Research Article

Evaluation of Biological Activities and Cytotoxicity of *Microporus vernicipes* PW17-173 and *Microporus xanthopus* PP17-16 Mushroom Extracts for Natural Cosmetics Applications

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ABSTRACT

Mushroom extracts, known for their rich content of bioactive compounds such as polysaccharides, phenolic acids, vitamins, and minerals, are increasingly recognized for their potential as cosmeceuticals. This study evaluated *Microporus vernicipes* PW17-173 and *Microporus xanthopus* PP17-16, collected from northeastern Thailand. The mushrooms were cultured in yeast malt broth, and four extracts were prepared using ethanol and ethyl acetate: culture broth ethyl acetate (BA), culture broth ethanol (BE), mycelium ethyl acetate (MA), and mycelium ethanol (ME). These extracts were assessed for antioxidant, anti-tyrosinase, and anti-inflammatory activities. The BA extracts from both species exhibited the highest antioxidant activity, followed by the ME and BE extracts. Anti-tyrosinase assays revealed low IC_{50} values for BA extracts, with *M. vernicipes* showing 0.591±0.013 mg/mL and *M. xanthopus* 0.335±0.055 mg/mL. In anti-inflammatory tests, all extracts from *M. vernicipes* demonstrated lower IC_{50} values compared to those from *M. xanthopus*. Cytotoxicity assays on human keratinocyte (HaCaT) cells indicated that the BA and ME extracts caused 50% cell damage at concentrations above 250 µg/mL, while BE extracts exhibited similar toxicity at concentrations above 250 µg/mL for *M. vernicipes* and 63 µg/mL for *M. xanthopus*. These findings suggest that *Microporus* species offer promising potential for cosmetic formulations due to their potent antioxidant, anti-tyrosinase, and anti-inflammatory properties.

Keywords: Antioxidant, Anti-tyrosinase, Anti-inflammatory, Cytotoxicity, Mushroom

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Introduction

Mushrooms are increasingly recognized for their valuable role in the cosmetic industry due to their bioactive compounds, which offer significant benefits for skin health and beauty. Rich in polysaccharides, polyphenols, terpenoids, and various secondary metabolites, mushrooms possess antioxidant, anti-inflammatory, anti-aging, and moisturizing properties, making them ideal components for modern skincare formulations [1, 2]. These attributes meet the growing demand for natural, skin-friendly, and environmentally sustainable cosmetic products. Antioxidants play a vital role in cosmetics by protecting the skin from free radical damage, which can be caused by environmental factors like UV radiation and pollution. Free radical damage accelerates skin aging, leading to wrinkles, fine lines, and reduced firmness. The DPPH radical scavenging assay is commonly employed to assess the antioxidant activity of ingredients. Compounds such as polyphenols, flavonoids, and vitamins (especially C and E) are known for their potent antioxidant effects. Mushrooms like *Ganoderma lucidum* (Reishi) and *Inonotus obliquus* (Chaga) exhibit high antioxidant activity, making them excellent ingredients for anti-aging products that reduce wrinkles and improve skin elasticity [3, 4]. Anti-tyrosinase activity is crucial, particularly in formulations aimed at reducing hyperpigmentation and promoting even skin tone. Tyrosinase is the key enzyme in melanin production, and its overactivity can lead to dark spots and uneven pigmentation. Natural tyrosinase inhibitors, such as kojic acid, arbutin, and extracts from mushrooms like *G. lucidum*, *Agaricus brasiliensis*, and *Cordyceps militaris* [5], inhibit melanin synthesis, making them valuable for brightening and depigmenting skincare products. Anti-inflammatory properties are particularly important for soothing irritated or inflamed skin, often associated with conditions such as acne, eczema, and dermatitis. Natural extracts with anti-inflammatory effects help reduce redness, calm irritation, and prevent further inflammation. The nitric oxide (NO) assay is used to evaluate an ingredient's ability to inhibit NO production, which is a mediator of inflammation [6]. Mushrooms like *Tremella fuciformis* (Snow Mushroom) and *Fomes fomentarius* contain polysaccharides and triterpenes that exhibit strong anti-inflammatory properties, making them beneficial in products for sensitive or acne-prone skin [7]. *T. fuciformis* is also renowned for its exceptional moisturizing abilities, surpassing hyaluronic acid in water retention, making it a suitable ingredient for hydrating serums and creams [8].

