ANTIOXIDANT, ANTIBACTERIAL AND ANTIBREAST CANCER (MCF-7) ACTIVITIES OF THE MUSHROOM *TYLOPILUS FELLEUS*

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Abstract

Tylopilus felleus is a wild mushroom distributed in Central Vietnam but has remained poorly characterized with respect to its chemical composition and biological activities. In this study, ethanol extract of *T. felleus* collected in Hue city was antioxidant capacity, antibacterial activity, anti-inflammatory effects on RAW 264.7 macrophages and cytotoxicity against MCF-7 breast cancer cells. GC-MS identified 13 compounds dominated by long chain fatty alcohols, fatty acids and sterols-particularly (R)-(-)-14-methyl-8-hexadecyn-1-ol, pentadecylic acid, stellasterol, and ergosterol derivatives.

Biologically, the extract demonstrated strong antioxidant activity (IC_{50} of DPPH = 20.41 µg/mL; ABTS = 23.86 µg/mL), moderate to strong antibacterial effects against Gram positive and Gram negative strains and potent NO inhibitory activity in LPS stimulated RAW264.7 cells (IC_{50} = 28.46 µg/mL), approaching dexamethasone. In addition, the extract showed clear cytotoxicity toward MCF-7 cells (IC_{50} = 7.6 µg/mL). These findings collectively highlight *T. felleus* from Hue, Vietnam as a rich source of bioactive compounds with promising antioxidant, antimicrobial, anti-inflammatory and anticancer properties, suggesting its potential application in functional food and natural therapeutic development.

Keywords: *Tylopilus felleus*; antibacterial, antioxidant; anti-inflammatory; GC–MS; RAW264.7; MCF-7 cells

1. Introduction

Medicinal mushrooms have long been recognized as a rich source of secondary metabolites with diverse bioactivities, including antibacterial, anti-inflammatory, antioxidant, and potential anticancer effects. The demand for natural compounds associated with functional foods has been steadily increasing in recent years (Rangel Vargas *et al*., 2021). Among them, the purple bolete *Tylopilus felleus* is notable for its characteristic bitterness and its documented bioactivities, such as selective inhibition of MCF-7 breast cancer cells (Šušaníková *et al*., 2018) and antibacterial effects against various Gram positive and Gram negative strains (Zade *et al*., 2025).

In addition to secondary metabolites such as polyphenols and flavonoids, characteristic mushroom sterols particularly ergosterol and ergosterol peroxide have been demonstrated to exert antioxidant, anti-inflammatory, and anticancer activities through modulation of signaling pathways associated with oxidative stress and inflammation (Rangsinth *et al*., 2023). However, despite the high commercial value of *T. felleus* in Hue, data on its nutritional composition, polysaccharide content, anti-inflammatory potential and other biological activities remain unpublished to date.

2. Materials and Methods

2.1. Research Materials

Fruit bodies of *T. felleus* were collected from the *Melaleuca cajuputi* forest area in Truoi Hamlet, Loc Hoa Commune, Hue City, during the period of July–September 2025. The specimens were morphologically identified and classified by MSc. Nguyen Viet Thang, Faculty of Biology, University of Sciences, Hue University. The samples were cleaned, dried at 45°C to constant weight, ground into fine powder and stored in dark glass containers at 4°C until use.

The bacterial strains used for antimicrobial testing included *Staphylococcus aureus* ATCC 25923, *Bacillus pumilus* ATCC 6633, *Escherichia coli* ATCC 25922, and *Salmonella typhi* ATCC 6539, provided by the Department of Microbiology, Hue Central Hospital. The MCF-7 breast cancer cell line (ATCC HTB-22) and the RAW264.7 macrophage cell line (ATCC TIB-71) were maintained and cultured according to ATCC guidelines and CLSI standards (2019).

Figure 1. *Tylopilus felleus*

2.2. Research Methods

2.2.1. Preparation of Extracts

Dried mushroom powder (200g) was extracted three times with 70% ethanol (1:10, w/v) at 50°C for 4h. The combined filtrates were concentrated under reduced pressure at 45°C to obtain the crude extract. The dried extract was dissolved in DMSO (10 mg/mL) to prepare the stock solution for biological assays (Klausen *et al*., 2023).

