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Bioactive potential of *Auricularia cornea*: Cytotoxicity, antioxidant activity, and beta-glucan content of mycelium and fruiting bodies

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Abstract

Auricularia cornea, an underexplored species within the medicinal *Auricularia* genus, was investigated for its bioactive potential, including cytotoxicity, antioxidant activity, and β-glucan content, promoting good health and well-being through natural nutraceuticals. This study compared three *A. cornea* isolates (MFLUCC 23-0084, MFLUCC 18-0346, and MFLUCC 18-0347) with commercial *Auricularia* spp. to evaluate their therapeutic and functional food applications. The methanolic extracts of mycelium were analyzed for free radical scavenging (DPPH assay), cytotoxicity against lung cancer cells (A549) and normal cells (Raw 264.7). β-Glucan content from fruiting bodies was also quantified by exploring sustainable fungal cultivation for high-value bioactive compounds. The results revealed strain-specific bioactivity: MFLUCC 23-0084 exhibited the highest antioxidant (IC_{50} 5,530 ± 1,039 µg/mL), while MFLUCC 18-0346 showed low cytotoxicity against A549 cells (IC_{50} 367.27 µg/mL). β-Glucan content varied significantly, with lab-cultivated isolates (12.14–13.22%) yielding less than *A. auricula* (18.86–24.94%). Low cytotoxicity to normal cells (IC_{50} >500 µg/mL) and moderate bioactivity suggest *A. cornea*'s suitability for functional foods, though strain optimization or extraction enhancements may be needed to rival leading species. This study positions *A. cornea* as a candidate for further development in sustainable nutraceuticals, through antioxidant applications and evaluating cultivation practices for enhanced β-glucan production.

Keywords – Bioactivity – good health – Jelly mushroom – Mushroom extracts – Polysaccharide

Introduction

Mushrooms of the genus *Auricularia* have long been celebrated for their culinary and medicinal value, particularly in traditional Asian medicine, where species like *Auricularia auricula-judae* and *A. polytricha* are prized for their immunomodulatory, antioxidant, and anticancer properties (Wasser 2017, Bandara et al. 2019). These effects are largely attributed to bioactive polysaccharides, especially β-glucans, which exhibit unique structures, such as (1→3)- and (1→6)-linked backbones, that interact with immune cells to enhance therapeutic responses (Xu

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et al. 2016, Vetvicka et al. 2019). Despite the well-documented potential of these species, the *Auricularia cornea*, a relatively phylogenetically close but understudied species, remains overlooked in scientific literature, even as the demand for new fungal bioresources in nutraceutical and pharmaceutical applications increases (Sari et al. 2017, Kalitukha et al. 2023).

The therapeutic potential of *Auricularia* spp. is closely tied to its cytotoxic effects against cancer cells and its ability to mitigate oxidative stress, a key driver of chronic diseases. Polysaccharides like β-glucans exert cytotoxicity by inducing apoptosis in tumor cells via mitochondrial pathways (Zhang et al. 2018) while sparing normal cells, a selectivity critical for drug development. Concurrently, their antioxidant capacity, measured through DPPH scavenging and metal chelation, neutralizes reactive oxygen species (ROS), reducing cellular damage related to aging and inflammation (Bandara et al. 2019). In pharmacological research, antioxidant capacity is routinely quantified through radical scavenging assays (DPPH, ABTS) and reducing power tests, which evaluate a compound's ability to neutralize free radicals and chelate pro-oxidant metals (Prior et al. 2005). Similarly, cytotoxicity screening typically employs viability assays like MTT that measure mitochondrial dehydrogenase activity in cancer cell lines, providing reliable indicators of apoptotic potential (Mosmann 1983, Fotakis & Timbrell 2006). These established methods will be crucial for characterizing the therapeutic profile of *A. cornea*. However, these properties vary with polysaccharide structure (e.g., branching patterns) and extraction methods, underscoring the need to characterize *A. cornea* bioactivity systematically.

