The Science of Nature (2026) 113:32
https://doi.org/10.1007/s00114-026-02089-0

RESEARCH

# In vitro antiproliferative activity on A549 and HT-29 cell lines and fatty acid profiling of *Agaricus bresadolanus* and *A. hortensis*

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Received: 5 January 2026 / Revised: 25 February 2026 / Accepted: 26 February 2026
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**Abstract**
The rising global burden of cancer has intensified the search for novel anticancer agents derived from natural sources, with mushrooms emerging as a rich source of bioactive metabolites. This study provides the first comparative evaluation of the toxic *A. bresadolanus* and the edible *A. hortensis* in terms of their fatty acid methyl ester (FAME) profiles and antiproliferative effects against human lung (A549) and colon (HT-29) carcinoma cell lines, alongside cytotoxicity toward normal 3T3 fibroblasts. GC–MS analysis revealed distinct lipid compositions between the two species, with *A. hortensis* exhibiting higher levels of unsaturated fatty acids (linoleic and oleic acids), whereas *A. bresadolanus* was enriched in sterol derivatives such as Δ7,22-ergostadienol. Both extracts inhibited cancer cell proliferation in a dose-dependent manner; however, *A. hortensis* demonstrated greater selectivity, preserving higher fibroblast viability relative to *A. bresadolanus*. The observed correlation between unsaturated fatty acid content and selective cytotoxicity suggests that lipid composition may contribute to the antitumor potential of mushroom extracts, though further studies are needed to establish causality. These findings identify *A. hortensis* as a candidate for further investigation as a source of lipid-derived bioactive compounds in future in vivo studies and provide a biochemical basis for the rational selection of *Agaricus* species in nutraceutical and pharmacological applications.

**Keywords** *Agaricus* spp. · Antiproliferative activity · Cytotoxicity · Fatty acid methyl esters (FAME) · lung cancer · colon cancer

## Introduction

The increasing incidence of cancer worldwide has driven the continuous search for novel anticancer agents, particularly from natural sources. Fungi, and in particular mushrooms, have emerged as a promising reservoir of bioactive compounds with potential antitumor properties (Kumar et al. 2021; Rauf et al. 2022). Several species of edible and medicinal mushrooms have been studied for their capacity to inhibit cancer cell proliferation, induce apoptosis, or modulate immune responses (Patel and Goyal 2012). However, relatively few studies have combined chemical profiling [such as fatty acid methyl ester (FAME) analysis] with comparative cytotoxicity across cancerous and noncancerous cell lines (Sefidabi et al. 2023; Hesham et al. 2025).

Within the genus *Agaricus*, many species are widely consumed (e.g., *Agaricus bisporus*) and studied for antitumor activity (Panda et al. 2022). On the other hand, certain species within the genus *Agaricus* are known or suspected

Communicated by Siddartha Yerramilli

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Published online: 06 March 2026

to possess toxic or indigestible properties. For instance, *A. bresadolanus* has been linked to reported cases of mushroom poisoning with variable clinical manifestations, and morphological and phylogenetic studies suggest close relationships with known toxic species, including *A. infidus* and *A. romagnesii* (Edwards and Leech 2014; Parra et al. 2023). *A. bresadolanus*, which differs from other *Agaricus* species by yellowing when crushed or damaged the fruitbodies, causes gastrointestinal syndrome (Cappelli 1984; Bresinsky and Besl, 1990). Comparisons between toxic and edible *Agaricus* species may provide insights into both the chemical determinants of antitumor activity and the associated safety margins. *A. hortensis* (Pers.) is recognized as an edible species, but to our knowledge, its anticancer potential has not been systematically explored in vitro. However, other *Agaricus* species, such as *A. bisporus* and *A. brasiliensis*, have demonstrated antitumor activity in various cancer cell lines (Kıvrak et al. 2020; Panda et al. 2022).