2.2.2. GC-MS Analysis

The *Tylopilus felleus* extract was dissolved in absolute ethanol (1 mg/mL) and filtered through a 0.45 µm PTFE membrane prior to analysis. GC-MS analysis was performed on an Agilent 7890A/5975C system equipped with a DB-XLB column (30 m × 0.25 mm × 0.25 µm). Helium (99.99%) was used as the carrier gas at a flow rate of 1 mL/min with a split ratio of 100:1. The oven temperature program ranged from 40-270°C with a heating rate of 4–20°C/min. The ion source temperature was set at 230°C with 70 eV ionization energy and mass spectra were recorded in the range of m/z 35–450. Compounds were identified by comparing their mass spectra with those in the NIST 2020 library (Rijia *et al*., 2024).

2.2.3. Antioxidant activity assays

- ABTS$^+$ radical scavenging assay: The $ABTS^+$ assay was performed according to Surveswaran *et al*. (2007). A mixture of 7 mM $ABTS^+$ and 2.45 mM $K_2S_2O_8$ in PBS buffer (1:1, v/v) was incubated in the dark for 16 h to generate the $ABTS^+$ radical. The resulting solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Sample solutions (100 µL; 20–100 µg/mL) were mixed with 3.9 mL of $ABTS^+$ solution and incubated for 30 min at 25°C, after which absorbance was measured at 734 nm. Radical scavenging activity was calculated as:

$$SA_{ABTS}(\%) = \frac{A_c - A_s}{A_c} \times 100$$

The IC_{50} (µg/mL) value was determined using a four-parameter logistic (4PL) nonlinear regression model.

- DPPH radical scavenging sssay: The DPPH assay was conducted following the method of Cai *et al*. (2003). 0.1 mM DPPH solution in methanol was mixed with the sample solution (20–100 µg/mL) at a ratio of 1:1 (v/v) and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm. Radical inhibition was calculated similarly to the ABTS assay and IC_{50} values (µg/mL) were obtained using the 4PL model.

2.2.4. Antibacterial activity

Antibacterial activity was evaluated following the method of Van den Berghe (1991) and CLSI guidelines (2019), with minor modifications. The test organisms included *Staphylococcus aureus* ATCC 25923, *Bacillus pumilus* ATCC 6633, *Escherichia coli* ATCC 25922, and *Salmonella typhi* ATCC 6539. Bacterial suspensions were adjusted to 0.5 McFarland (~10^8 CFU/mL) and spread onto Mueller Hinton agar plates. Wells (6 mm diameter) were punched into the agar, and 50 µL of extract at concentrations of 25, 50 and 100 µg/well was applied. DMSO (≤1%) served as the negative control, and ciprofloxacin (5 µg/disc) was used as the positive control. Plates were incubated at 35°C for 24h and the inhibition zone diameter (mm) was measured. Minimum inhibitory concentrations (MICs) were determined using the tube dilution method according to CLSI guideline M07.

2.2.5. *In vitro* anti-inflammatory activity in RAW 264.7 macrophages

The anti-inflammatory activity of *T. felleus* extract was assessed by its ability to inhibit nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophages. Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin and maintained at 37°C under 5% CO_2. RAW264.7 cells were seeded at 1×10^5 cells/well in 96-well plates and incubated overnight for stabilization.

Cells were then treated with varying concentrations of *T. felleus* extract (100-3.125 µM) and stimulated with LPS (1 µg/mL) for 24 h. Two control groups were included: a negative control (cells without LPS and without sample) and a positive control (dexamethasone, 10 µM) (Jang et al., 2020).

After incubation, 50 µL of the culture supernatant was mixed with 50 µL of Griess reagent and incubated for 10 min at room temperature. Absorbance was measured at 540 nm. Nitrite levels were quantified using a $NaNO_2$ standard curve and NO inhibition (%) was calculated as:

$$\text{NO inhibition (\%)} = \frac{A_{\text{LPS}} - A_{\text{sample}}}{A_{\text{LPS}}} \times 100$$

where A_{LPS}represents the absorbance of the LPS-stimulated control group and A_{sample}represents the absorbance of extract-treated cells. The IC_{50} value for NO inhibition was determined using a four-parameter logistic (4PL) nonlinear regression model.

2.2.6. Cytotoxicity assay (MCF-7 cells)

The cytotoxic effect of *T. felleus* extract was evaluated using the MTT assay described by Mosmann (1983), with slight modifications. Human breast cancer MCF-7 cells (ATCC HTB-22) were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin and maintained at 37°C in a 5% CO_2 incubator. The extract was dissolved in DMSO (≤1%) to prepare a stock solution of 20 mg/mL, which was then diluted to obtain a concentration range of 0.5-128 µg/mL in 96-well plates.