Preliminary studies suggest that *A. cornea* shares biochemical traits with its more famous relatives, including a comparable β-glucan content and antioxidant activity (Vaithanomsat et al. 2019). However, critical gaps persist in understanding strain-specific variability, optimal extraction methods, and bioactivity mechanisms. For instance, while *A. auricula-judae* polysaccharides have demonstrated antitumor effects through NK cell activation (Zhang et al. 2018), and *A. heimuer* hydrocolloids show exceptional moisture retention for cosmetic use (Kalitukha et al. 2023), similar applications for *A. cornea* remain speculative. Moreover, the influence of cultivation conditions on *A. cornea*'s β-glucan yield, such as substrate composition or extraction solvents (e.g., methanol vs. water), has yet to be systematically evaluated, despite evidence that these factors significantly impact bioactivity in related species (Vaithanomsat et al. 2019).

This study addresses these gaps by comprehensively profiling three *A. cornea* isolates (MFLUCC 23-0084, MFLUCC 18-0346, and MFLUCC 18-0347) for their cytotoxic, antioxidant, and β-glucan properties. Using standardized assays (DPPH scavenging, MTT cytotoxicity tests, anti-cancer assay, and enzymatic β-glucan quantification), we compare their bioactivity among different strains of *Auricularia* spp. Our objectives are threefold: (1) to evaluate strain-specific differences in bioactivity, (2) to compare β-glucan yields, and (3) to compare *A. cornea* with established species like *A. auricula* and *A. polytricha* for potential nutraceutical or biomedical use. By clarifying these aspects, this work not only elevates *A. cornea* from obscurity but also provides a framework for characterizing other underexplored medicinal mushrooms.

Materials & Methods

Crude extraction

Five-agar plugs of fully grown *Auricularia cornea* strains MFLUCC 23-0084, MFLUCC 18-0346, and MFLUCC 18-0347 (Fig. 1) were individually inoculated in potato dextrose broth (PDB) 200 mL, incubated at 28 ºC, 120 rpm for 2 months or until there is no sugar could be detected in the media. Mycelium was harvested and then soaked with 30 mL HPLC-grade ethyl acetate (EtOAc, $CH_3{-}COO{-}CH_2{-}CH_3$), methanol (CH_3OH), using a homogeniser (Ultra-turraxP®), and water (H_2O). The process was carried out in three replicates. The mycelium of our mushroom strains was soaking for three days for the first extraction of each solvent. The liquid layer and the solid layer were separated. The solid layer was again added with solvents, and the mixture was soaked for three days each time for the second and third extractions. Subsequently, the solid layer was discarded. The liquid layers were collected and then transferred to a round bottle, the liquid was

frozen, and then it was freeze-dried. The remaining solid (powdery) component is the crude extract. This will be used for further laboratory analyses. Ethyl acetate extracts yielded negligible quantities and were excluded from further assays.

Fig. 1 – (a) The basidiocarp of *A. cornea* MFLUCC 18-0346. (b) The basidiocarp of *A. cornea* MFLUCC 18-0347. (c) The basidiocarp of *A. cornea* MFLUCC 23-0084.

Antioxidant assay

2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity (DPPH)

The DPPH radical scavenging activity was measured according to the method described by Xiao et al. (2019). Briefly, 100 µL of 0.2 mM DPPH radical solution in ethanol was added to 100 µL of the extract solutions at different concentrations. The mixture was vortexed and incubated in the dark for 30 minutes, after which the absorbance was measured at 517 nm (INFINITE M NANO). Ascorbic acid served as the positive control. The IC_{50} value, defined as the sample concentration required to reduce the absorbance of DPPH by 50%, was graphically determined. The percentage inhibition was calculated using the following equation.

Scavenging activity (%) = $[(A_0 – A_1)/A_0]$ x100

where, A_0 is the absorbance of the control, and A_1 is the absorbance of the samples (Xiao et al. 2019).