Fatty acids and their derivatives are among the classes of metabolites known to influence cell membrane composition, signaling pathways, oxidative stress, and apoptosis in cancer cells (Comba et al. 2011). Profiling of fatty acid methyl esters (FAMEs) in mushroom extracts can demonstrate correlations between specific fatty acid components and observed biological effects. For instance, Lee et al. (2022) identified linoleic and oleic acids as the main antiproliferative constituents in the ethanol extract of *Oudemansiella tomentosa*. Integrating FAME analysis with cell-based assays allows researchers to link chemical composition with functional antiproliferative outcomes (Nan et al. 2025), providing a comprehensive understanding of the bioactive properties of mushroom extracts.

In the present study, we aimed to evaluate and compare the antiproliferative effects of *A. bresadolanus* (a toxic species) and *A. hortensis* (an edible species) against human lung carcinoma (A549) and colon carcinoma (HT-29) cell lines, while assessing cytotoxicity toward normal 3T3 fibroblast cells to gauge selectivity. In parallel, we performed FAME analysis on both mushroom extracts to characterize their fatty acid composition and explore possible relationships between fatty acid profiles and anticancer activity. To our knowledge, this is the first comparative investigation combining FAME profiling with selective cytotoxicity assays for these two *Agaricus* species in lung and colon cancer models.

This study contributes uniquely to the current body of knowledge by providing the first integrative evaluation of two phylogenetically related but biologically distinct *Agaricus* species—one toxic and one edible—in terms of both their lipid-derived metabolites and antiproliferative effects. The findings are expected to broaden our understanding of the biochemical diversity and pharmacological potential within the genus *Agaricus*. Furthermore, by correlating FAME profiles with selective cytotoxicity in cancer versus normal cells, this research may guide the future isolation of specific bioactive fatty acids or secondary metabolites responsible for anticancer activity. In a broader perspective, such comparative analyses can inform the rational selection of mushroom species for nutraceutical, pharmaceutical, or toxicological applications, ultimately paving the way for future in vivo studies on mushroom-derived anticancer agents.

## Materials and methods

### Fungal material collection and preparation

The fungal species *A. bresadolanus* Bohus (toxic) and *A. hortensis* Pers. (edible) were collected from mixed pine-oak forest clearings with grassy areas in the Zeytin Alan region of Köyceğiz, Muğla, Turkey, on December 13, 2022 (Voucher No: A7087 and A7086, respectively). Fresh fruiting bodies were immediately dried overnight in a fan-assisted oven at 35 °C to prevent microbial degradation. Once dried, the samples were finely ground using liquid nitrogen to preserve thermolabile bioactive compounds (Şen et al. 2012).

### Cell lines

The human lung carcinoma epithelial cell line A549 (ATCC, CCL-185™), human colorectal adenocarcinoma cell line HT-29 (ATCC, HTB-38™) and NIH/3T3 fibroblast (ATCC, CRL-1658™) used in the current work were commercially available.

### Fatty acid methyl ester (FAME) analysis

Lipid extraction and fatty acid methyl ester (FAME) analysis were carried out using a modified hexane extraction and transesterification method. Briefly, 10.00 g of dried mushroom material was extracted with 50 mL of hexane at room temperature for three days. After complete solvent removal using a rotary evaporator, 100 mg of the resulting hexane extract was transferred into a 20 mL glass tube and diluted with 10 mL of hexane. The mixture was vortexed for 5 min, followed by the addition of 100 µL of 2 N KOH in methanol to initiate transesterification. The tube was tightly capped, vortexed for 3 min, and then centrifuged at 4000 rpm for 10 min. The upper phase was filtered through a Macherey-Nagel Chromafil Xtra PTFE-20/25 (0.20 µm) syringe filter and analyzed using an Agilent 7890 A gas chromatograph coupled with a 5975 C mass selective detector (MSD). Separation was achieved on a J&W HP-5MS UI column (30 m

× 0.25 mm × 0.25 µm) using helium as the carrier gas at a constant flow rate of 1.8 mL/min. The oven temperature program was set as follows: 110 °C for 1 min, ramped at 10 °C/min to 180 °C (held for 10 min), then increased at 10 °C/min to 280 °C and maintained for 30 min, resulting in a total run time of 58 min. The injector was maintained at 250 °C in split mode (split ratio of 50:1). The mass spectrometer was operated in scan mode (35–450 m/z) with an electron multiplier voltage of 1200 V, source temperature of 230 °C, and quadrupole temperature of 150 °C. Chromatograms obtained from the analysis were used to determine the fatty acid composition of the mushroom extracts. Detection is done with 5975 C mass detector and MSD Chem Station E.02.02 Data Analysis Software. Wiley 08 (W8N08.L) and Wiley 12 (W12N20.L) and NIST 08 Mass Spectral Library with MS Search and AMDIS Deconvolution software (Kıvrak et al. 2020).