Cells were seeded at 1×10^4 cells/100 µL per well and treated with the extract for 24 h. Subsequently, 10 µL of MTT solution (5 mg/mL) was added, followed by a 4 h incubation. The medium was removed and formazan crystals were dissolved in 100 µL of absolute DMSO. Absorbance was measured at 570 nm using a microplate reader (Biotek Instruments). Ellipticine (0.01 mM) was used as the positive control, while DMSO served as the negative control. IC_{50} values (µg/mL) were calculated using a four-parameter logistic (4PL) nonlinear regression model in TableCurve 2D v4.0.

2.2.7. Statistical Analysis

All experiments were performed in triplicate (n = 3). Results are expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA, with differences considered significant at $p < 0.05$.

3. Results and discussion

3.1. Chemical composition of Tylopilus felleus

The chemical constituents of the *T. felleus* ethanol extract were analyzed using GC-MS. Mass spectra were compared with the NIST 2020 library for compound identification. A total of 13 compounds were detected, grouped into three major classes: fatty acids, lipid esters, and sterols (Figure 2; Table 1). The dominant compound was (R)-(-)-14-methyl-8-hexadecyn-1-ol, accounting for more than half of the total peak area, followed by pentadecylic acid, stellasterol and ethyl palmitate. These compounds are commonly reported in macrofungi and are associated with antibacterial, antioxidant, and lipid-modulating activities (Casillas Vargas *et al*., 2021; Erbiai *et al*., 2023).

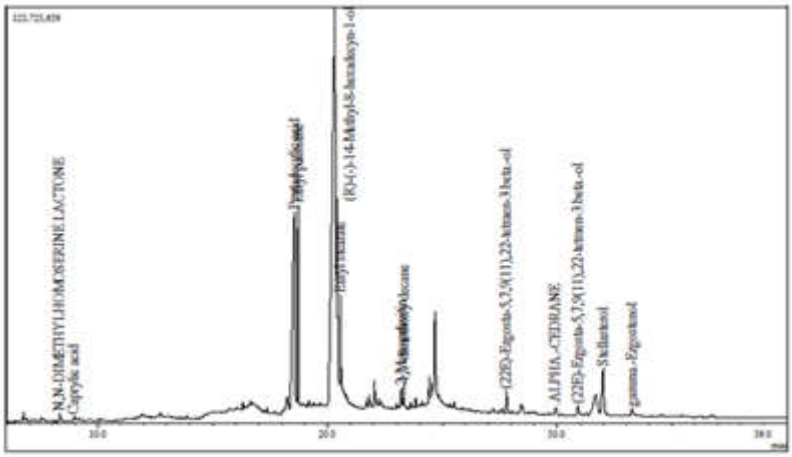

Figure 2. GC–MS chromatogram of the ethanol extract of *T. felleus*.

Table 1. Major chemical constituents of *Tylopilus felleus* extract identified by GC–MS.

Compound	Peak area (%)	Biological activity
N,N-Dimethylhomoserine lactone	0.38	Microbial signaling molecule; quorum-sensing regulator (Li *et al*., 2012).
Caprylic acid	0.21	Antibacterial, membrane-disrupting activity (Huang *et al*., 2011).
Pentadecylic acid	14.62	Antioxidant, antibacterial, anti-inflammatory (Casillas-Vargas *et al*., 2021).
Ethyl palmitate	6.04	Anti-inflammatory, lipid regulation, tissue protection (Ali *et al*., 2018).
(R)-(−)-14-Methyl-8-hexadecyn-1-ol	53.76	Antioxidant and antiproliferative activity (Verma *et al*., 2022).
Ethyl stearate	2.10	Antibacterial, anti-inflammatory, membrane protection (Erbiai *et al*.,2023).
2-Monopalmitin	0.54	Membrane-stabilizing properties.
1,1-Dimethoxy decane	0.48	Lipid intermediate with structural functions (Sarikahya et al., 2021).
Ergosta-5,7,9(11),22-tetraen-3β-ol	3.35	Anticancer and anti-inflammatory (Ikram et al., 2025).
α-Cedrane	0.40	Potent antibacterial and anti-inflammatory terpenoid (Kim et al., 2017).
Ergosta-5,7,9(11),22-tetraen-3β-ol	2.98	Anticancer and anti-inflammatory (Ikram et al., 2025).
Stellasterol	10.84	Antioxidant, apoptosis induction (Ikram et al., 2025).
γ-Ergostenol	4.30	Anti-inflammatory, neuroprotective (Ikram et al., 2025).

