Pharmaceutical Sciences Asia

Pharm Sci Asia 2025; 52(1), 100-109

DOI:10.29090/psa.2025.01.24.2036

Research Article

Enhancing Cosmetic Formulations with *Pleurotus cystidiosus* Extract: A Study on Anti-Photoaging and Moisturizing Potential

Pariya Atawong[1,2], Wannaporn Sagasae[3], Jongrak Attarat[3], Worasak Kaewkong[3], Worawut Kriangkrai[1,2,*]

1 Center of Excellence for Natural Health Product Innovation and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand
2 Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand
3 Department of Biochemistry, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

ABSTRACT

Pleurotus cystidiosus, distinguished for its nutritional and biomedical attributes due to a rich repository of bioactive compounds such as antibacterial properties, antioxidant effects, and antihypertensive agents, presents promising therapeutic functionalities for cosmetic applications. This study investigated the anti-photoaging and moisturizing potential of *P.cystidiosus* extracts (PCE) for cosmetic application, addressing a gap in scientific exploration regarding the use of *P.cystidiosus* in cosmetic formulations. Through an extraction process with 60% saturation using ammonium sulfate, the results show protein yields of 72% for PCE1, 85% for PCE2, and 100% for PCE3, respectively. This demonstrates the method's efficiency with high protein yields and a rich presence of short molecular weight peptides, as confirmed by the Lowry assay and electrophoresis. These extracts demonstrated antioxidant efficacy, assessed via 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,20 -azino-bis-3- ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assays, alongside anti-tyrosinase activity, primarily attributed to their low molecular weight fractions (<3 kDa). PCE3 demonstrated a high protein content in comparison to both PCE1 and PCE2. Therefore, PCE3 was assessed its effect on cell viability was examined through a UV exposure test. PCE3 exhibited UV radiation-protective effects against photoaging in Human keratinocytes (HaCaT) cells at 100 µg/mL concentration, suggesting its potential for sunscreen and anti-aging product integration. This study investigated the skin moisturizing effect of PCE in cosmetic formulations through snake skin moisturizing studies and water contact angle measurements, revealing an improvement in skin wettability. This enhancement in hydration highlights the critical role of PCE in formulating moisturizing products, which are integral to skincare routines.

Keywords:
Pleurotus cystidiosus; Cosmetic Applications; Anti-Photoaging; Skin Moisturizing Effect; Human Keratinocytes (HaCaT) Cells

1. INTRODUCTION

Pleurotus cystidiosus (*P. cystidiosus*) has emerged as an edible mushroom of interest due to its nutritional and biomedical properties, attributed to its bioactive compounds with diverse therapeutic functions[1,2]. Mushrooms produce many proteins and peptides with various biological effects[3]. A common method involves extracting protein isolates using an alkaline solution. The protein is precipitated at its isoelectric pH or ammonium sulfate-induced precipitation. This method could recover up to 36% of the protein content[4,5]. Salting out is a cost-effective and environmentally simple method for protein separation, preserving protein integrity compared to organic solvent precipitation[6,7].The Mushroom proteins are increasingly used in cosmetics for their beneficial properties in skincare and haircare formulations[8] Additionally, the long history of mushroom use and their derivatives supports their safety for human health.

***Corresponding author:**
***** Worawut Kriangkrai Email: Worawutkr@nu.ac.th

However, there is limited documentation on the use of *P. cystidiosus* extract as an ingredient in cosmetic products. Considering this gap in published research, this study aimed to assess the safety, anti-photoaging activity, and skin moisturizing effect of *P. cystidiosus* extract for potential incorporation into cosmetic formulations.

2. MATERIALS AND METHODS

2.1. Materials

P. cystidiosus, sourced from mushroom farms in Phitsanulok, Thailand. Ammonium sulfate and sodium chloride were procured from Elago Enterprises Pty Ltd., N.S.W., Australia. Super enzymes were procured from Now Foods., Glen Ellyn., USA. Human keratinocytes (HaCaT) were graciously provided by the National Center for Genetic Engineering and Biotechnology. Tyrosinase (EC 1.14.18.1) was purchased from Sigma-Aldrich, USA. Snake skin™ Dialysis Tubing 10,000 MWCO was purchased by Thermo Fisher Scientific Inc., Rockford, USA. L-3, 4-dihydroxyphenylalanine (L-dopa), Trolox, Kojic acid, Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), and other chemicals used in the study were analytical grade and obtained from Sigma-Aldrich USA.

2.2. Preparation of *P. cystidiosus* extract (PCE)

The extraction of *P. cystidiosus* was conducted following modified procedures previously described[9]. Initially, 3 kg of fresh fruiting body of *P. cystidiosus* was shredded and blended with 0.15 M NaCl in a 1:2 (w/v) ratio. The resulting mixture was then centrifuged (Model 5922, KUBOTA, Tokyo, Japan) at 15,300 × g at 4°C for 30 min, resulting in 7 L of supernatant, which was subsequently collected for the protein precipitation. Precipitation was achieved by 60% saturation with ammonium sulfate. The precipitate was collected and resuspended in distilled water at a volume ratio of 3:50 (v/v), resulting in 700–750 ml of protein solution, and underwent repeated centrifugation. The protein suspension of 650–700 ml, was dialyzed at intervals of 6 h over 1-3 days to eliminate salt, followed by freeze-drying of PCE, which was stored at -80°C (Model 905, Thermo Fisher Scientific Inc., Marietta, USA) until further analysis.