## Extraction procedure

For extraction, 1 g of powdered material from each species was mixed with 10 mL of 99% ethanol (Merck KGaA, Darmstadt, Germany). The mixtures were subjected to ultrasonic agitation combined with intermittent vortexing to enhance solubilization. Each extraction cycle lasted 30 min, followed by centrifugation at 4000 rpm for 4 min at a controlled temperature. The supernatants were collected via filtration, and the extraction procedure was repeated twice to ensure maximal recovery of bioactive constituents. All filtrates from each species were pooled and concentrated under a chemical fume hood to obtain crude extracts. The resulting crude extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions at a concentration of 200 mg/mL stock solutions. For experimental applications—including cytotoxicity, cell migration, and antiproliferative assays—working solutions were freshly prepared in Dulbecco's Modified Eagle Medium (DMEM) (adapted from Abdul Ghafoor et al. 2022).

## Determination of cytotoxic dose

The cytotoxic effects of the mushroom extracts were evaluated in vitro using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, following the method described by Mosmann (1983). NIH/3T3 fibroblast cells were used to assess cytotoxicity. Cells were counted using the Trypan Blue exclusion method and seeded into 96-well microplates at a density of 1 × 10^4 cells per well in 200 µL of culture medium. Plates were incubated for 24 h at 37 °C in a humidified atmosphere containing 5% $CO_2$.

After incubation, the culture medium was removed, and cells were treated with varying concentrations of mushroom extracts prepared in DMEM for 24 h. Following treatment, wells were carefully emptied, washed twice with 100 µL of Dulbecco's Phosphate Buffered Saline (D-PBS), and replenished with fresh medium (100 µL per well) containing 10 µL of MTT solution. Plates were then incubated for 3 h at 37 °C under 5% $CO_2$ and 98% humidity. After incubation, the medium was removed, and 100 µL of DMSO was added to each well to dissolve the formazan crystals. Plates were shaken at room temperature for 25 min, and the absorbance was measured at 540 nm (Abdul Ghafoor et al. 2022; Yeniocak et al. 2025). Cell viability was calculated relative to the untreated control, which was considered 100%. The half-maximal inhibitory concentration ($IC_{50}$) representing the extract concentration causing 50% cell death was determined using statistical analysis (Mosmann 1983). The percentage of viable cells was calculated using the following formula:

$$(\%) = [100 \times (Sample_{abs})/(Control_{abs})]$$

$$Sample_{abs} : \ Absorbance\ in\ extract - treated\ wells$$

$$Control_{abs} : \ Absorbance\ of\ control\ wells\ .$$

## Determination of the antiproliferative effects of agaricus mushroom extracts on A549 lung and HT-29 colon cancer cell lines

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The antiproliferative effects of the mushroom extracts on A549 lung and HT-29 colon cancer cell lines were also evaluated in vitro using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, following the method described by Mosmann (1983). Cells were maintained at 37 °C in a humidified incubator containing 5% $CO_2$ using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin-streptomycin) with high glucose content. Cells were seeded into 96-well plates at a density of $1 \times 10^4$ cells per well and incubated for 24 h under the same conditions. After initial incubation, each well was treated with 200 µL of fresh DMEM containing varying concentrations of the fungal extracts and incubated for an additional 24 h. Following treatment, wells were carefully emptied and washed twice with 100 µL of Dulbecco's Phosphate Buffered Saline (D-PBS). Subsequently, 10 µL of MTT solution and 100 µL of fresh medium were added to each well, and plates were incubated for 3 h at 37 °C under 5% $CO_2$ and 98% humidity. After incubation, the medium was removed, 100 µL of DMSO was added to dissolve formazan crystals, and plates were gently shaken