The choice of solvent is critical for extracting specific bioactive compounds from mushrooms. Ethanol and ethyl acetate are widely used in natural product research due to their efficacy in isolating a broad spectrum of bioactive substances. Ethanol, a polar solvent, is effective in extracting polar compounds such as phenolics, flavonoids, and polysaccharides, while ethyl acetate, a less polar solvent, is more suited for extracting non-polar or less polar compounds like terpenoids and lipids. Utilizing these solvents either separately or in combination can optimize the recovery of bioactive compounds, depending on the desired properties of the extract [9, 10]. *Microporus* is a non-edible polypore mushroom belonging to the family Polyporaceae. Though species within this genus are lesser-known, they exhibit significant pharmacological activities [11]. Research has indicated that *Microporus* species hold potential for cosmetic use due to their antioxidant, anti-inflammatory, and antibacterial properties [12]. Studies on *Microporus vernicipes* and *Microporus xanthopus* suggest that their bioactive components could contribute to cosmetic formulations, offering innovative solutions for skincare by harnessing the unique properties of these fungi [2]. However, most research has focused on extracting compounds from their fruiting bodies, with limited exploration of biological

activities derived from cultures. This study aimed to evaluate the antioxidant, anti-tyrosinase, and anti-inflammatory properties of mycelial and culture broth extracts using ethanol and ethyl acetate as solvents. Additionally, cytotoxicity assays were conducted on keratinocyte cell lines to assess the safety of these extracts for potential cosmetic applications, given the critical role of keratinocytes in maintaining skin health and integrity.

Materials and Methods

Mushroom cultivation and extraction

Pure cultures of two mushroom isolates, *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16, obtained from our previous study [13] and deposited at the Department of Microbiology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand, were initially cultured on potato dextrose agar (PDA) for seven days. Following this incubation period, the cultures were transferred to 1.5 L of yeast malt broth in 5 L Erlenmeyer flasks, with three flasks prepared per isolate. The mushroom cultures were incubated at 30°C under static conditions for six weeks. The mycelium was then harvested from the culture broth, washed with tap water to remove any residual medium, air-dried, and further dried in an oven at 45°C for 24 hours. Once dried, the mycelium was ground using a blender. For extraction, three gram of the ground mycelium was separately treated with 100 mL of 95% ethanol and 100 mL of ethyl acetate on a shaker for 24 hours. The solvents were then collected, and the extraction process was repeated three times. The collected solvents were pooled and concentrated using a rotary evaporator. The resulting crude extracts from the mycelium, designated as ME (ethanol extract) and MA (ethyl acetate extract), were stored at 4°C for further analysis. CAS numbers of chemicals used in this study are shown in Table 1.

For the culture broth extraction, ethyl acetate was added to the culture broth at a 1:1 (v/v) ratio and extracted three times. The extracted solvent was concentrated using a rotary evaporator to obtain the ethyl acetate extract (BA). The remaining culture broth was then extracted with 95% ethanol at the same ratio overnight, followed by centrifugation to collect the ethanol extract (BE). All extracts (ME, MA, BA, and BE) were subsequently used to evaluate their biological activities.

Table 1 CAS numbers of chemical substances used in this study.

Chemicals	CAS number
2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)	30931-67-0
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	1898-66-4
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)	298-93-1
Ethanol	64-17-5
Ethyl acetate	141-78-6
Ethylenediaminetetraacetic acid (EDTA)	60-00-4
Ferrous chloride	7758-94-3
Gallic acid	149-91-7
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	53188-07-1
Methanol	67-56-1
Nitric oxide	10102-43-9
L-tyrosine	60-18-4

Antioxidant activities

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

The antioxidant activity of the extracts was determined using the DPPH radical scavenging assay, as described by Wangsawat et al. (2021), with slight modifications [14]. Briefly, two-fold serial dilutions of the extracts were prepared in methanol (MeOH) in 96-well plates. To each well, 150 μL of 2 mM DPPH in MeOH was added and mixed gently. The mixtures were then incubated for 30 minutes at room temperature, after which the absorbance was measured at 517 nm using a microplate reader. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a positive control, while MeOH served as the blank. The percentage of DPPH scavenging activity was calculated using the following equation. IC_{50} value was the concentration of the extract required to scavenge 50% of DPPH radicals.

DPPH scavenging activity (%) = [(A- (B-C)/A] ×100

Where A is the absorbance of the control (DPPH solution), B is the absorbance of the extract, C is the absorbance of the blank (MeOH).

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay

The antioxidant activity of the extracts was also assessed using the ABTS assay, as described by Wangsawat et al. (2021) [14]. The extracts were subjected to two-fold serial dilutions in a 96-well plate using distilled water. To each well, 176 μL of ABTS solution, adjusted to an absorbance of 0.7 at 734 nm, was added. The mixtures were incubated at room temperature for 6 minutes, after which the absorbance was measured at 734 nm. Trolox was used as the positive control. The ABTS scavenging activity (%) and IC_{50} values were subsequently calculated.

ABTS scavenging activity (%) = [(A- (B-C)/A] ×100

Where A is the absorbance of the control (ABTS solution), B is the absorbance of the extract, C is the absorbance of the blank (distilled water).