The PEC was hydrolyzed with a 10:1 super enzyme: 5 mM potassium phosphate. The hydrolysis was performed at 37°C for 24 h at pH 6.8 by using protease (5% enzyme/substrate), and then the protein sample was heated at 95°C for 20 min. To stop the reaction. The protein hydrolysate was then prepared using the following steps: centrifugation to separate the hydrolysate from any remaining solids, leading to the collection of the supernatant. The supernatant was then subjected to purification using ultrafiltration membranes with molecular cut-off less than or equal to 3- kDa which is called PCH (≤3kDa). The filtered supernatant was freeze-dried to yield the protein hydrolysates. PCH (≤3kDa) was stored at -20°C (Model SF-C997, SANYO Commercial Solutions (Thailand) Co., Ltd., Thailand) until required for further analysis.

2.3. Specification of PCE

2.3.1. Protein content

The protein content adapted from the Lowry assay method was performed as previously described[10]. This method was used for measuring the protein levels of the PCE in 96-well plates. After the preparation of Solution, A (100 mL of distilled water with 5 g of $CuSO_4$ $5H_2O$) and Solution B (1 L of distilled water with 21.2 g of Na_2CO_3, 4 g of NaOH), Solution C (1 mL of Solution A and 50 mL of Solution B) were prepared, followed by Solution D (10 mL of Folin-Ciocalteu phenol reagent and 10 mL of DI water). The sample preparation procedure entailed the dissolution of PCE and PCH (≤3kDa) in deionized water, resulting in the formation of a protein solution derived from PCE and PCH (≤3kDa). A 20 µL sample of PCE and PCH (≤3kDa) were added to 200 µL of solution C, and the solution was left at room temperature (25±2 °C) for 15 min. Then 20 µL of Solution D was added to the solution, which was left for a further 30 min at room temperature (25±2 °C). The absorbance of the sample was measured at 750 nm using a microplate reader (Biotek, Synergy HT, Biotek Instruments, Winooski, USA), and the protein concentration was estimated using a BSA standard curve from the same plate.

2.3.2. Electrophoresis

The electrophoretic profile of the PCE was conducted following modified procedures previously described[11]. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-Page) analysis was used to distinguish between proteins based on their molecular weights of 6.5–270 kDa and 3-260 kDa. Each PCE protein concentration at 10 µg/30 µL was loaded onto the gel. The supernatant of each sample was boiled at 95°C for 15 min before gel electrophoresis. The electrophoresis was run at 120V for 2 h using General Electric Healthcare Bioscience diagnostic equipment, EPS301. The BLUltra pre-stained protein marker was used to identify and compare the molecular weight of the proteins. The gel was tinted with silver and a Coomassie brilliant blue (R250) stain. To document and analyze the protein bands, a gel documentation (GelDoc) system and the Image J analysis software were utilized.

2.4. The bioactivity of PCE

2.4.1. Determination of total phenolic content of the PCE

To determine the total phenolic content (TPC) within the PCE, the Folin–Ciocalteu assay was employed[12], with adaptations for 96-well microplates[13]. The sample was dissolved with deionized water for a concentration of 1 mg/mL. Subsequently, 25 µL of this prepared solution was combined with 25 µL of Folin–Ciocalteu reagent, diluted threefold, and 200 µL of ultrapure water. Following a 5 min incubation, 25 µL of a saturated sodium carbonate solution (10.6 g/100 mL) was introduced to the mixture. The microplate underwent 20 s of shaking. The resultant mixture was allowed to stand for 60 min in a dark environment at a temperature of 25°C. The absorbance was measured at λ = 725 nm using a microplate reader (Biotek, Synergy HT, Biotek Instruments, Winooski, USA). The total phenolic content was quantified and expressed in milligrams of gallic acid equivalents per milliliter.

