attributed to the differing extraction methods and extractants utilized in the experiment [13,14]. Furthermore, it has been noted that the substrate utilized for mushroom cultivation and the maturation stages of fruiting bodies may affect the antioxidant content in mushrooms, potentially resulting in variations in antioxidant activity among the same mushroom across different studies [42].

Boonsong et al. [13] studied the antioxidant activity of five edible mushrooms in Thailand (*L. edodes*, *V. volvacea*, *P. sajor-caju*, *A. auricular-judae*, and *P. eous*) using three different extractants (ethanol, diethyl ether, and distilled water). The findings indicated that ethanol served as the most effective extractant, with *L. edodes* exhibiting the highest antioxidant activity, followed by *V. volvacea*, *P. eous*, *P. sajor-caju*, and *A. auricular-judae*, in that order. The findings contrast with our study, which indicated that *L. edodes* and *A. auricular-judae* extracted with ethanol exhibited comparable antioxidant activity. Despite the application of the same

extractant, this inconsistency may be due to differences in the extraction method and other factors, as mentioned earlier. Turning now to the comparison of antioxidant activity and total phenolic content, antioxidant activity was observed to increase with total phenolic content in this study. A group of mushrooms that had high total phenolic content exhibited high antioxidant activity. For instance, *L. edodes* and *P. ostreatus*, classified within the group with the highest total phenolic content, exhibited significant antioxidant activity. In contrast, *T. fuciformis* and *F. velutipes*, placed in the low total phenolic content group, displayed low antioxidant activity, as expected. Thus, it can be summarized that there is a positive relationship between total phenolic content and antioxidant activity. This finding is consistent with the results of Boonsong et al. [13], Cheung et al. [14], Heleno et al. [45], and Hussein et al. [42], who reported that the antioxidant activity of mushrooms is positively correlated with their phenolic compound content.

3.3 Anticancer activity of edible mushroom extracts on HT-29 cells

To assess the anticancer effect of mushroom extracts on colon cancer cells, six concentrations of a two-fold serial dilution of edible mushroom extracts (5 to 0.156 mg/mL) were incubated with HT-29 cells for 48 h, and the MTT assay was used to assess cell viability. The results, as illustrated in Figure 4, indicated that all mushroom extracts enhanced the anticancer effect in a dose-dependent manner. Upon comparing the IC_{50} values across all mushroom extracts, it was evident that these extracts could be classified into three groups (Figure 5). The first group demonstrated a significant anticancer effect, characterized by low IC_{50} values: *P. ostreatus* (0.11 mg/mL), *A. auricular-judae* (0.15 mg/mL), *P. djamor* (0.20 mg/mL), and *P. sajor-caju* (0.26 mg/mL). The second group, exhibiting a moderate anticancer effect, consisted of *T. fuciformis* (0.49 mg/mL) and *V. volvacea* (0.53 mg/mL). The third group, demonstrating a low anticancer effect, included *F. velutipes* (0.94 mg/mL) and *L. L. edodes* (1.038 mg/mL). The results suggest that the observed impact may be attributed to the elevated levels of total phenolic content and antioxidant activity present in these mushroom extracts (Table 1).

Interestingly, *A. auricular-judae*, an under-researched species, exhibited a more potent anticancer effect than the more extensively studied mushrooms, such as *L. edodes*. While *L. edodes* exhibited high total phenolic content and antioxidant activity, it demonstrated low anticancer activity. Similarly, Elhusseiny et al. [46] indicated that aqueous extracts of *L. edodes* exhibited significant antioxidant activity while displaying only moderate cytotoxicity against various cancer cell lines. However, this discrepancy may be attributed to several factors. First, although phenolic compounds are well known for their antioxidant properties through free radical scavenging, not all phenolic constituents exhibit direct cytotoxic effects on cancer cells. The type of phenolic compounds present in *L. edodes* may contribute more to antioxidant activity than to mechanisms associated with antiproliferation of cancer cells, such as apoptosis induction or cell cycle arrest. Nam et al. [47] highlighted that phenolic compounds contribute significantly to the antioxidant activity in *L. edodes*, but their anticancer activity is context-dependent and influenced by the type of compound and cellular targets. Roszczenko et al. [48] indicated that while antioxidants, such as phenolics, protect against oxidative stress, anticancer effects require mechanisms like apoptotic induction or cell-cycle arrest, which are not generally provided by all phenolics. Second, the cancer cell line used in this study may not be sensitive to the specific bioactive compounds in *L. edodes*, highlighting the importance of cell-type specificity in anticancer evaluations. Silva et al. [49] reported that elevated levels of phenolic content and antioxidant activity exhibit significant potential for cancer prevention; however, the response is determined by the specific type of phenolic and the type of cancer cells involved. This result suggests that high phenolic content and antioxidant activity do not necessarily translate to strong anticancer properties, particularly if the compounds do not induce apoptosis or inhibit cell proliferation.