Ferrous ion chelating activity

The chelating activity of the extracts was evaluated using a ferrous ion chelating assay, as described by Gulcin & Alwasel (2022) [15]. The extracts were subjected to two-fold serial dilutions with distilled water in 96-well plates. To each well, 2 mM ferrous chloride solution in distilled water was added, followed by the addition of 60 µL of 5 mM ferrozine. The mixture was thoroughly mixed and incubated at room temperature for 10 minutes, after which the absorbance was measured at 562 nm. All tests were performed in triplicate, with ethylenediaminetetraacetic acid (EDTA) serving as the positive control. The IC_{50} value was then calculated.

Total phenolic content

Total phenolic content (TPC) was measured using the Folin-Ciocalteu method, as described by Khoddami et al. (2013) [16]. TPC was calculated by comparing the results to a gallic acid standard curve and expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated using a nitric oxide (NO) radical scavenging assay, following a modified protocol by Inkanuwat et al. (2019) [17]. The extracts were mixed with 10 mM sodium nitroprusside in phosphate-buffered saline and incubated at room temperature for 150 minutes. After incubation, 50 µL of Griess reagent was added, and the mixture was incubated at room temperature for 30 minutes. Absorbance was measured at 540 nm using a microplate reader. The percentage of NO inhibition and IC_{50} values were calculated as follows:

$$\text{NO inhibition } (\%) = [(A_{control} - A_{blank}) - (A_{sample} - A_{background})/(A_{control}-A_{blank})] \times 100$$

Where $A_{control}$ represents the absorbance of the control, A_{sample} the absorbance of the sample, $A_{background}$ the absorbance of the background, and A_{blank} the absorbance of blank.

Anti-tyrosinase activity

The anti-tyrosinase activity of the extracts was evaluated following the method described by Ma et al. (2021), with slight modifications [18]. Two-fold serial dilutions of the extracts were prepared in 96-well plates using DMSO. Each well received 40 µL of the extract, which was then mixed with 40 µL of 25 mM phosphate buffer (pH 6.8) and 40 µL of tyrosinase (100 units/mL). The mixture was incubated at 37°C for 10 minutes. Following incubation, 120 µL of the enzyme substrate, L-tyrosine at a concentration of 2.76 M, was added, and the mixture was incubated again at 37°C for 30 minutes. Absorbance was then measured at

475 nm. Ascorbic acid at 1 mg/mL served as the positive control. The percentage of tyrosinase inhibition was calculated using the following equation:

Tyrosinase inhibition (%) = [(A-B) − (C-D)/A-B] × 100

Where A is the absorbance of the control (L-Tyrosine with tyrosinase), B is the absorbance of the blank (L-Tyrosine), C is the absorbance of the extract (L-Tyrosine with tyrosinase and the extract), and D is the blank for the extract (L-Tyrosine with the extract).

Cytotoxicity of the extracts against HaCaT cell lines

HaCaT cells were seeded in 96-well tissue culture plates at a concentration of 8.0×10^3 cells/well in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The cells were incubated at 37°C in a 5% CO_2 atmosphere. Cytotoxicity of the extracts on HaCaT cells was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [19]. After seeding the cells at a density of 8.0×10^3 cells/mL for 24 h, they were treated with varying concentrations of the extracts (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250 and 500 μg/mL) for 24 h. Cell viability was assessed by adding 100 μL of MTT of each well. After 2 h incubation, the medium was removed, and the formazan crystals were dissolved in 50 μL of 100% DMSO. Absorbance was measured at 570 nm using a microplate reader, and the percentage of cell viability was calculated.

Statistical analysis

The significant differences among the extracts were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test for pairwise comparison with the positive control. A *p*-value of less than 0.05 was considered statistically significant.

Results

Mushroom cultivation and extraction yields

Microporus vernicipes PW17-173 and *M. xanthopus* PP17-16 were cultured in YM broth (Figure 2), and the extracts were obtained from both the mycelium and culture broth, resulting in four fractions: MA, ME, BA, and BE. Among these, the ethyl acetate extract from the culture broth (BE) yielded the highest quantities, with 45.20 mg/mL from *M. vernicipes* PW17-173 and 43.20 mg/mL from *M. xanthopus* PP17-16 (Table 2). Ethanolic extracts, both from the mycelium (ME) and the culture broth (BE), consistently produced higher yields compared to their ethyl acetate counterparts. Notably, the ethyl acetate extracts from the mycelium (MA) were lower than 1 mg/g dry weight, and therefore excluded from further analysis.

Table 2 Extraction yields of *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16 extracts using ethyl acetate and ethanol solvents.