2.4.2. DPPH radical scavenging activity

The DPPH assay was performed to evaluate the radical scavenging ability (RSA) of PCE[14]. The DPPH reagent was prepared by dissolving 0.1 mM DPPH in methanol. The sample contained 20 µL of the solution mixture and three protein concentrations (50, 100, and 250 µg/mL). PCE 100 µL of DPPH reagent was added to a microplate. The reaction was incubated for 30 min in the dark at room temperature (25±2 °C). The absorbance of the resulting solution was measured at 517 nm using a microplate reader (Biotek, Synergy HT, Biotek Instruments, Winooski, USA). The result was expressed as 0.5 mg/mL as a positive control of Trolox. A low absorbance of the reaction mixture indicated a high free-radical-scavenging activity. The capability to scavenge radicals in the DPPH was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{OD517}_{\text{blank}} - \text{OD517}_{\text{sample}})}{\text{OD517}_{\text{blank}}} \times 100 \quad (1)$$

2.4.3. ABTS radical scavenging activity

The $ABTS^{\cdot+}$ scavenging activity was performed as previously described [14]. The ABTS reagent was prepared by dissolving 7 mM ABTS reagent in distilled water. The solution contained 20 µL of samples, consisting of three concentrations (50, 100, and 250 µg/mL) comprising a variety of protein concentrations of PCE. Additionally, 2.45 mM potassium persulphate, and 7 mM ABTS were added to the microplate. The mixture was incubated for 30 min in the dark at room temperature (25±2 °C). The absorbance of the resulting solution was measured at 734 nm using a microplate reader (Biotek, Synergy HT, Biotek Instruments, Winooski, USA). The results were expressed as 0.5 mg/mL as a positive control of Trolox. A low absorbance of the reaction mixture indicated a high free-radical-scavenging activity. The capability to scavenge the radical of the ABTS was calculated using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = \frac{(\text{OD734}_{\text{blank}} - \text{OD734}_{\text{sample}})}{\text{OD734}_{\text{blank}}} \times 100 \quad (2)$$

2.4.4. Anti-tyrosinase activity

The Anti-tyrosinase activity of the PCE was performed as previously described[15]. A sum of 5 mM L-dopa 120 µL was drawn and then mixed in a 5 mM potassium phosphate buffer solution (pH 6.8) of 70 µL. Tyrosinase (EC 1.14.18.1) 15 µL and the PCE were added during the reaction. The kojic acid was used as a positive control for anti-tyrosinase activity. After incubation at 37°C for 20 min, the amount of DOPA-chrome produced in the reaction mixture was determined at 475 nm using a microplate reader (Biotek, Synergy HT, Biotek Instruments, Winooski, USA). The capability for anti-tyrosinase activity was calculated using the following equation:

$$\text{Tyrosinase inhibition (\%)} = \frac{(\text{OD475}_{\text{control}} - \text{OD475}_{\text{sample}})}{\text{OD475}_{\text{control}}} \times 100 \quad (3)$$

2.5. *In vitro* cytotoxicity determination

The *in vitro* cytotoxicity determination of the PCE was conducted following modified procedures previously described[16]. Human keratinocytes (HaCaT) cells were plated in DMEM containing 10% FBS (Gibco, Thermo Fisher Scientific Inc., Massachusetts, U.S.), and 1% Antibiotic/Antimycotic (Gibco, Thermo Fisher Scientific Inc., Massachusetts, U.S.) (100U/mL) in a 5% CO_2 atmosphere at 37 °C. The effects of PCE on HaCaT cell cytotoxicity and cell viability were measured using the MTT value. Then, cells were seeded on a 96-well culture plate at 2,000 cells/well and incubated for 24 h. The cells were treated with PCE at various concentrations for 24 h. After incubation, 10 µL of the MTT solution (5 mg/mL in PBS) were added to each well. After subsequent incubation for 3 h, the purple-colored precipitates were obvious. The supernatant was washed, and the formazan precipitates were solubilized with the addition of 100 µL DMSO (Sigma-Aldrich, Singapore) per well. Then, after 10 min of incubation, absorbance was obtained at 540 nm using a microplate reader (Biotek, Synergy HT, Biotek Instruments, Winooski, USA). The cell viability was assessed using the following equation.

$$\text{Cell viability (\%)} = \frac{(\text{OD540}_{\text{control}} - \text{O540}_{\text{treated}})}{\text{OD540}_{\text{control}}} \times 100 \quad (4)$$

2.6. The assessment of UV light source and its effect on cell viability through UV exposure test

UV lamps emitting UVA (320-400 nm) were employed for irradiation, utilizing an *in vitro* UVGL-25 Compact UV lamp model L118304 (Analytik Jena GmbH+Co. KG., Cambridge, UK) equipped with a sensor calibrated at 365 nm to measure consistent power output in units of J/cm². Exposure duration was calculated using the equation:

$$\text{Dose (J/cm}^2\text{)} = \text{exposure time (s)} \times \text{output intensity (W/cm}^2\text{)}. \quad (5)$$

Rigorous quality control procedures were implemented to assess lamp performance and exposure time accuracy before each use to account for potential fluctuations in power output.

The protective and therapeutic effects of PCE on cell viability were assessed using the MTT assay. For both experiments, cells were initially seeded into 96-well culture plates at a density of 2,000 cells/well and cultured for 24 h. To evaluate the protective effect, cells were treated with PCE at low and high concentrations for either 24 or 48 h prior to UV exposure. Following treatment, the cells were exposed to UV radiation at intensities of 0, 1.59, and 3.18 J/cm². The therapeutic effect of PCE was assessed using a slightly different approach. After UV exposure at the same intensities (0, 1.59, and 3.18 J/cm²), cells were treated with PCE at low and high concentrations for 24 or 48 h. This post-exposure treatment distinguished the therapeutic evaluation from the protective assessment, where treatment occurred before UV exposure.