Anti-cancer assay

The anticancer activity was evaluated using A549 lung cancer cells. The cytotoxicity assay was performed following established procedures (Ma et al. 2013, Phonemany et al. 2025) with modifications. Briefly, cells were seeded in 96-well tissue culture plates at a density of 4×10^4 cells per well in 100 µL of culture medium and allowed to adhere for 24 hours (or until cell confluency reached >80%) in a 5% CO_2 incubator at 37 °C. Crude extracts from three strains of *A. cornea* were dissolved in 10% DMSO, and 100 µL of each extract was added to the wells. After 24 hours of incubation, 100 µL of 0.5% MTT solution was added to each well and incubated for 4 hours. Subsequently, 100 µL of sterile DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader (INFINITE M NANO). Cytotoxicity was expressed as the half-maximal inhibitory concentration (IC_{50}), calculated from the dose-response curve. Cisplatin was used as a positive control. The IC_{50} values were determined by analyzing the linear regression of the concentration-response data.

Cytotoxicity assay

The cytotoxicity of *A. cornea* extracts was evaluated using Raw 264.7 cells via the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were seeded at a density of 4×10^4 cells per well (100 µL/well) in a 96-well plate and incubated for 24 hours (5% CO_2, 37°C) until > 80% confluence. Subsequently, 100 µL of the mushroom extract, dissolved in 10% DMSO, was added to each well and incubated for an additional 24 hours. After treatment, 100 µL of 0.5% MTT solution was added, followed by 4 hours of incubation. The formazan crystals were then dissolved in 100 µL of sterile DMSO, and absorbance was measured at 570 nm using a

microplate reader (INFINITE M NANO). Cytotoxicity was expressed as the half maximum inhibitory concentration (IC_{50}), calculated from a dose-response curve. Cisplatin was used as a positive control, and multiple concentrations were tested to determine the IC_{50} value.

Beta-glucan determination

All mushroom strains were obtained from the previous experiment/market, and all strains have already been confirmed by molecular identification. All strains of *Auriculria* spp. have been ground using a blender to obtain powder form. Beta-glucan levels from samples were determined with the "Mushroom and yeast β-glucan" assay kit (K-YBLG, Megazyme International, Bray, Ireland) following the provided protocol. The method involved two key steps: total glucan quantification via acid hydrolysis with sulfuric acid and subsequent enzymatic treatment with exo-1,3-β-glucanase and β-glucosidase, with the resulting glucose measured using a glucose oxidase/peroxidase reagent. α-glucans were separately quantified by hydrolyzing starch-like glucans with glucoamylase and similarly detecting the glucose. β-glucan content was then calculated as the difference between the glucose from total glucan hydrolysis and that from α-glucan hydrolysis. The measurement of total glucans, α-glucans, D-glucose in oligosaccharides, sucrose, and free D-glucose contents was measured spectrophotometrically at 510 nm. The β-glucan calculation was performed using an Excel spreadsheet from the manufacturer (Megazyme© International Ireland Ltd, 2013).

Results

Antioxidant activity

The methanolic extracts of *A. cornea* strains exhibited antioxidant activity in the DPPH radical scavenging assay (Figure 2). Strain MFLUCC 23-0084 showed the highest activity with an IC_{50} of 5,530 ± 1,390 µg/mL, followed by MFLUCC 18-0346 (7,120 ± 590 µg/mL) and MFLUCC 18-0347 (7,410 ± 330 µg/mL) (Table 1). Water extracts displayed no significant antioxidant effects.