for 25 min at room temperature. Absorbance was measured at 540 nm using a microplate reader (Abdul Ghafoor et al. 2022; Yeniocak et al. 2025). Cell inhibition was calculated relative to the untreated control. The half-maximal inhibitory concentration ($IC_{50}$) representing the extract concentration causing 50% cell death was determined using statistical analysis (Mosmann 1983). The percentage of inhibition was calculated using the following formula:

$$(\%) = [100 \times (Sample_{abs})/(Control_{abs})]$$

$$Sample_{abs} : \ Absorbance\ in\ extract\ treated\ wells$$

$$Control_{abs} : \ Absorbance\ of\ control\ wells$$

All experiments were performed in quadruplicate, with eight technical replicates per experiment. For all cell culture assays, negative control groups containing only culture medium were included to monitor and prevent potential contamination.

### Statistical analysis

The data were expressed as mean ± standard deviation (SD) of at least three independent experiments. Microsoft Excel was used for $IC_{50}$ determination. Dose-dependent inhibition data were subjected to a two-stage regression analysis. Regression was conducted using the LINEST function in Microsoft Excel, from which the slope, intercept, coefficient of determination ($R^2$), and associated standard errors were derived.

## Results

### FAME analysis of *A. bresadolanus* and *A. hortensis*

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GC–MS analysis identified differences in the fatty acid methyl ester (FAME) compositions of *A. bresadolanus* and *A. hortensis* (Table 1). The total identified compounds constituted 96.86% of the total extract composition in *A. bresadolanus* and 98.00% in *A. hortensis*.

In *A. hortensis*, palmitic acid methyl ester (C16:0) and linoleic acid methyl ester (C18:2n6c) were the predominant components, constituting 36.50% and 28.41% of the total fatty acids, respectively. Oleic acid methyl ester (C18:1n9c) comprised 12.94%, and stearic acid methyl ester (C18:0) comprised 9.07% of the total fatty acids. Minor constituents, including pelargonic (C9:0), pentadecanoic (C15:0), and arachidic acid (C20:0) methyl esters, were present at concentrations below 3%.

**Table 1** Fatty acid methyl ester (FAME) composition of *A. bresadolanus* and *A. hortensis* determined by GC–MS analysis. Values are given as mean ± SD ($n = 3$, $p < 0.05$). "n.d.", not detected

| No | Fatty acid methyl ester (FAME) | RT | A. bresadolanus | A. hortensis |
|---|---|---|---|---|
| 1 | Pelargonic acid (C9:0) | 6,905 | n.d. | 2,398 |
| 2 | Pentadecanoic acid methyl esters (C15:0) | 19,630 | n.d. | 1,174 |
| 3 | Palmitoleic acid methyl esters (C16:1) | 21,975 | n.d. | 1,321 |
| 4 | Palmitic acid methyl esters (C16:0) | 22,930 | 28,548 | 36,501 |
| 5 | cis-10-Heptadecenoic acid methyl esters (C17:1) | 25,826 | 1,627 | n.d. |
| 6 | Heptadecanoic acid methyl esters (Margaric acid) (C17:0) | 26,103 | 0,650 | 1,843 |
| 7 | Linoleic acid methyl esters (C18:2n6c) | 27,917 | 28,979 | 28,411 |
| 8 | Oleic acid methyl esters (C18:1n9c) | 28,211 | 3,696 | 12,940 |
| 9 | Elaidic acid methyl esters (C18:1n9t) | 28,381 | n.d. | 1,698 |
| 10 | Stearic acid methyl esters (C18:0) | 29,208 | 6,320 | 9,066 |
| 11 | Docosane | 31,900 | 3,031 | n.d. |
| 12 | Arachidic acid methyl esters (C20:0) | 35,056 | 1,271 | 2,649 |
| 13 | Eicosanoic | 42,760 | 2,260 | n.d. |
| 14 | Ergosta-5,7,9(11), 22-Tetraen-3-ol | 53,876 | 3,659 | n.d. |
| 15 | Delta 7,22-Ergostadienol | 55,016 | 16,815 | n.d. |
| 16 | 5,6-Dihydroergosterol | 55,030 | n.d. | n.d. |
| 17 | 7,22-Ergostadienone | 55,476 | n.d. | n.d. |
| 18 | Campesterol | 56,316 | n.d. | n.d. |

Values are presented as the mean of three parallel measurements ($n = 3$). ($p < 0.05$).