2.7. Preparation of hydrating water formulations

Hydrating water formulations were prepared by combining hydrophilic substances in water. Mix water with butylene glycol, polyethylene glycol 400, and glycerin. PCE and phenoxyethanol were added, and the solution was then homogenized. All three formulations included varying percentages of components (see Table 1).

Table 1. Ingredients and functions of substances in the hydrating water formulations.

Ingredients	Formulation (%w/w)			Function
	F1	F2	F3	
Water	90.00	79.00	89.96	Solvent
Butylene glycol	4.00	8.89	4.00	Humectant
Polyethylene glycol 400	4.00	8.89	4.00	Humectant
Glycerin	1.00	2.22	1.00	Humectant
Phenoxyethanol	1.00	1.00	1.00	Preservative
PCE	-	-	0.04	Active ingredient

2.8. Snake skin moisturizing study

2.8.1. The water loss in snake skin

The assessment of weight loss in snake skin to determine the efficacy of hydrating water formulations was conducted following modified procedures previously described[17]. Snake skins (Thermo Fisher Scientific; 10,000 MWCO) were cut into 2x2 cm² segments, immersed in water, and maintained at 4°C. After 24 h, the snake skin was dried on filter paper to eliminate residual water. Each sample received 10 g of deionized water, with 25 µL of hydrating water formulations added. Following 30 min air-drying, the samples were weighed. Percent weight loss was assessed at predetermination times (0 – 8 h) under ambient conditions. This experimental procedure was replicated six times to ensure experimental reliability.

2.8.2. Contact angle measurement

The drop methodology was employed to determine contact angles on both applications snake skin treated with hydrating water was conducted following modified procedures previously described[18]. Contact angle measurements were carried out using a DSA25 Drop Shape Analyzer (A.KRÜSS Optronic GmbH, Hamburg, Germany). The snake skin was affixed onto glass slides, following which 80 µL of the hydrating water was applied and left to dry overnight at room temperature (25±2 °C). Contact angle measurements were conducted using a liquid-phase droplet volume of 10 µL of water. Reported values represent the average of six samples.

2.9. Accelerated stability

The powder of PCE and hydrating water were placed in sealed glass bottles and stored for accelerated stability tests. The powder of PCE and hydrating water were evaluated initially (t_0 = 24 h) and 90 days after exposure to different temperature conditions: 4 ± 2°C, ambient temperature (25±2 °C), and 45 ± 2°C. The organoleptic test (pH, appearance, and organoleptic characteristics) was used as evaluation parameters of stability.

2.10. Statistical analyses

Data were presented as mean ± SD of independent experiments. Statistical analysis was conducted using the R Project for Statistical Computing (version R-4.4.1, Auckland, New Zealand). Analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons, was performed, with significance set at $\rho<0.05$.

3. RESULTS AND DISCUSSION

3.1. *P. cystidiosus*

3.1.1. Specification of PCE

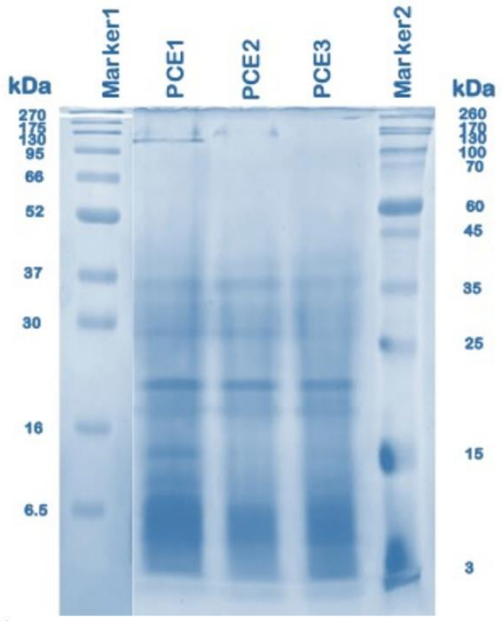
PCE extraction was performed to facilitate industrial upscaling for cosmetic ingredient production. The characteristics of PCE were assessed for each batch. *P. cystidiosus* was subjected to extraction in three batches to ensure consistency in characteristics.

The powder of PCE exhibited a fine brown texture. The percentage yields of PCE were determined as 0.08% for PCE1, 0.06% for PCE2, and 0.04% for PCE3 of the fresh fruiting body of *P. cystidiosus*. PCE3 underwent a longer dialysis process, resulting in a higher total protein content (100.00%), while the total protein content percentages of PCE1 and PCE2 were 72.00% and 85.00%, respectively, as shown in Figure 1A. The lower total protein content observed in PCE1 and PCE2 compared to PCE3 indicated the presence of residual salts from the extraction procedure. This result is corroborated by the conductivity measurements of the protein solutions before the freeze-drying process. PCE1 and PCE2 displayed conductivity values of approximately 2500 µS/cm, while PCE3 showed a significantly lower conductivity of 549.37 ± 0.25 µS/cm. The extended dialysis time used in the PCE3 approach demonstrates the potential for improving protein yield. Previous research suggested that pre-extraction salt removal before protein extraction explained the decline in conductivity of the alkali-soluble protein fraction from seaweed pulp[6].