Species	Isolate	Mycelium extracts (mg/g dry weight)		Culture broth extracts (mg/L)	
		Ethyl acetate (MA)	Ethanol (ME)	Ethyl acetate (BA)	Ethanol (BE)
M. vernicipes	PW17-173	< 1	20.10	12.30	45.20
M. xanthopus	PP17-16	< 1	19.50	10.40	43.20

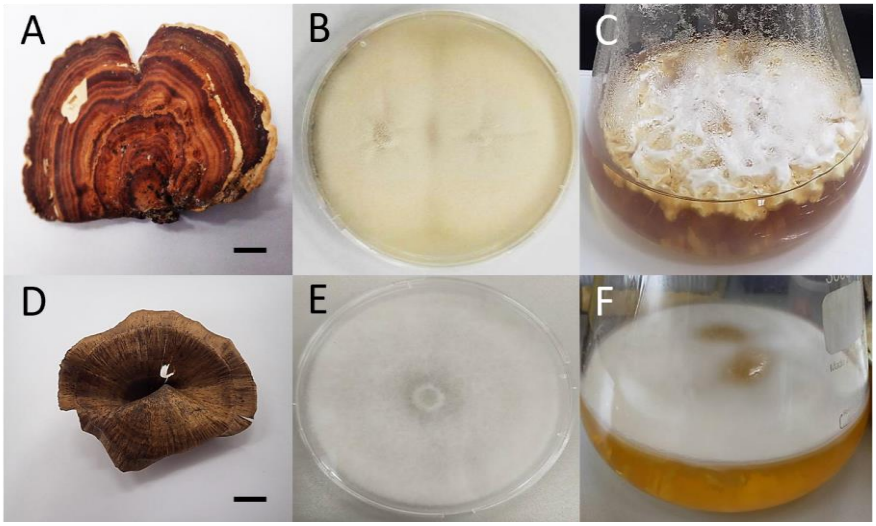

Figure 1 Mushroom samples and cultures. A) fruiting body of *M. vernicipes* PW17-173 (Bar = 1 cm), B-C) cultures of *M. vernicipes* PW17-173 on PDA and YM media, D) fruiting body of *M. xanthopus* PP17-16 (Bar = 1 cm), E-F) cultures of *M. xanthopus* PP17-16 on PDA and YM media.

Antioxidant activities and total phenolic compounds

The extracts were analysed for their antioxidant activities using various assays. Among the fractions, the culture broth ethyl acetate extract (BA) demonstrated the strongest antioxidant activity, showing the lowest IC_{50} values across all three analytical methods: DPPH assay, ABTS assay, and chelating activity, in both species (Figure 2, Table 3). Specifically, the BA extract from *M. vernicipes* PW17-173 exhibited IC_{50} values of 0.784±0.076 mg/mL for the DPPH assay, 1.188±0.126 mg/mL for the ABTS assay, and 1.680±0.003 mg/mL for chelating activity. Similarly, BA extracts from *M. xanthopus* PP17-16 showed comparable results, with IC_{50} values of 0.901±0.028 mg/mL for the DPPH assay, 1.447±0.004 mg/mL for the ABTS assay, and 2.236±0.007 mg/mL for chelating activity. Notably, the ethanolic extracts from the culture broth (BE) exhibited stronger antioxidant activities in the DPPH and ABTS assays compared to the mycelium ethanolic extracts (ME). However, the ME extracts displayed higher antioxidant activity in the chelating assay. Total phenolic content (TPC) analysis revealed significant differences between the species and extracts (Figure 2D). TPC values for *M. vernicipes* PW17-173 ranged from 4.830±0.189 to 71.623±1.677 mg GAE/g extract, with the highest value observed in the BA extract (71.623±1.677 mg GAE/g extract). In contrast, TPC for *M. xanthopus* PP17-16 ranged from 1.811±0.680 to 2.881±0.663 mg GAE/g extract, indicating much lower phenolic content.

Table 3 Antioxidant activities and total phenolic content of *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16 extracts.

Species	Isolate	Antioxidant activities (IC_{50} mg/mL)									Total phenolic content (mg GAE/g extract)		
		DPPH assay			ABTS assay			Chelating assay					
		BA	BE	ME	BA	BE	ME	BA	BE	ME	BA	BE	ME
M. vernicipes	PW17-173	0.784± 0.076bA	1.188± 0.126bB	1.68± 0.003bC	0.416± 0.053bA	1.708± 0.018bB	1.614± 0.48bB	4.146± 0.139bA	10.306 ± 0.168bB	5.758± 0.015bA	71.623 ± 1.677aA	4.83± 0.189aC	5.774± 0.189aB
M. xanthopus	PP17-16	0.901± 0.028cA	1.447± 0.004cB	2.236± 0.007cC	0.633± 0.015cA	3.42± 0.179cC	2.947± 0.077cB	5.467± 0.072cA	11.743 ± 0.165cB	6.234± 0.001cA	2.314± 0.288bB	1.811± 0.68bA	2.881± 0.663bB
Trolox		0.004± 0.001a	0.004± 0.001a	0.004± 0.001a	0.019± 0.002a	0.019± 0.002a	0.019± 0.002a	-	-	-	-	-	-
EDTA		-	-	-	-	-	-	0.035± 0.001a	0.035± 0.001a	0.035± 0.001a	-	-	-

Note: BA = culture broth ethyl acetate extract, BE = culture broth ethanolic extract, ME = mycelium ethanolic extract. Values are expressed as the mean ± SD of triplicate experiments. Different lowercase letters (a–c) indicate statistically significant differences ($p < 0.05$). Uppercase letters (A–B) within a row represent statistically significant differences ($p < 0.05$).