In contrast, *A. bresadolanus* contained comparable proportions of palmitic (28.55%) and linoleic (28.98%) acids, as well as sterol derivatives including Δ7,22-ergostadienol (16.81%) and ergosta-5,7,9(11),22-tetraen-3-ol (3.66%), which were not detected in *A. hortensis*.

## Cytotoxicity and antiproliferative effects

The cytotoxic and antiproliferative activities of *A. bresadolanus* and *A. hortensis* extracts were evaluated in A549 (lung carcinoma), HT-29 (colon carcinoma), and normal 3T3 fibroblast cells using the MTT assay. Both extracts inhibited cancer cell proliferation in a dose-dependent manner, with varying effects on cancer versus normal cells (Figs. 1, 2, 3, 4, 5 and 6).

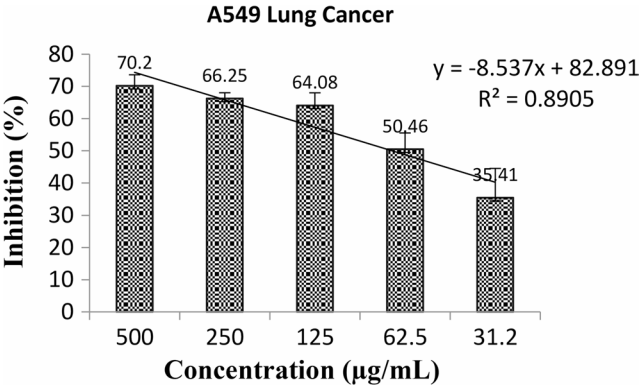



**Fig. 1** The antiproliferative activity of the extract of *A. bresadolanus* on A549 lung cancer cells. Data points represent mean inhibition percentages. Error bars represent mean ± SD from three independent experiments. ($p < 0.01$)

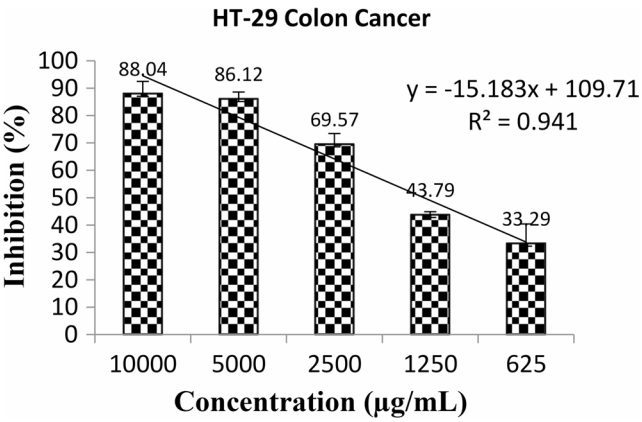



**Fig. 2** The antiproliferative activity of the extract of *A. bresadolanus* on HT-29 colon cancer cells. Data points represent mean inhibition percentages. The results given as mean ± SD from three independent experiments. ($p < 0.01$)

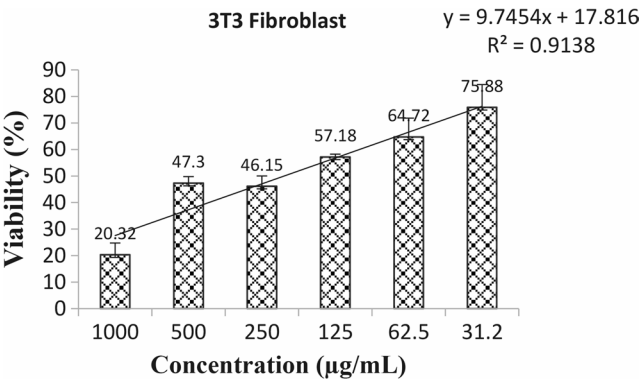



**Fig. 3** The cytotoxicity of the extract of *A. bresadolanus* on 3T3 fibroblast cells. Data points represent mean viability percentages. The results given as mean ± SD from three independent experiments. ($p < 0.01$)