3.1.2. Profile of protein from PCE

To ensure the consistency and quality of protein extraction in each batch, the molecular weight pattern is crucial. The protein profile of PCE was assessed using SDS-PAGE, revealing bands spanning from 3 to 270 kDa. Predominantly, protein bands below 40 kDa were observed in PCE1, PCE2, and PCE3, as depicted in Figure 1B. Notably, PCE demonstrated uniform protein profiles across all batches, suggesting its viability as a standard for prospective industrial-scale manufacturing.

(A)



(B)

Protein from	Conductivity (µS/cm)	% yield		Total protein content in PCE (%)
		PCE	Protein in PCE	
PCE1	2505.22 ± 1.67	0.08	0.06	72.00
PCE2	2513.58 ± 1.73	0.06	0.05	85.00
PCE 3	549.37 ± 0.25	0.04	0.04	100.00

Figure 1. The percentage of yields and protein (A) and protein profile (B) of PCE.

3.2. The bioactivity of PCE

3.2.1. Antioxidant activity

There's a rising interest in adding antioxidants to cosmetics due to market demand and ingredient advancements. Bioactive peptides from animal and plant protein hydrolysates are recognized for effectively combating oxidative stress[19]. In this study, the antioxidant activity of the protein extracted from *P. cystidiosus* was determined by DPPH and ABTS assays. The percentage of DPPH and ABTS radical scavenging activity, or % antioxidant index, was determined for PCE1, PCE2, PCE3, and PCH (≤3kDa) at concentrations of 50, 100, and 250 µg/mL, with Trolox serving as the reference standard at 500 µg/mL, as shown in Table 2. The DPPH radical scavenging activity of PCE indicates low radical scavenging activity across the three batches initially, but an increase is observed with higher concentrations of PCE. A similar pattern was observed in the ABTS assay

However, notable antioxidant activity was observed for the PCH (≤3 kDa). This suggests that proteins with a molecular weight below 3 kDa displays superior antioxidant activity compared to those with higher molecular weights. Previous investigations illustrated that the hydrolysate fraction of protein, with a low molecular weight (less than 3 kDa), exhibited greater scavenging activities against DPPH• and •OH compared to fractions with molecular weight > 3 kDa[20].

3.2.2. Anti-tyrosinase activity

Skin pigmentation is induced by melanin production from melanocytes due to UV radiation exposure. Tyrosinase plays an important role in converting tyrosine into melanin. Inhibiting tyrosinase activity is a property of a substance that can disrupt melanin production, reducing skin pigmentation[21, 22]. In this study, the tyrosinase inhibitory percentages for PCE1, PCE2, PCE3, and PCH (≤3 kDa) were evaluated at concentrations of 50, 100, and 250 µg/mL, with kojic acid at 100 µg/mL as the reference standard (Table 2). The inhibitory activity was determined using a standard tyrosinase inhibition assay. The results indicated that PCE exhibited no tyrosinase inhibition, whereas kojic acid at 100 µg/mL demonstrated 82.66 ± 1.03% inhibition. Kojic acid at concentrations of 0.125–1.0 mg/mL achieved 91.23–99.00% inhibition, likely due to its higher concentration of low molecular weight proteins. In contrast, PCH at 50-250 µg/mL demonstrated higher tyrosinase inhibitory activity (49.89% to 63.41%). Compared to the tyrosinase inhibitory activities of hot water extracts from *P. ostreatus* fruiting bodies (0.125-1.0 mg/mL)[23] which ranged from 9.60% to 49.60%, PCH showed superior inhibition, suggesting the efficacy of small proteins. This aligns with findings from previous research indicating that low molecular weight proteins exhibit stronger tyrosinase inhibitory activity compared to larger counterparts[24]. Additionally, prior studies have highlighted a positive correlation between peptide antioxidant activity and their inhibitory effects against lipoxygenase (LOX), tyrosinase activity, and xanthine oxidase (XOD) activity[25-27].

Table 2. The DPPH, ABTS radical scavenging activity, and inhibitory tyrosinase activity of PCE.