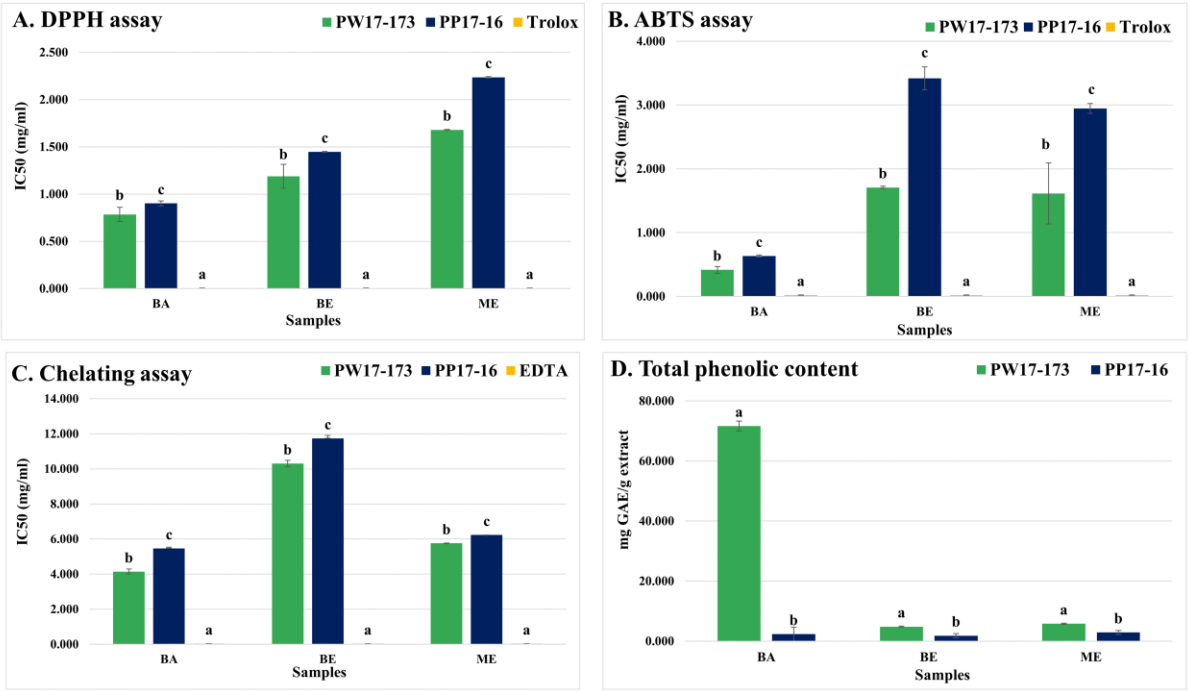

Figure 2 Antioxidant activities and total phenolic content of *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16 extracts. A) DPPH assay, B) ABTS assay, C) Chelating agent, and D) Total phenolic content. BA = culture broth ethyl acetate extract, BE = culture broth ethanolic extract, ME = mycelium ethanolic extract. Values are expressed as the mean ± SD of triplicate experiments. Different lowercase letters (a–c) indicate statistically significant differences ($p < 0.05$).

Anti-inflammatory activity and anti-tyrosinase activity

The extracts from *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16 demonstrated notable anti-inflammatory effects, as indicated by their ability to inhibit NO production, a key inflammatory mediator [20]. The extracts from *M. vernicipes* PW17-173 exhibited stronger anti-inflammatory activity, with IC_{50} values ranging from 1.550±0.045 to 2.282±0.016 mg/mL, compared to *M. xanthopus* PP17-16, which ranged from 4.995±0.298 to 6.618±1.016 mg/mL (Table 4). Among all the tested extracts, the ethanolic extract from *M. vernicipes* PW17-173 mycelium exhibited the most potent activity with an IC_{50} of 1.550±0.045 mg/mL, while the ethanolic extract from *M. xanthopus* PP17-16 culture broth showed the highest activity for this species at 4.995±0.298 mg/mL.

Table 4 Anti-inflammatory activity of *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16 extracts.