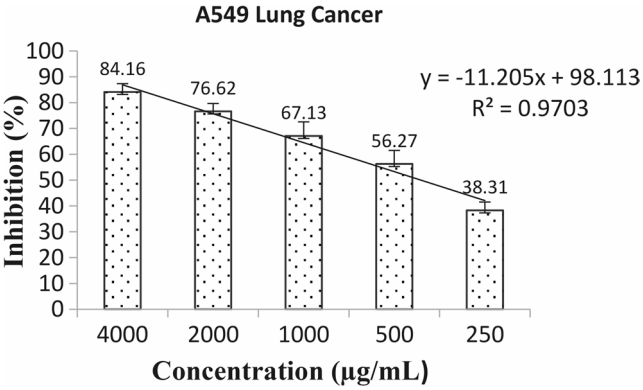



**Fig. 4** The antiproliferative activity of the extract of *A. hortensis* on A549 lung cancer cells. Data points represent mean inhibition percentages. The results given as mean ± SD from three independent experiments. ($p < 0.01$)

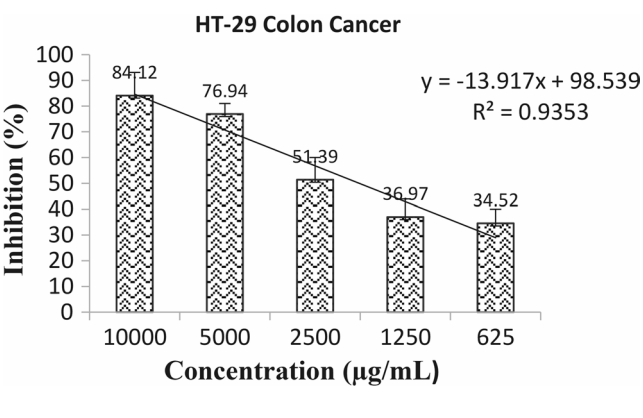



**Fig. 5** The antiproliferative activity of the extract of *A. hortensis* on HT-29 colon cancer cells. Data points represent mean inhibition percentages. The results given as mean ± SD from three independent experiments. ($p < 0.01$)

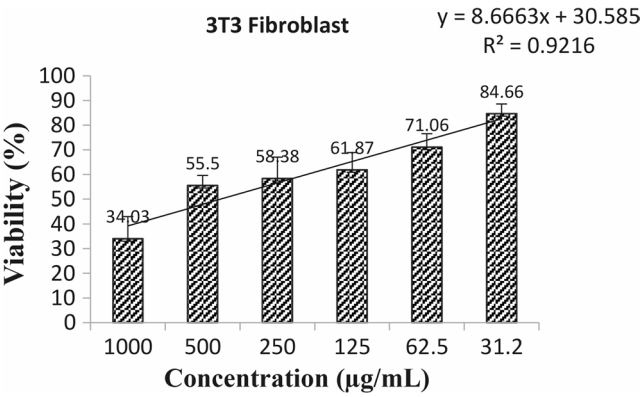



**Fig. 6** The cytotoxicity of the extract of *A. hortensis* on 3T3 fibroblast cells. Data points represent mean viability percentages. The results given as mean ± SD from three independent experiments. ($p < 0.01$)

At the highest tested concentrations, *A. bresadolanus* inhibited A549 cell proliferation by 70.2% (500 µg/mL) and HT-29 cell proliferation by 88.04% (10,000 µg/mL) (Figs. 1 and 2). Under the same conditions, normal 3T3 fibroblast viability was reduced to 20.32% at 1000 µg/mL (Fig. 3).

The extract of *A. bresadolanus* exhibited concentration-dependent growth inhibition against A549 lung cells (Fig. 1). Linear regression analysis showed a concentration-dependent relationship for all tested cell lines (Figs. 1, 2, 3, 4, 5 and 6).