Sterols were present in notable amounts, including stellasterol, γ-ergostenol, and ergosta-5,7,9(11), 22-tetraen-3β-ol. These molecules are integral components of fungal membranes and have been shown to modulate NF-κB and Wnt/β-catenin pathways, contributing to anti-inflammatory effects and apoptosis induction in cancer cell lines (Kobori *et al*., 2006; Nilkhet *et al*., 2024). Additionally, minor constituents such as caprylic acid, 2-monopalmitin, and 1,1-dimethoxy decane were detected. Short-chain fatty acids like caprylic acid are known for their strong membrane-disruptive antibacterial activity (Huang *et al*., 2011), while monoacylglycerol derivatives contribute to lipid membrane stability. Although α-cedrane occurred at low abundance, it is recognized as a potent anti-inflammatory and antibacterial terpenoid (Kim *et al*., 2016).

Overall, the combination of fatty alcohols, fatty acids, and sterols in the *T. felleus* extract suggests a synergistic contribution to its antioxidant, antibacterial and anti-inflammatory properties.

3.5. Antioxidant activity

The free radical scavenging capacity of the *T. felleus* extract was evaluated using the DPPH and ABTS assays, with ascorbic acid serving as the positive control. As shown in Figure 3, radical-scavenging efficiency increased in a concentration dependent manner, reaching maximum values of 88.62% (DPPH) and 84.82% (ABTS) at 100 µg/mL. Ascorbic acid exhibited a scavenging rate of 94.12%. Nonlinear four parameter logistic (4PL) regression indicated IC_{50} values of 20.41 ± 1.25 µg/mL (DPPH) and 23.86 ± 1.14 µg/mL (ABTS), while ascorbic acid showed an IC_{50} of 14.06 ± 0.93 µg/mL (Table 2).

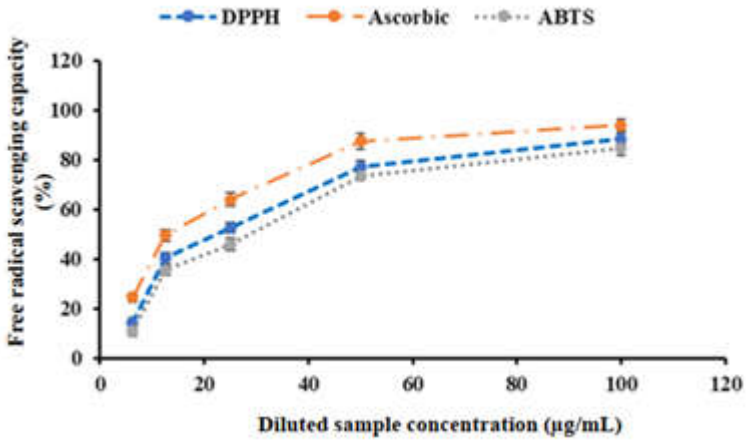

Figure 3. Radical-scavenging activity of *T. felleus* extract and the positive control.

Table 2. IC_{50} values and nonlinear regression parameters for antioxidant assays.

Method	IC_{50} (µg/mL, Mean ± SD)	R^2	Model	p-value
DPPH	20.41 ± 1.25	0.993	4PL	< 0.001
ABTS	23.86 ± 1.14	0.992	4PL	< 0.001
Ascorbic acid	14.06 ± 0.93	0.995	4PL	< 0.001

The results demonstrate that the *T. felleus* extract exhibits strong antioxidant activity, approximately 1.4 fold weaker than ascorbic acid, confirming its notable free radical scavenging potential. Compared with the findings of Yetgin *et al*., (2019), where methanolic extracts of *T. felleus* from Türkiye had an IC_{50} of approximately 31.42 µg/mL (DPPH), the Hue-derived sample shows substantially higher antioxidant potency. This suggests a positive influence of tropical humid ecological conditions in Central Vietnam on secondary metabolite accumulation.

Moreover, the antioxidant activity of *T. felleus* is comparable to that of other medicinal mushrooms, such as *Suillus* spp. (IC_{50} = 23–35 µg/mL; Judžentienė et al., 2025) and *Ganoderma lucidum* (IC_{50} = 22–25 µg/mL; Ha *et al*., 2023), further supporting its pharmacological potential as a bioactive mushroom species. Additionally, the presence of ergosterol, α-cedrane, and other terpenoids may enhance cellular protection against oxidative stress through membrane interactions and modulation of intracellular signaling. These factors collectively contribute to the strong correlation observed between high reducing compound content and the notable antioxidant properties of *T. felleus* from Hue, highlighting its potential applications in natural pharmaceuticals and functional foods.