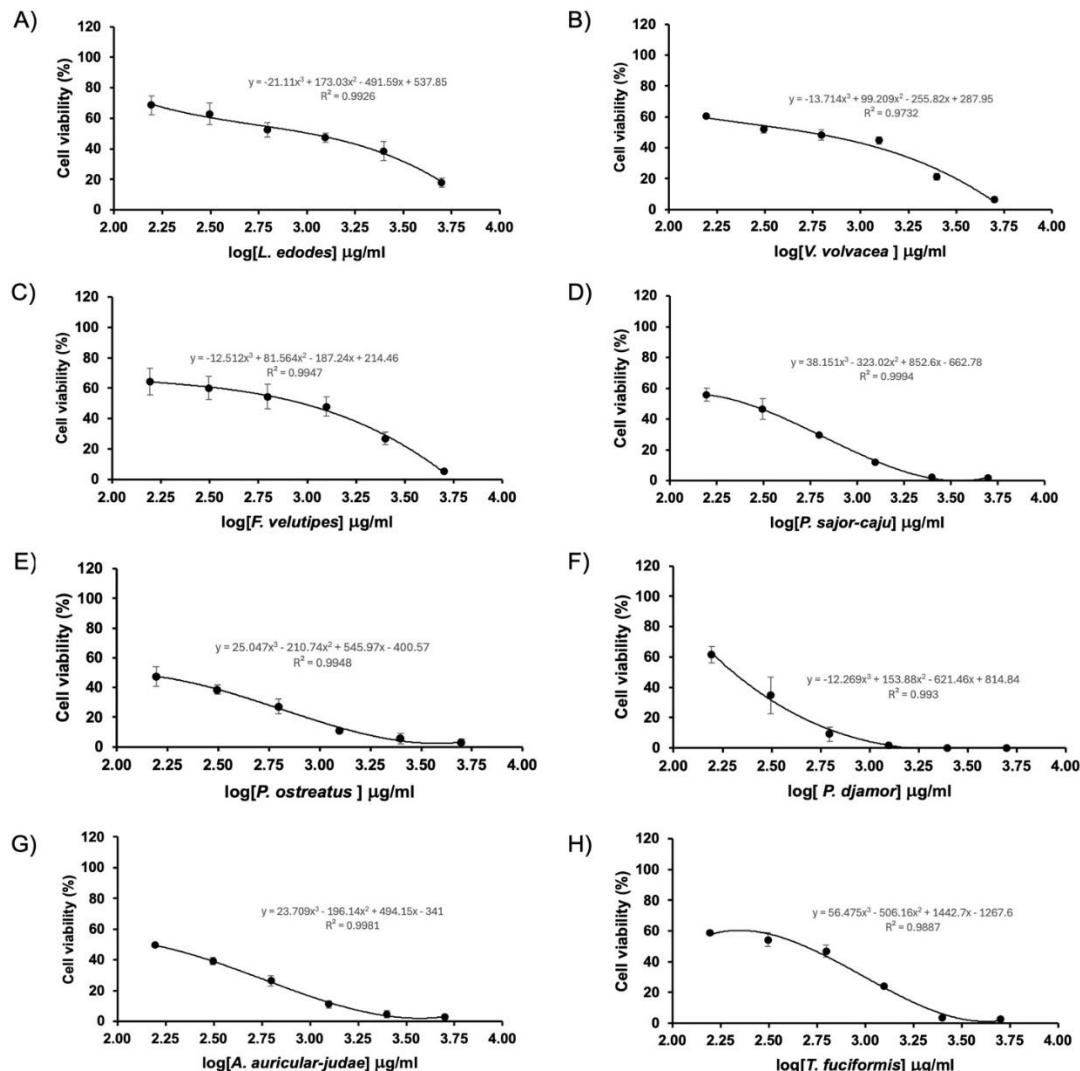


Figure 4. Cell viability of HT-29 cells toward eight mushroom extracts. Varying concentrations of eight mushroom extracts were tested with HT-29 cells by using the MTT assay. *L. edodes* (A), *V. volvacea* (B), *F. velutipes* (C), *P. sajor-caju* (D), *P. ostreatus* (E), *P. djamor* (F), *A. auricular-judae* (G), and *T. fuciformis* (H). Values presented are mean \pm SEM derived from three independent experiments.