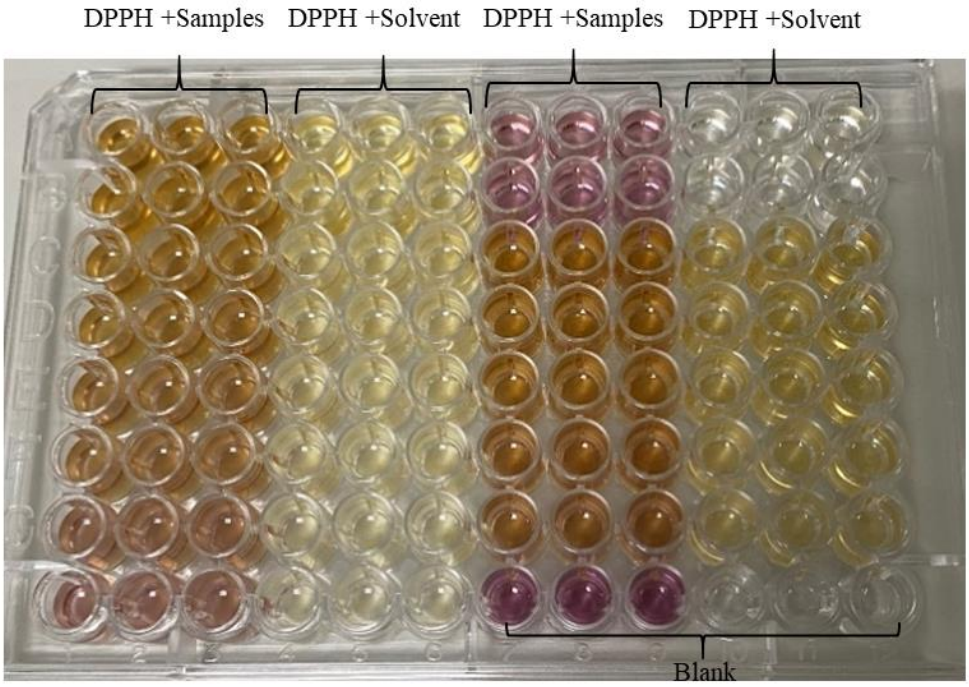

Fig. 2 – The DPPH assay performed in a 96-well plate. The purple color indicates no antioxidant activity, and orange to dark orange indicates antioxidant activity.

Table 1 Antioxidant activities of *A. cornea* of different strains in terms of DPPH radical scavenging activities (IC_{50} (µg/mL)).

Treatments	IC_{50} values of inhibition (µg/mL)
MFLUCC 23-0084	5,530 ± 1,390
MFLUCC 18-0347	7,410 ± 330
MFLUCC 18-0346	7,120 ± 590
Ascorbic acid	6.91 ± 0.20

Results are expressed as means ± SD, n = 3.

Cytotoxicity and anticancer activity

The results showed that all *A. cornea* strains demonstrated low cytotoxicity against Raw cells 264.7 normal cells, with IC_{50} values exceeding 500 µg/mL. Only MFLUCC 18-0346 showed very low activity against A549 lung cancer cells, with an IC_{50} of 367.27 µg/mL, while the other strains showed no cytotoxicity at the tested concentrations (Table 2).

Table 2 Inhibition against raw cells (264.7) and lung cancer (A549) cells from *A. cornea* extracts.

Treatments	IC_{50} values of inhibition (µg/mL)	
	Raw cells (264.7)	Lung cancer cells (A549)
MFLUCC 23-0084	524.46	Inactive
MFLUCC 18-0347	584.08	Inactive
MFLUCC 18-0346	716.39	367.27
Positive control (Cisplatin)	6.42	5.63

Beta-glucan determination

The β-glucan content of *A. cornea* strains MFLUCC 18-0346 and MFLUCC 18-0347 was 12.14 ± 1.09% and 13.22 ± 1.18%, respectively (Table 3). Total glucan content ranged from 13.27 ± 1.53% to 14.46 ± 1.00%, with α-glucans contributing 1.13–1.26%.

Table 3 Comparison of glucan contents of *Auricularia* spp.

Mushroom strains	Total Glucan (%) Average	Alpha Glucan (%) Average	Beta Glucan (%) Average	Remarks
A. cornea (MFLUCC 18-0346)	13.27 ± 1.53	1.13 ± 1.11	12.14 ± 1.09	This experiment
A. cornea (MFLUCC 18-0347)	14.46 ± 1.00	1.26 ± 1.25	13.22 ± 1.18	This experiment
A. thailandica (MFLU 16-2122)	19.01± 1.03	1.35± 0.98	17.66± 2.11	This experiment
A. auricula	~25.53*	0.59 ± 0.04	24.94 ± 0.91	Setyawan et al. (2024)
A. heimuer	18.77 ± 1.47	0.53 ± 0.13	18.86 ± 0.71	Kalitukha et al. (2023)

*The value obtained from the sum of alpha-glucan and beta-glucan, as this value was not reported in the original article.