3.6. Antibacterial activity

The antibacterial activity of the *T. felleus* extract was evaluated at four concentrations (100, 75, 50 and 25 µg/mL) against *Staphylococcus aureus* ATCC 25923, *Bacillus pumilus* ATCC 6633, *Escherichia coli* ATCC 25922, and *Salmonella typhi* ATCC 6539. Ciprofloxacin (5 µg/disc) served as the positive control, whereas DMSO (≤1%) was used as the negative control. The inhibitory effects of the extract are shown in Figure 4.

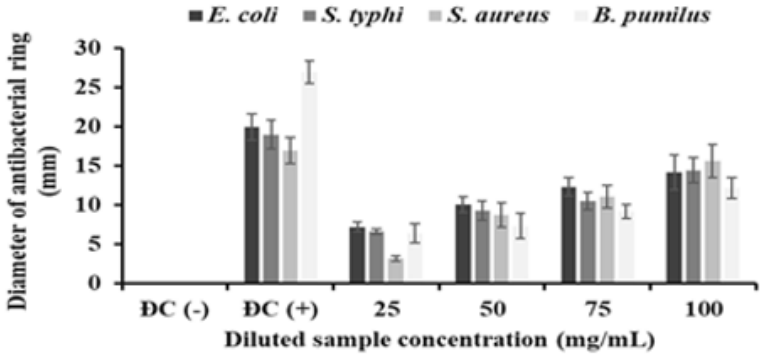

Figure 4. Antibacterial activity of *T. felleus* extract compared with controls.

The extract inhibited the growth of both Gram positive and Gram negative bacteria in a concentration dependent manner. At 100 µg/mL, the strongest activity was observed against *S. aureus* (15.63 ± 0.29 mm), followed by *S. typhi* (14.48 ± 0.27 mm) and *E. coli* (14.01 ± 0.19 mm), whereas *B. pumilus* exhibited lower susceptibility (12.13 ± 0.25 mm). Although the inhibition zones were smaller than those of ciprofloxacin (17–28 mm), the clear dose dependent increase in activity confirms the extract's genuine antibacterial potential.

These results are consistent with the findings of Yetgin *et al*., (2019), who reported inhibition zones of 10–13 mm against *E. coli* and *S. aureus* for methanolic extracts of *T. felleus* collected in Türkiye. However, the Hue derived extract exhibited stronger antibacterial activity, suggesting that tropical humid ecological conditions may enhance the accumulation of lipophilic and sterol based metabolites.

Key constituents identified via GC-MS such as pentadecylic acid, ethyl palmitate, ergosterol and α-cedrane are known to disrupt phospholipid membranes, inhibit protein-synthesis enzymes, or increase bacterial membrane permeability (Dunnick *et al*., 1997). These mechanisms support the role of lipophilic compounds in *T. felleus* as potential natural broad-spectrum antibacterial agents.

3.7. *In vitro* anti-inflammatory activity in RAW264.7 macrophages

The anti-inflammatory activity of the *T. felleus* extract was evaluated based on its ability to inhibit lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW264.7 macrophages. The results showed a dose dependent suppression of NO (Figure 5). At 100 µg/mL, the extract achieved 90.12% inhibition, while dexamethasone the positive control reached 95.78% at the same concentration.

Using a four parameter logistic (4PL) regression model, the IC_{50} of the *T. felleus* extract was calculated as 28.46 µg/mL, compared with 26.37 µg/mL for dexamethasone, indicating that the NO inhibitory potency of the mushroom extract is comparable to that of the standard anti-inflammatory drug.

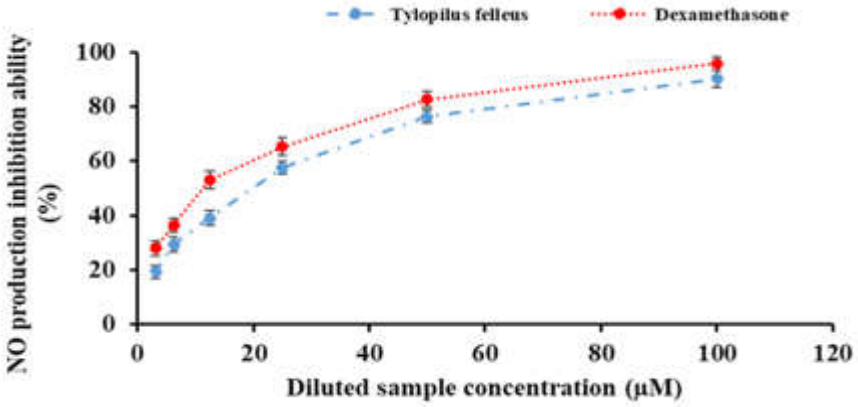

Figure 5. Nitric oxide inhibition (%) of *T. felleus* extract and dexamethasone in RAW264.7 macrophages.