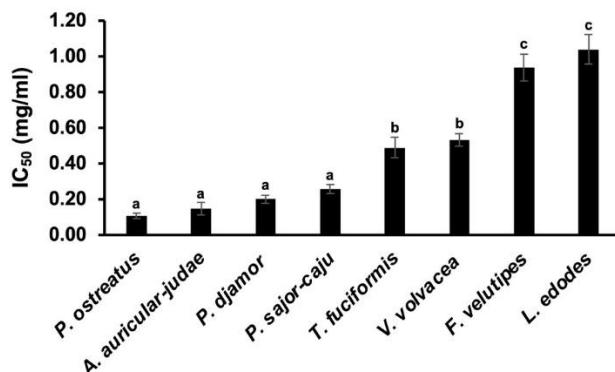


Figure 5. Half maximal inhibitory concentration (IC₅₀) of eight mushroom extracts. These data were obtained from Figure 4, which was the concentration of each mushroom extract at 50% viability of HT-29 cells. Values presented are mean \pm SEM derived from three independent experiments. Bars with different letters (a, b, c) indicate statistically significant differences from each other ($P < 0.05$).

To date, edible mushrooms have demonstrated potential anticancer properties in various studies; however, specific research on their effects on colon cancer cells is limited. The extracts of *L. edodes* demonstrated minimal impact on the proliferation of HT-29 cells, in contrast to *P. ostreatus*, which notably inhibited their growth [50]. The dichloromethane extract of *A. auricular-judae* showed potent antitumor effects against HT-29 cells, with an IC₅₀ of 0.73 mg/mL [51]. An aqueous extraction of *P. ostreatus* was shown to inhibit cell proliferation in COLO-205 colon cancer cells with an IC₅₀ of 0.812 mg/mL [52]. Compounds isolated from *P. djamor*, such as ergosta-5,7,22-trien-3 β -ol, have shown significant cytotoxic effects against HT-29 cells [53]. Lectins extracted from *P. ostreatus* exhibited anticancer activity toward HT-29 cells with an IC₅₀ of 0.27 mg/mL [54]. Lentinan from *L. edodes* demonstrated significant anticancer effects on HT-29 cells by inhibiting cell proliferation, reducing migration, and inducing cell death [55]. The ethanol extract of *V. volvacea*, when combined with cetuximab, significantly reduced cell viability in HT-29 cells [56].

3.4 HPLC Analysis of phenolic compounds in mushroom extracts

Phenolic compounds have been reported to exhibit antioxidant and anticancer activities. Thus, in this experiment, we analyzed the quantities of three specific phenolic compounds (gallic acid, tannic acid, and caffeic acid) using HPLC techniques to evaluate the relationship between bioactive substances in edible mushrooms and their antioxidant activity, as well as their effect on inhibiting the growth of HT-29 cells. The results are shown in Table 2. Gallic acid was present in the minimum quantity among the phenolic components detected in the edible mushroom extracts. *L. edodes* exhibited the highest concentration of gallic acid (0.118 mg/mL), which contains more gallic acid than other types of mushrooms by more than three times, whereas *P. ostreatus* showed no detectable levels of gallic acid. The mushrooms with the highest concentration of tannic acid were *P. sajor-caju* at 11.69 mg/mL, followed by *F. velutipes* at 6.94 mg/mL, *A. auricular-judae* at 4.19 mg/mL, and *V. volvacea* at 3.27 mg/mL, while *L. edodes* showed no detectable levels of tannic acid. *A. auricular-judae* possessed the most excellent content of caffeic acid (335.32 mg/mL), which demonstrated a significantly higher concentration of caffeic acid relative to other mushroom extracts. This aligns with the findings of Oke & Aslim [43], who reported that *A. auricular-judae* exhibited significantly higher concentrations of caffeic acid compared to other mushrooms. Palacios et al. [57] discovered that *P. ostreatus* contained the highest concentration of gallic acid when compared to other mushrooms, while no caffeic acid was detected. This contrasts with our finding, in which gallic acid was not detected; however, a small amount of caffeic acid was detected in *P. ostreatus*. The concentration of phenolic compounds in mushrooms can fluctuate based on various factors, including the mushroom species, cultivation nutrients, growing conditions, the growth stage of the mushrooms, and the age of the fresh samples [58,59].