*A. hortensis* also inhibited cancer cell proliferation in a dose-dependent manner (Figs. 4 and 5). In A549 cells, inhibition reached 68.44% at 500 µg/mL, while HT-29 inhibition was 82.17% at 10,000 µg/mL. In 3T3 fibroblasts, cell viability was 75.88% at 31.2 µg/mL and 64.72% at 62.5 µg/mL (Fig. 6).

The extract of *A. hortensis* exhibited concentration-dependent growth viability 3T3 fibroblast cells (Fig. 6). Treatment with 31.2–1000 µg/mL resulted in viability rates ranging from 34.0% to 84.6%. Significant viability (>50%) was achieved at concentrations ≥ 500 µg/mL.

The $IC_{50}$ values of *A. bresadolanus* and *A. hortensis* on A549, HT-29, and 3T3 cell lines are given in Table 2.

The $IC_{50}$ values for each extract and cell line are presented in Table 2. *A. bresadolanus* exhibited $IC_{50}$ values of 93.94 µg/mL against A549 cells, 1551.1 µg/mL against HT-29 cells, and 207.13 µg/mL against 3T3 fibroblasts. A. hortensis exhibited $IC_{50}$ values of 486.49 µg/mL against A549 cells, 2435.81 µg/mL against HT-29 cells, and 542.36 µg/mL against 3T3 fibroblasts.

## Discussion

In this study, we compared two phylogenetically related but biologically divergent *Agaricus* species—*A. bresadolanus* (toxic) and *A. hortensis* (edible)—in terms of their FAME (fatty acid methyl ester) profiles and their antiproliferative/cytotoxic effects on cancer (A549, HT-29) and normal (3T3) cell lines.

The FAME analysis revealed that *A. hortensis* contains a higher proportion of unsaturated fatty acid methyl esters such as linoleic and oleic acids, while *A. bresadolanus* contains sterol derivatives (e.g. Δ7,22-ergostadienol) not detected in the edible species. Whether these compositional differences are associated with the observed cytotoxic and selectivity profiles requires further investigation. These differences may suggest distinct lipid metabolic profiles between the two species, potentially linked to their divergent biological effects and toxicological properties.

**Table 2** $IC_{50}$ values of *A. bresadolanus* and *A. hortensis* on A549, HT-29, and 3T3 cell lines

| Mushrooms | Cell lines | | |
|---|---|---|---|
| | A549 | HT-29 | 3T3 |
| *A. bresadolanus* | 93.94 ± 5.29 | 1551.1 ± 0.04 | 207.13 ± 5.12 |
| *A. hortensis* | 486.49 ± 1.58 | 2435.81 ± 6.94 | 542.36 ± 6.61 |

Multiple studies have emphasized that fungal lipids—including free fatty acids, sterols, and other lipid derivatives—can influence cell membrane fluidity, signaling pathways, oxidative stress responses, and programmed cell death (Nandi et al. 2024). In particular, mushroom oils are known to include linoleic acid, which plays a key role in membrane integrity and signaling (Tan et al. 2024). The presence of sterols and unusual lipid derivatives in *A. bresadolanus* may contribute to membrane perturbation and generalized cytotoxicity, rather than selective antitumor activity.

Moreover, mushrooms are widely recognized as sources of bioactive compounds (polysaccharides, terpenoids, sterols, phenols, and lipids) with antitumor potential (Zade et al. 2025). The integrative approach of comparing FAME profiles with cell-based responses, as in our study, may generate hypotheses about which lipid classes could be associated with cytotoxic or antiproliferative effects.

Edible wild mushrooms have been reported to contain higher proportions of unsaturated fatty acids (Bulam et al., 2021). The FAME profile of *A. hortensis* is consistent with this trend, while the sterol-rich composition of *A. bresadolanus* differs markedly. Further studies are needed to determine whether these compositional differences are functionally related to the observed selectivity patterns.

In cell culture assays, *A. bresadolanus* inhibited A549 and HT-29 cell proliferation (70.2% at 500 µg/mL and 88.0% at 10,000 µg/mL, respectively) and reduced 3T3 fibroblast viability to 20.3% at 1000 µg/mL. These results indicate that the cytotoxic effect was not selective for cancer cells.