These results demonstrate that *T. felleus* exhibits strong anti-inflammatory activity through suppression of LPS-induced NO production in RAW264.7 cells. The IC_{50} value (28.46 µg/mL) being very close to that of dexamethasone (26.37 µg/mL) highlights the notable inhibitory effect, even though the extract is a crude preparation.

The observed dose dependent response from 3.125 to 100 µg/mL aligns with reported inhibition of iNOS expression and reduction of nitrosative stress in various sterol and polysaccharide rich medicinal mushrooms. Key sterols identified in *T. felleus*, including ergosterol, γ-ergosteno and Δ5,7,22-trien sterols, are known modulators of NF-κB and PI3K/AKT pathways, leading to iNOS downregulation and decreased NO synthesis (Nilkhet *et al*., 2024; Kobori *et al*., 2007).

3.8. Cytotoxic activity against MCF-7 breast cancer cells

The cytotoxic activity of the *Tylopilus felleus* extract was assessed using the MTT assay in MCF-7 human breast cancer cells, with ellipticine serving as the positive control (Mosmann, 1983). The extract showed a clear dose dependent inhibitory effect on cell viability. At 128 µg/mL, the extract achieved 86.17% inhibition, whereas ellipticine reached 95.42% inhibition (Figure 7). The lowest cell viability values observed (10-15%) further indicate the strong cytotoxic potential of the extract (Figure 6).

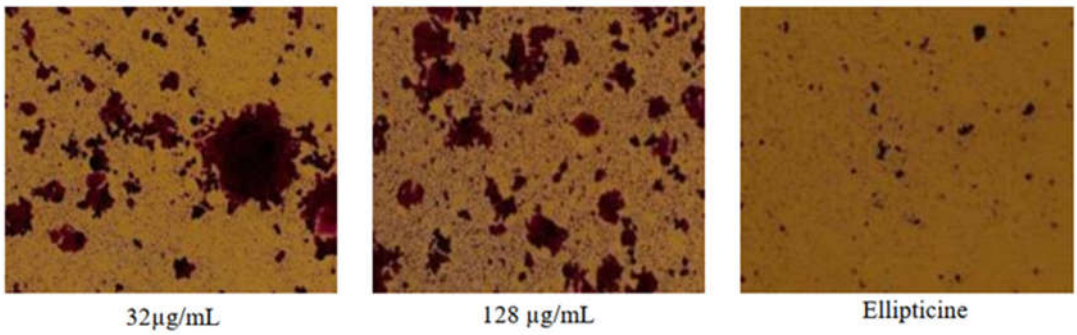

Figure 6. Morphology of MCF-7 cells following treatment with *T. felleus* extract compared with the positive control.

IC_{50} values were determined by linear interpolation around the 50% inhibition point. The *T. felleus* extract exhibited an IC_{50} of 7.6 ± 0.3 µg/mL (95% CI: 7.1–8.1), while ellipticine showed a stronger effect with an IC_{50} of 4.3 ± 0.2 µg/mL (95% CI: 3.9–4.7). Both dose response curves displayed excellent goodness of fit with $R^2 > 0.99$ using a four parameter logistic (4PL) regression model in TableCurve 2D v4.

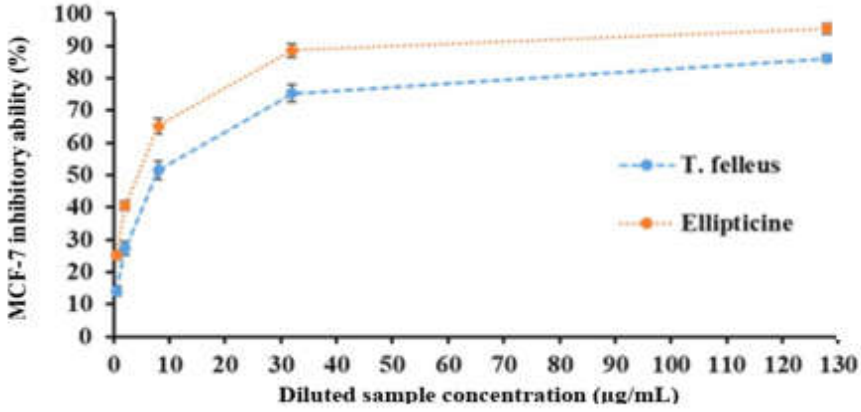

Figure 7. Dose response curves of *T. felleus* extract and ellipticine in MCF-7 cells.