Species	Isolate	Anti-inflammatory activity (IC50 mg/mL)		
		ME	BA	BE
M. vernicipes	PW17-173	1.550±0.045bA	1.967±0.066bB	2.282±0.016bC
M. xanthopus	PP17-16	6.314±0.539cB	6.618±1.016cB	4.995±0.298cA
Ascorbic acid		0.287±0.002a	0.287±0.002a	0.287± 0.002a

Values are expressed as the mean ± SD of triplicate experiments. Different lowercase letters (a–c) within a column indicate statistically significant differences ($p < 0.05$). Uppercase letters (A–B) within a row represent statistically significant differences ($p < 0.05$).

The anti-tyrosinase activity of all extracts, expressed as IC_{50} values, is presented in Table 5. The BA extracts of both mushrooms exhibited notable anti-tyrosinase activity, with *M. xanthopus* PP17-16 showing an IC_{50} of 0.335±0.055 mg/mL and *M. vernicipes* PW17-173 exhibiting 0.591±0.013 mg/mL. The ME extracts of *M. vernicipes* PW17-173 demonstrated particularly strong activity, with an IC_{50} of 0.262±0.013 mg/mL, outperforming the *M. xanthopus* PP17-16 extract, which had an IC_{50} of 1.044±0.008 mg/mL. Additionally, the BE extracts showed *M. vernicipes* PW17-173 to have lower IC_{50} values (1.044±0.008 mg/mL) compared to *M. xanthopus* PP17-16 (1.954±0.010 mg/mL). Ascorbic acid, used as a positive control, demonstrated the strongest anti-tyrosinase activity, with an IC_{50} of 0.050±0.002 mg/mL across all extracts.

Table 5 Anti-tyrosinase activity of *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16 extracts.

Species	Isolate	Anti-tyrosinase activity (IC_{50} mg/mL)		
		ME	BA	BE
M. vernicipes	PW17-173	1.262±0.013cB	0.591±0.013cA	1.317±0.025bB
M. xanthopus	PP17-16	1.044±0.008bB	0.335±0.055bA	1.954±0.010cC
Ascorbic acid		0.050±0.002a	0.050±0.002a	0.050±0.002a

Values are expressed as the mean ± SD of triplicate experiments. Different lowercase letters (a–c) within a column indicate statistically significant differences ($p < 0.05$). Uppercase letters (A–B) within a row represent statistically significant differences ($p < 0.05$).

Cytotoxicity of the extracts against HaCaT cells using MTT assay

The cytotoxicity of the extracts was assessed using HaCaT cells, a biologically relevant model for evaluating potential skin irritants. The extracts were tested at varying concentrations, and cell viability remained at 100% for most extracts within the range of 3.9 μg/mL to 62.5 μg/mL, indicating non-toxic to the cells. However, the BE extract of *M. xanthopus* PP17-16 demonstrated higher toxicity, with cell viability reducing to 62.5 μg/mL (Figure 3). At a concentration of 125 μg/mL, the BE extract led to a 50% reduction in cell viability, indicating a more pronounced cytotoxic effect compared to other extracts. In contrast, the ME extract from *M. vernicipes* PW17-173 showed higher biocompatibility, maintaining 90% and 70% cell viability at concentrations of 250 μg/mL and 500 μg/mL, respectively. These findings suggest that while most extracts exhibited low toxicity at standard concentrations, the BE extract of *M. xanthopus* PP17-16 may pose a higher risk of irritation or cytotoxicity at relatively lower doses.