*A. hortensis* also suppressed cancer cell proliferation in a dose-dependent manner, while 3T3 cell viability ranged from 64 to 75% over the tested concentrations. This differential response suggests that *A. hortensis* may be less toxic to normal cells under these in vitro conditions, though further studies are required to assess selectivity.

The obtained $IC_{50}$ values demonstrated that both extracts exhibited concentration-dependent inhibition, albeit with different potencies. The extract of *A. bresadolanus* showed stronger cytotoxic activity, particularly against the A549 lung cancer cell line ($IC_{50}$ = 93.94 µg/mL). In contrast, the *A. hortensis* extract, while exhibiting relatively higher $IC_{50}$ values against both A549 and HT-29 colon cancer cells, displayed significantly lower toxicity towards normal 3T3 fibroblast cells ($IC_{50}$ = 542.36 µg/mL).

Edible mushroom extracts have been reported to exhibit selective antitumor effects through various mechanisms including apoptosis induction, cell cycle arrest, oxidative stress, and immunomodulation (Roszczenko et al. 2024; Hasan

et al. 2025). The lipid composition of *A. hortensis*, together with other potential bioactive compounds, may contribute to its differential effects on cancer versus normal cells observed in this study. Overall, *A. hortensis* demonstrated a more balanced cytotoxic profile, inhibiting tumor cell growth effectively while preserving normal cell viability to a greater extent than *A. bresadolanus*. These findings align with the FAME results, where the higher unsaturated fatty acid content of *A. hortensis* may contribute to its selective antiproliferative effects. However, the specific mechanisms remain to be elucidated.

Several limitations of this study should be acknowledged. First, as an in vitro investigation, these findings cannot be directly extrapolated to in vivo conditions, and the concentrations tested may not be physiologically relevant. Future animal studies are necessary to evaluate potential therapeutic applications. Second, the use of single cell lines for each cancer type limits generalizability; testing additional cell lines would provide a more comprehensive understanding. Third, while FAME analysis identified compositional differences, the extracts are complex mixtures containing other bioactive compounds (e.g., polysaccharides, phenolics) not characterized here. Therefore, the observed effects cannot be attributed solely to the identified lipids, and the associations remain correlational rather than causal. Fourth, the MTT assay does not elucidate mechanisms of cell death; further studies incorporating apoptosis and oxidative stress analyses are needed. Despite these limitations, this study provides a foundation for understanding the differential biological activities of these *Agaricus* species.

## Conclusion

This study presents the first comparative evaluation combining FAME profiling and selective cytotoxicity assays of *A. bresadolanus* and *A. hortensis* in lung and colon cancer cell models. Our results show that *A. hortensis*, with a lipid profile richer in unsaturated fatty acid methyl esters, exhibits substantial antiproliferative activity against cancer cells while preserving greater viability in normal fibroblasts compared to *A. bresadolanus*. In contrast, *A. bresadolanus* exerts potent cytotoxic effects but with limited selectivity.

These findings suggest that lipid composition may play a contributory role in the selective anticancer potential of mushroom extracts. *A. hortensis* emerges as a promising candidate for further bioactive compound isolation, mechanism-of-action studies, and in vivo validation. By integrating chemical profiling with functional assays, this work lays the groundwork toward the rational selection of mushroom species for nutraceutical or pharmaceutical development and points to future directions for mechanistic and translational research.

**Author contributions** Hakan Allı contributed to the collection and taxonomic verification of mushroom specimens and participated in manuscript preparation. Aysel Uğur and Nurdan Saraç supervised the study, guiding the experimental design and cell culture investigations; additionally, Nurdan Saraç contributed to the writing and critical revision of the manuscript. İrem Demir and Sevil Yeniocak performed experimental procedures involving cell culture. Ergun Kaya contributed to data acquisition and interpretation, as well as manuscript drafting. İbrahim Kıvrak carried out the fatty acid methyl ester (FAME) analysis. All authors reviewed and approved the final version of the manuscript.

**Funding** Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). Declaration. The authors declare that they have not any funding for this study.

**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Competing interests** The authors declare no competing interests.

**Ethical Approval** This article does not present studies involving human or animal subjects.

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