Table 2. HPLC analyzed the quantity of phenolic compounds in eight mushroom extracts.

| Samples | Phenolic compounds (mg/mL) | | |
|---------------------------|-------------------------------|-------------------------------|---------------------------------|
| | Gallic acid | Tannic acid | Caffeic acid |
| <i>A. auricular Judae</i> | 0.02 \pm 0.002 ^c | 4.19 \pm 0.65 ^c | 335.32 \pm 94.45 ^a |
| <i>F. velutipes</i> | 0.01 \pm 0.001 ^d | 6.94 \pm 0.77 ^b | 1.00 \pm 0.01 ^b |
| <i>L. edodes</i> | 0.12 \pm 0.003 ^a | 0.00 ^f | 1.98 \pm 0.13 ^b |
| <i>P. djamor</i> | 0.03 \pm 0.002 ^b | 0.79 \pm 0.05 ^e | 2.65 \pm 0.13 ^b |
| <i>P. ostreatus</i> | 0.00 ^e | 1.05 \pm 0.11 ^e | 1.07 \pm 0.01 ^b |
| <i>P. sajor-caju</i> | 0.02 \pm 0.00 ^c | 11.69 \pm 0.15 ^a | 1.20 \pm 0.08 ^b |
| <i>T. fuciformis</i> | 0.02 \pm 0.003 ^c | 0.97 \pm 0.04 ^e | 1.05 \pm 0.04 ^b |
| <i>V. volvacea</i> | 0.01 \pm 0.002 ^d | 3.27 \pm 0.19 ^d | 6.06 \pm 0.43 ^b |

Data were expressed as mean \pm SEM.

Means with different letters (a, b, c, d, e, f) within a column of each group are statistically significant differences from each other (P<0.05).

Interestingly, no clear relationship was observed between the concentrations of gallic acid, tannic acid, and caffeic acid and the anticancer activity of the mushroom extracts against HT-29 cells. For instance, *L. edodes*, which exhibited the lowest anticancer activity, contained the highest amount of gallic acid among the eight

mushroom extracts. In contrast, *P. ostreatus*, *A. auricular-judae*, *P. djamor*, and *P. sajor-caju*, which demonstrated strong antiproliferative effects, had very low gallic acid levels. Additionally, when considering the amount of tannic acid, it was found that *F. velutipes*, which belongs to the low anticancer activity group, has a higher amount of tannic acid than other mushrooms in the high anticancer activity group, except for *P. sajor-caju*. Furthermore, *A. auricular-judae*, categorized among the group exhibiting elevated anticancer activity, contained the highest concentration of caffeic acid, whereas other mushrooms in this group possessed markedly lower levels of caffeic acid. These findings suggest that phenolic acid content alone is not a determining factor for anticancer activity in these mushroom extracts. Other bioactive components, such as polysaccharides, terpenoids, or synergistic interactions among various compounds, may contribute more significantly to the observed biological effects. This result is consistent with previous studies in other plants, which reported that the presence of phenolic acids does not always directly translate to high anticancer activity [60–63].

4. Conclusions

This study evaluated total phenolic content, antioxidant properties, and the anticancer activity of eight edible mushroom extracts. According to their cytotoxic effects against HT-29 cells, the mushrooms could be classified into three groups. The first group, exhibiting pronounced anticancer activity, included *P. ostreatus*, *A. auricular-judae*, *P. djamor*, and *P. sajor-caju*. The second group, demonstrating moderate anticancer effect, comprised *T. fuciformis* and *V. volvacea*. The third group, showing a low anticancer effect, included *F. velutipes* and *L. edodes*. A positive relationship was generally observed between total phenolic content, antioxidant activity, and anticancer efficacy, supporting the role of phenolic compounds in mediating bioactivity. It is worth noting that *L. edodes* presented an exception to this trend, as it exhibited high total phenolic content and antioxidant activity but low anticancer activity. It can be suggested that while phenolic compounds and antioxidant potential may contribute to anticancer effects, other factors or bioactive compounds are likely involved in mediating the anticancer effects. Moreover, not all phenolic constituents exhibit direct antiproliferative effects on cancer cells. These findings suggest that edible mushrooms could serve as promising natural sources of antioxidants and anticancer agents. The results may support future development of mushroom-derived nutraceuticals for colon cancer prevention. Further research is required to identify the bioactive compounds and unravel the molecular mechanisms, such as apoptosis or cell cycle arrest, that contribute to their anticancer effects. Specifically, further studies should focus on identifying the specific compounds or mechanisms unique to *L. edodes* to better understand why it exhibits potent antioxidant but weak anticancer activity. A statistical correlation analysis would also be necessary in the future to confirm the relationship between phenolic content and biological activities. Moreover, it would be beneficial to evaluate the activity of these extracts against various cancer cell lines, as well as non-cancerous normal cell lines, to assess their selectivity and toxicity. Future *in vivo* studies are also needed to verify the anticancer effects of the extracts in an animal model.

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