		Protein concentration (µg/ml)			
		50	**100**	**250**	
DPPH radical scavenging activity (%)	**PCE1**	3.12 ± 0.42	7.99 ± 0.81	13.43 ± 0.57	-
	PCE2	3.76 ± 0.42	9.64 ±0.83	10.12 ± 0.96	-
	PCE3	0.00±0.00	2.57±0.73	6.70±0.61	-
	PCH (≤3 kDa)	16.12±1.11	18.39±0.61	24.24±1.17	-
	Trolox 500 µg/ml	-	-	-	81.98±0.28
ABTS radical scavenging activity (%)	**PCE1**	81.98±0.28	6.98±0.34	8.06±0.75	-
	PCE2	6.49±0.32	6.88±0.15	7.03±0.37	-
	PCE3	9.31±0.17	11.26±0.53	16.99±0.57	-
	PCH (≤3 kDa)	24.24±0.50	26.41±0.68	32.14±2.13	-
	Trolox 500 µg/ml	-	-	-	89.21±0.17
Inhibitory tyrosinase activity (%)	**PCE1**	nd	nd	nd	-
	PCE2	nd	nd	nd	-
	PCE3	nd	nd	nd	-
	PCH (≤3 kDa)	49.89±1.69	58.21±1.28	63.41±0.25	-
	Kojic acid 100 µg/ml	-	-	-	82.66±1.03

Results are given as mean ±standard deviation, and nd: not detected.

3.2.3. Total phenolic content of the PCE

Total phenolic content reflects the concentration of phenolic compounds in an extract, with higher levels typically associated with enhanced antioxidant[28-30] and tyrosinase inhibitory effects[31, 32]. The undetectable total phenolic contents were observed in PCE, indicating that the antioxidant and anti-tyrosinase activities described in sections 3.2.1-3.2.2 may not correlate with phenolic content in these extracts.

3.3. *In vitro* cytotoxicity of PCE

Skin products must undergo toxicity testing to prevent diseases and allergic reactions. Measuring cell viability in response to PCE is essential for product safety evaluation. This information is crucial for selecting appropriate concentrations and developing high-quality products from PCE in the future.

In this cytotoxicity assessment, HaCaT cells were exposed to varying concentrations (100 - 5000 µg/mL) of protein from PCE for 24, 48, and 72 h. As shown in Figure 2, increasing PCE concentration resulted in decreased cell viability of HaCaT cells. Concentrations exceeding 500 µg/mL exhibited clear cytotoxic effects at all time points. However, the concentration of 100 µg/mL appeared safe for HaCaT cells, with cell viability percentages at 24, 48, and 72 h being 86.71±5.33%, 85.32±4.54%, and 105.73±6.86%, respectively. Consequently, the concentration of 100 µg/mL of PCE was deemed suitable for further investigation as a cosmetic ingredient in this study.

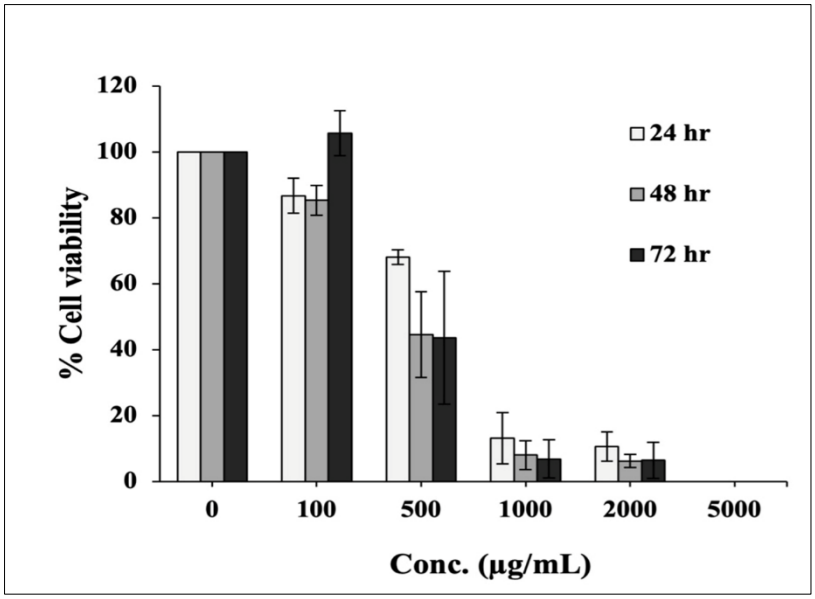


Figure 2. The percentage of cytotoxicity attributed to the PCE.

3.4. Effect of PCE on cell viability before-after UV exposure

The protective and therapeutic effects of PCE were evaluated on HaCaT cells exposed to UV radiation at doses of 0, 1.59, and 3.18 J/cm² for 24 and 48 h (Figure 3). Treatment with PCE at a concentration of 100 µg/mL prior to UVA exposure at doses of 1.59 and 3.18 J/cm² resulted in increased cell viability compared to control cells at 24 h post-UVA radiation (Figure 3A), suggesting its potential as an anti-photoaging agent. However, prolonged UVA exposure (Figure 3B) showed no significant difference compared to the untreated group. Therapeutic effects (PCE treatment after UVA exposure), exhibited in Figure 3C and 3D, did not show superior effects compared to the untreated group. These results indicated that PCE at a concentration of 100 µg/mL may offer protection against UVA-induced epidermal damage in keratinocytes. These findings align with a previous study[33] where aqueous extracts of *Pleurotus ostreatus* and *Hericium erinaceus* demonstrated positive DPPH and ABTS radical-scavenging activity. The extracts promoted procollagen type I production and reduced matrix metalloproteinase-1 and elastase activity in human dermal fibroblast cells exposed to UVA damage.