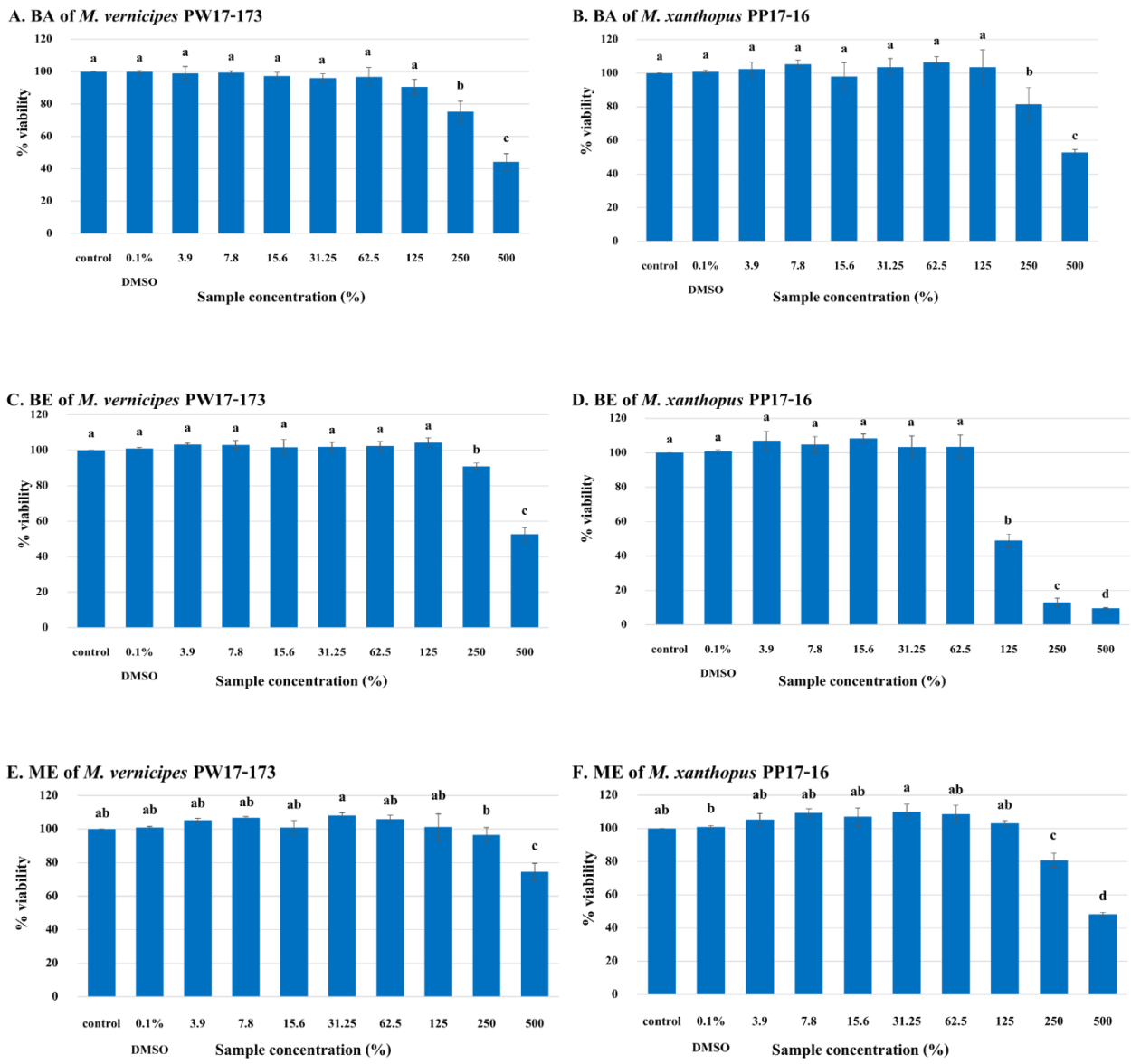

Figure 3 Cytotoxicity of *Microporus vernicipes* PW17-173 (A–C) and *Microporus xanthopus* PP17-16 (D–F) extracts against HaCaT cells using MTT assay. A, D) BA extracts, B, E) BE extracts, and C, F) ME extracts. Values are expressed as the mean ± SD of triplicate experiments. Different lowercase letters (a–d) within a column indicate statistically significant differences ($p < 0.05$).

Discussion

Microporus vernicipes PW17-173 and *M. xanthopus* PP17-16 were selected for metabolite extraction from both mycelium and culture broth using ethanol and ethyl acetate. Ethanolic extracts produced significantly higher yields, likely due to ethanol's polarity, which facilitates the extraction of a broader spectrum of bioactive compounds, including phenolic compounds and polysaccharides. In contrast, ethyl acetate, being less polar, demonstrated reduced efficiency in extracting hydrophobic compounds [10]. The mycelium ethyl acetate (MA) extracts of both mushroom species were limited, possibly due to the lower concentration of low-polarity metabolites in the mycelia. Thus, ethanol proved

to be the more suitable solvent for mycelium extracts. Moreover, cultivation conditions—such as medium type, aeration, and growth duration—were found to significantly influence fungal growth and metabolite production [21]. All fractions of mushroom extracts displayed significant antioxidant activity, with *M. vernicipes* PW17-173 showing the highest levels in its BA extract (IC_{50} = 0.784±0.076 mg/mL, measured by DPPH assay), which also had the greatest total phenolic content (71.623±1.677 mg GAE/g extract). For *M. xanthopus* PP17-16, the BA extract demonstrated the strongest antioxidant activity across all assays, indicating that the ethyl acetate fraction from the culture broth is particularly rich in bioactive compounds contributing to antioxidant potency. Notably, our study highlights the effectiveness of cultured mushroom extracts, contrasting with previous research, which primarily focused on fruiting body extracts [12, 22]. For example, Juliette-Ornely et al. (2018) extracted metabolites from *M. xanthopus* basidiomes using aqueous, ethanol-aqueous, and ethanol solvents, yielding IC_{50} values ranging from 12.09 to 35 μg/mL in DPPH assays [22]. Similarly, Fernando et al. (2016) extracted *M. vernicipes* basidiomes with ethanol, reporting an IC_{50} value of 552.70±1.38 μg/mL and a total phenolic content of 7.4±1.12 μg GAE/mg [23]. However, our study focused on extracting bioactive compounds from cultured mycelium and broth, which may produce metabolites distinct from those in fruiting bodies. Additionally, the culture extracts are of natural origin and hold significant potential for scaling up using fermentation techniques. Utilizing culturable mushrooms also contributes to species conservation by reducing the need for wild collection, thereby aiding in their preservation before they become endangered. The highest antioxidant activities were achieved using ethyl acetate extracts. These results underscore the strong antioxidant potential of mushroom-derived extracts, with both the source material and solvent selection playing critical roles in bioactivity. Furthermore, certain extracts, such as the BE extracts from *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16, exhibited strong antioxidant activity in the DPPH and ABTS assays but lower activity in the ferrous ion chelating assay. This variation can be explained by the different antioxidant mechanisms these assays evaluate. While the DPPH and ABTS assays measure the ability of an extract to donate electrons or hydrogen atoms to neutralize free radicals, the ferrous ion chelating assay assesses the extract's capacity to bind metal ions like Fe^{2+}, which catalyze reactive oxygen species (ROS) formation via the Fenton reaction [24]. The lower activity in the chelating assay suggests that while the extracts are potent free radical scavengers, they are less effective at sequestering metal ions. This highlights the presence of diverse bioactive compounds within the extracts, each with distinct antioxidant properties.