These results indicate that the *T. felleus* extract exhibits potent cytotoxic activity, being only ~1.7 fold less active than ellipticine. Compared with the findings of Yetgin *et al*., (2019), where methanolic extracts of the same species yielded an IC_{50} of ~38 µg/mL in HeLa cells, the markedly lower IC_{50} observed in this study suggests that 70% ethanol is more effective in extracting bioactive cytotoxic constituents. Sterols and triterpenoids identified by GC-MS including ergosterol, γ-ergostenol, and stellasterol have been reported to induce apoptosis through NF-κB suppression and caspase-3 activation, mechanisms similar to those described for sterols isolated from *Ganoderma lucidum* (Ha *et al*., 2023).

Overall, these findings highlight the strong anticancer potential of *T. felleus* as a natural source of bioactive compounds capable of inhibiting human breast cancer cell proliferation, reinforcing the medicinal value of this fungal species endemic to Vietnam.

Conclusions

This study provides a comprehensive assessment of the chemical composition, nutritional value and biological activities of *T. felleus* collected in Hue, Vietnam. GC-MS analysis revealed that the ethanol extract contains a diverse profile of lipophilic compounds and characteristic sterols including (R)-(-)-14-methyl-8-hexadecyn-1-ol, pentadecylic acid, stellasterol, and ergosterol derivatives which are known for their antioxidant, antimicrobial and immunomodulatory properties.

Regarding biological activities, the extract exhibited strong antioxidant capacity in both DPPH and ABTS assays, with EC_{50} values similar to those of established medicinal fungi. Broad-spectrum antibacterial effects were observed against both Gram positive and Gram negative bacteria. Remarkably, the extract demonstrated potent anti-inflammatory activity in LPS-stimulated RAW264.7 macrophages (IC_{50} = 28.46 µg/mL), nearly comparable to dexamethasone. In addition, significant cytotoxicity was recorded against MCF-7 breast cancer cells (IC_{50} = 7.6 µg/mL). These biological effects suggest that synergistic interactions among sterols, fatty acids, and polysaccharides may contribute to the overall bioactivity of the extract.

Overall, the findings confirm *T. felleus* as a valuable natural resource rich in antioxidant, antimicrobial, anti-inflammatory and anticancer constituents, while also possessing notable nutritional attributes. These results support the potential application of *T. felleus* in functional foods and natural therapeutic products, and provide a scientific basis for future studies focusing on compound isolation and mechanistic evaluation.