3.5. Hydrating water containing PCE

A hydrating water product is a water-based skincare product that aims at replenishing moisture levels in the skin for a hydrated, plump, and refreshed complexion. In this study, the hydrating water containing PCE was prepared to assess its in vitro moisturizing effect in a cosmetic product and the final product's stability. From Table 1, formulations F1 and F2 (without PCE) containing 9% and 20% humectant, respectively, exhibited clear liquid solutions. The addition of PCE led to a transparent yellow liquid appearance. Stability tests of the three formulations were conducted under accelerated conditions at 4 ± 2°C, ambient temperature (25±2 °C), and 45 ± 2°C for 90 days. None of the formulated hydrating waters exhibited changes in pH, color, or odor. Subsequently, the hydrating water containing PCE was further evaluated for its moisturizing properties in a snake skin moisturizing study and water contact angle test.

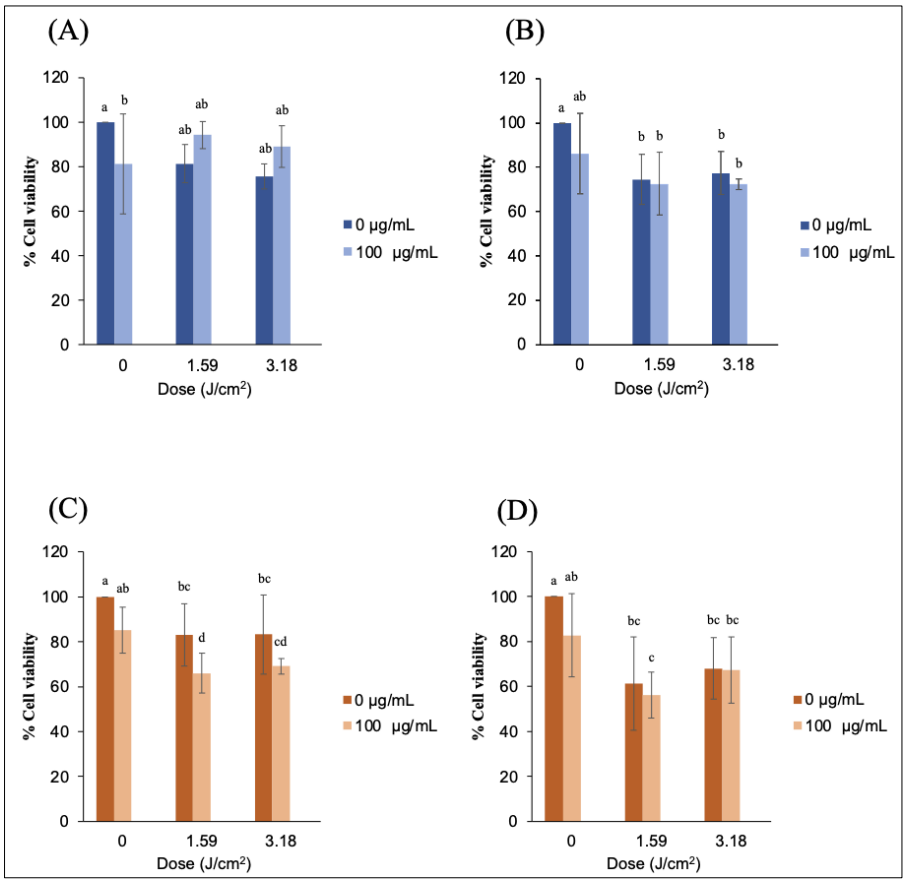


Figure 3. Effects of PCE on HaCaT cell viability before and after UV exposure: A) pre-UV (protective) at 24 h, B) pre-UV (protective) at 48 h, C) post-UV (therapeutic) at 24 h, D) post-UV (therapeutic) at 48 h.

3.6. The water loss of snake skin treated with hydrating water

Hydrating water is frequently employed to enhance the moisturizing properties of the skin by attracting moisture into the skin. The assessment of weight loss of snake skin can explain the efficacy of hydrating water in moisturizing properties[17]. Error! Reference source not found.A illustrates the rate of water loss from snake skin surfaces treated with hydrating waters. The untreated snake skin displayed the highest percentage of water loss. Upon application of the hydrating waters (F1, F2, and F3), the rate of water loss without statistical significance. At the 8 h mark, F1, F2, and F3 significantly demonstrated a lower percentage of water loss. This suggests the positive moisturizing effect of hydrating water containing PCE. This aligns report that high molecular weight proteins from animal and plant sources exhibit excellent moisturizing properties[34].