A clear distinction in anti-inflammatory activity was observed between the two species, with *M. vernicipes* PW17-173 exhibiting a higher concentration of bioactive compounds capable of modulating inflammatory responses. The difference was most dominant in the ME, where *M. vernicipes* PW17-173 showed an IC_{50} value of 1.550±0.045 mg/mL, while *M. xanthopus* PP17-16 was most dominant in the BE, with an IC_{50} value of 4.995±0.298 mg/mL. These differences may be attributed to the types of mushrooms studied. These results are consistent with earlier studies, which highlight the potential of mushroom-derived extracts as natural anti-inflammatory agents. For instance, mushrooms, including *Microporus* species, are known to contain bioactive compounds such as polysaccharides, terpenoids,

and phenolics [11], which exert anti-inflammatory effects by inhibiting pro-inflammatory mediators like nitric oxide (NO) and cytokines [25]. Herawati et al. (2021) explored the phytochemical profile of wild-growing *Microporus* species, revealing that *M. xanthopus* extracts obtained using chloroform contained alkaloids, flavonoids, steroids, and triterpenoids, while methanol extracts contained alkaloids, flavonoids, and steroids [26]. Furthermore, Obama-Engonga et al. (2018) found that ethanol extracts from the fruiting body of *M. xanthopus* contained alkaloids, flavonoids, and coumarins, which have been associated with anti-inflammatory activities [27]. These findings underscore the importance of solvent choice in optimizing the extraction of bioactive compounds and point to the potential of *Microporus* extracts in therapeutic applications for inflammation-related conditions.

The study also revealed promising tyrosinase inhibitory properties in both mushrooms, with *M. vernicipes* PW17-173 again exhibiting superior activity. Although none of the extracts matched the potency of the positive control (ascorbic acid), the BA extracts from both species showed significant tyrosinase inhibition, suggesting their potential for inclusion in cosmetic formulations aimed at managing hyperpigmentation disorders. The variation in IC_{50} values across different extracts underscores the importance of selecting appropriate extraction methods and solvents to enhance bioactivity. Future research should focus on isolating the active compounds responsible for this inhibition, with a view toward developing natural tyrosinase inhibitors for cosmetic use. The observed differential cytotoxicity among the extracts highlights the importance of carefully selecting extracts for cosmetic or therapeutic use. The higher cytotoxicity of the BE extract may be attributed to specific cytotoxic compounds, necessitating further chemical characterization. Conversely, the ME extract of *M. vernicipes* PW17-173 demonstrated lower toxicity at higher concentrations, making it a more promising candidate for skin-related applications. Further research is essential to identify the active compounds responsible for these effects, as well as to conduct *in vivo* studies to ensure the safety and efficacy of these extracts in cosmetic formulations. Further investigation is warranted to isolate and identify the specific compounds responsible for these effects, as well as to explore their applicability in health and cosmetic formulations.

Conclusions

This study explored the bioactive properties of *M. vernicipes* PW17-173 and *M. xanthopus* PP17-16 extracts obtained from mycelium and culture broth using ethanol and ethyl acetate. Ethanolic extracts yielded higher extraction amounts, while ethyl acetate extracts exhibited strong antioxidant, anti-inflammatory, and tyrosinase inhibition activities. *M. vernicipes* PW17-173 showed greater antioxidant and anti-inflammatory activities, whereas *M. xanthopus* PP17-16 demonstrated stronger tyrosinase inhibition. Cytotoxicity varied among extracts, with the *M. vernicipes* PW17-173 ME extract showing lower toxicity. These findings highlight the therapeutic and cosmetic potential of *Microporus* species, though further research is needed on specific bioactive compounds.

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