References

1. Rangel Vargas E., Rodriguez J. A., Domínguez R., Lorenzo J. M., Sosa M. E., Andrés S. C., Rosmini M., Pérez-Alvarez J. A., Teixeira, A., & Santos E. M. (2021). Edible Mushrooms as a Natural Source of Food Ingredient/Additive Replacer. *Foods (Basel, Switzerland)*, *10*(11), 2687. https://doi.org/10.3390/foods10112687
2. Šušaníková, I., Kvasnicová, A., Brzková, Ž., Ďuriška, O., & Mučaji, P. (2018). New biological findings of ethanol and chloroform extracts of fungi S*uillellus rubrosanguineus* and *Tylopilus felleus*. *Interdisciplinary toxicology*, *11*(3), 204–208. https://doi.org/10.2478/intox-2018-0018
3. Zade, S., Upadhyay, T. K., Rab, S. O., Sharangi, A. B., Lakhanpal, S., Alabdallah, N. M., & Saeed, M. (2025). Mushroom-derived bioactive compounds pharmacological properties and cancer targeting: a holistic assessment. *Discover oncology*, *16*(1), 654. https://doi.org/10.1007/s12672-025-02371-z
4. Rangsinth, P., Sharika, R., Pattarachotanant, N., Duangjan, C., Wongwan, C., Sillapachaiyaporn, C., Nilkhet, S., Wongsirojkul, N., Prasansuklab, A., Tencomnao, T., Leung, G. P., & Chuchawankul, S. (2023). Potential Beneficial Effects and Pharmacological Properties of Ergosterol, a Common Bioactive Compound in Edible Mushrooms. *Foods (Basel, Switzerland)*, *12*(13), 2529. https://doi.org/10.3390/foods12132529
5. Klausen, S. J., Falck-Ytter, A. B., Strætkvern, K. O., & Martin, C. (2023). Evaluation of the Extraction of Bioactive Compounds and the Saccharification of Cellulose as a Route for the Valorization of Spent Mushroom Substrate. *Molecules (Basel, Switzerland)*, *28*(13), 5140. https://doi.org/10.3390/molecules28135140
6. Rijia, A., Krishnamoorthi, R., Rasmi, M., Mahalingam, P. U., & Kim, K. S. (2024). Comprehensive Analysis of Bioactive Compounds in Wild *Ganoderma applanatum* Mushroom from Kerala, South India: Insights into Dietary Nutritional, Mineral, Antimicrobial, and Antioxidant Activities. *Pharmaceuticals (Basel, Switzerland)*, *17*(4), 509. https://doi.org/10.3390/ph17040509
7. Surveswaran, S., Cai, Y., Corke, H., & Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry, 102*, 938–953. https://doi.org/10.1016/j.foodchem.2006.06.033
8. Cai, Y. Z., Luo, Q., Sun, M., & Corke, H. (2003). Antioxidant activity of betalains from plants in the Amaranthaceae. *Journal of Agricultural and Food Chemistry, 51*(8), 2288–2294. https://doi.org/10.1021/jf020837w
9. Van den Berghe, D. A., & Vlietinck, A. J. (1991). Screening methods for antibacterial and antiviral agents from higher plants. In K. Hostettmann, A. Marston, M. Maillard, & M. Hamburger (Eds.), *Methods in Plant Biochemistry: Assays for Bioactivity* (Vol. 6, pp. 47–69). Academic Press.
10. Clinical and Laboratory Standards Institute. (2019). *Performance standards for antimicrobial susceptibility testing* (29th ed.; CLSI supplement M100). CLSI.
11. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4
12. Casillas Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., Del Valle, R. G., Carballeira, N. M., & Sanabria-Ríos, D. J. (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next generation of antibacterial agents. *Progress in Lipid Research, 82*, 101093. https://doi.org/10.1016/j.plipres.2021.101093
13. Erbiai, E. H., Amina, B., Kaoutar, A., Saidi, R., Lamrani, Z., Pinto, E., Esteves da Silva, J. C. G., Maouni, A., & Pinto da Silva, L. (2023). Chemical characterization and evaluation of antimicrobial properties of the wild medicinal mushroom *Ganoderma lucidum* growing in northern Moroccan forests. *Life, 13*(5), 1217. https://doi.org/10.3390/life13051217
14. Huang, C. B., Alimova, Y., Myers, T. M., & Ebersole, J. L. (2011). Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Archives of Oral Biology, 56*(7), 650–654. https://doi.org/10.1016/j.archoralbio.2011.01.011

15. Nilkhet, S., Vongthip, W., Lertpatipanpong, P., Prasansuklab, A., Tencomnao, T., Chuchawankul, S., & Baek, S. J. (2024). Ergosterol inhibits the proliferation of breast cancer cells by suppressing AKT/GSK-3β/β-catenin pathway. *Scientific Reports, 14*(1), 19664. https://doi.org/10.1038/s41598-024-70516-1

16. Kobori, M., Yoshida, M., Ohnishi-Kameyama, M., & Shinmoto, H. (2007). Ergosterol peroxide from an edible mushroom suppresses inflammatory responses in RAW264.7 macrophages and growth of HT-29 colon adenocarcinoma cells. *British Journal of Pharmacology, 150*(2), 209–219. https://doi.org/10.1038/sj.bjp.0706972

17. Kim, J. H., Hwang, D. K., Moon, J. Y., Lee, Y., Yoo, J. S., Shin, D. H., & Lee, H. S. (2017). Multiple UDP-glucuronosyltransferase and sulfotransferase enzymes are responsible for the metabolism of verproside in human liver preparations. *Molecules, 22*(4), 670. https://doi.org/10.3390/molecules22040670

18. Yetgin, A., Canlı, K., Akata, I., & Altuner, E. (2017). *In vitro* antimicrobial activity screening of *Tylopilus felleus* and determination of the ethanol extract composition by gas chromatography/mass spectrometry. *Journal of Microbiology, Biotechnology and Food Sciences*, 6(6), 1346–1350.

19. Ha, H. T., et al. (2023). Study on chemical compositions, antioxidants and intracellular protective effects of five selected *Ganoderma lucidum* varieties in Vietnam. *International Journal of Food Science & Technology*. https://doi.org/10.1111/ijfs.16694

20. Judžentienė, A., & Šarlauskas, J. (2025). Comparative research of antioxidant, antimicrobial, antiprotozoal and cytotoxic activities of edible *Suillus* sp. fruiting body extracts. *Foods, 14*(7), 1130. https://doi.org/10.3390/foods14071130

21. Dunnick, J. K., & O'Leary, W. M. (1970). Correlation of bacterial lipid composition with antibiotic resistance. *Journal of Bacteriology, 101*(3), 892–900. https://doi.org/10.1128/jb.101.3.892-900.1970