Discussion

The present study elucidates the bioactive potential of *Auricularia cornea*, revealing significant strain-specific variability in antioxidant capacity, low toxicity, and β-glucan content. This study positions *A. cornea* as a promising but underexplored candidate within the genus *Auricularia*, warranting further investigation for the safety and nutraceutical applications.

The superior DPPH radical scavenging activity of *A. cornea* MFLUCC 23-0084 (IC_{50} 5,530 ± 1,390 µg/mL) over other isolates aligns with studies on *A. auricula-judae*, where methanolic

extracts outperformed aqueous extracts due to higher solubility of phenolic compounds (Sari et al. 2017). This suggests that the antioxidant effects of *A. cornea* may likewise stem from polyphenols or other non-polar metabolites, though further HPLC-MS analysis is needed to identify specific contributors. However, the inactivity of water extracts contrasts with *A. heimuer*, where hot-water extraction yielded potent antioxidants (Kalitukha et al. 2023). This discrepancy may reflect differences in polysaccharide solubility or cell wall structure between species.

The low toxicity obtained from MFLUCC 18-0346 against A549 lung cancer cells (IC_{50} 367.27 μg/mL), alongside minimal effects on Raw cells 264.7 (IC_{50} >500 μg/mL). The inactivity of *A. cornea* MFLUCC 23-0084 and MFLUCC 18-0347 against A549 cells underscores strain-specific bioactivity, possibly related to variations in β-glucan branching patterns (Xu et al. 2016). In this study, follow the pattern seen in *Phellinus linteus*, where minor structural differences in β-glucans dramatically altered anticancer effects (Kim et al. 2021). This study revealed that while the extracts of *A. cornea* demonstrated low activity against cancer cells, they also exhibited relatively low cytotoxic effects on Raw 264.7 cells as compared to Cisplatin. This observation is significant as it supports their potential for safe applications. Future studies should isolate fractions to pinpoint active constituents and assess synergies with clinical agents.

Additionally, the β-glucan content of lab-cultivated *A. cornea* isolates (12.14–13.22%) over other *Auricularia* spp. highlighting the impact of growth conditions. Similar disparities were reported by Sari et al. (2017) reported similar disparities, who attributed low yields in laboratory strains to suboptimal substrates (e.g., lack of lignocellulosic additives). Notably, wild *Auricularia* spp. often outperform cultivated ones due to environmental stressors that stimulate polysaccharide production (Sari et al. 2017). This invites exploration of stress induction (for example, light exposure, nutrient limitation) or solid-state fermentation with agricultural waste (for example, rice bran) to enhance yields.

The moderate bioactivity of *A. cornea* β-glucans compared to *A. auricula* may reflect structural differences. For instance, *A. auricula-judae* immunomodulatory effects correlate with (1→3)-linked backbones and 1,6-branching (Xu et al. 2016), while the hydrocolloid properties of *A. heimuer* depend on the placement of the acetyl group (Kalitukha et al. 2023). NMR analysis of *A. cornea* β-glucans could reveal whether its lower activity stems from shorter chains or fewer branches. Alternatively, the presence of α-glucans (1.49–1.55% in isolates) may dilute bioactive effects, as these polymers lack immune-stimulating properties (Volman et al. 2010).

This study establishes *A. cornea* as a species of significant yet underexplored bioactive potential, with strain-specific variations dictating its antioxidant, cytotoxic, and β-glucan profiles. The superior antioxidant capacity of *A. cornea* MFLUCC 23-0084 (IC_{50} 5,530 μg/mL), together with *A. cornea* MFLUCC 18-0346 selective cytotoxicity against A549 cells (IC_{50} 367.27 μg/mL), positions *A. cornea* as a dual-purpose candidate for nutraceutical and biomedical applications, while its low toxicity to normal cells (RAW 264.7 IC_{50} >500 μg/mL) underscores its safety. However, modest β-glucan yields reveal critical gaps in cultivation optimization that must be addressed through substrate engineering or stress induction strategies. Future research should prioritize structural characterization of active compounds, scale-up protocols for industrial production, and in vivo validation of therapeutic effects, particularly in wound healing and adjuvant cancer therapy, to fully harness the potential of this species.

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