3.7. The water contact angle of hydrating water on snake skin surfaces

The water contact angle is an important parameter for assessing skin wettability after treatment with cosmetic products. A lower water contact angle indicates better wetting properties[35], potentially improving spreadability and absorption on the skin. The snake skin treated with hydrating water exhibited a significant reduction in water contact angle (Figure 4B). Notably, the inclusion of PCE in the F3 formulation resulted in the lowest water contact angle value, indicating enhanced skin wettability conferred by PCE. The protein's low molecular weight could serve two functions: first, as a humectant; it binds water from the lower layers of the epidermis to the stratum corneum, and also as an occlusive as it reduces trans-epidermal water loss[36].

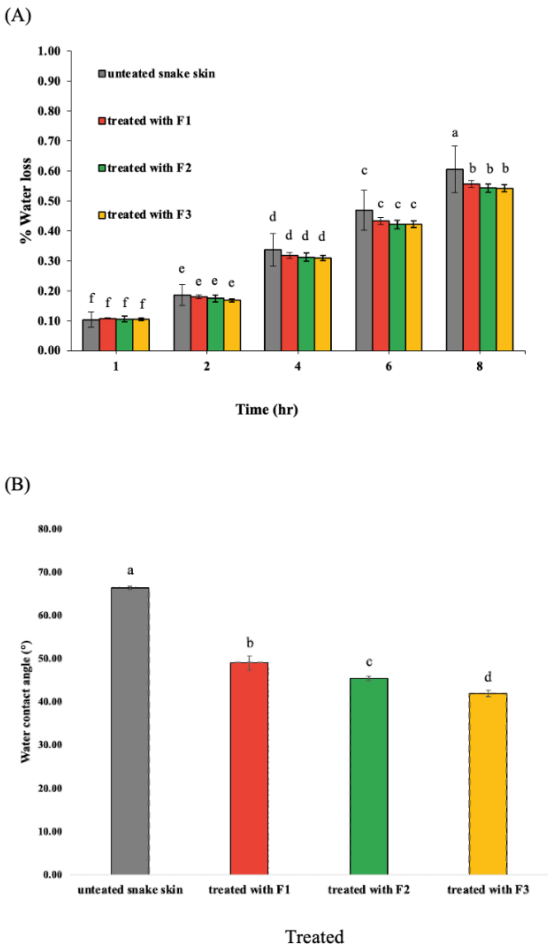


Figure 4. The percentage of water loss (A) and water contact angle (B) of snake skin surfaces treated by hydrating water on snake skin surfaces.

4. CONCLUSIONS

In conclusion, *Pleurotus cystidiosus* extracts (PCE) exhibit potential for cosmetic applications, particularly in anti-photoaging and moisturizing formulations. PCE displayed promising characteristics in protein profile, with high protein content. PCE also demonstrated antioxidant and anti-tyrosinase activities, attributed to its low molecular weight (≤3 kDa). Cytotoxicity assessments indicated the safety of PCE at a concentration of 100 µg/mL, supporting its potential for cosmetic use. In UV radiation tests, the protective effect against photoaging in keratinocytes was observed, suggesting its potential as a sunscreen and anti-aging ingredient. The incorporation of PCE into hydrating water formulations could enhance its moisturizing properties, as evidenced by water loss of snake skin and water contact angle test. Hydrating water containing PCE showed good stability after 3 months. These results show that PCE could be an addition to skincare products. It offers benefits like antioxidants, anti-photoaging effects, and moisturization, which can help keep the skin healthy and vibrant.

5. ACKNOWLEDGEMENTS

This research was made possible in part with generous grants from the Faculty of Pharmaceutical Sciences, and Medical Science, Naresuan University, Thailand (Basic research fund: 2022, grant no. R2565B022) and from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Thailand.

Author contribution

PA: Conceptualization, Methodology, Investigation, Formal Analysis, Visualization, Writing – original draft.
WS: Methodology
JA: Data curation, Validation, Formal analysis, Visualization, Writing – Review and Editing.
WK: Data curation, Validation, Formal analysis, Visualization, Writing – Review and Editing.
WKR: Conceptualization, Methodology, Validation, Formal analysis, Writing – review and editing, Supervision, Resources, Project administration, Funding acquisition.

Conflict of interest

none to declare

Funding

This research was made possible in part with generous grants from Naresuan University, Thailand (Basic research fund: 2022, grant no. R2565B022) and from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Thailand.

Ethics approval

The research protocol adhered to the guidelines of the Naresuan University Institutional Review Board and received approval from the Committee for Biosafety at Naresuan University (NUIBC MI 66-10-41).

Article info:

Received April 5, 2024
Received in revised form September 17, 2024
Accepted October 4, 2024

REFERENCES

1. Rahman M, Hossain A, Rahman M, Bashir N, Mia R. Study on Maturity Level of *Pleurotus Cystidiosus*-2 Maple Oyster Mushroom Emphasized on Organoleptic Taste and Nutrient Content. J. Nutr. Sci. 2021;6(153):2.
2. Elsner P. Antimicrobials and the skin physiological and pathological flora. Biofunctional Textiles and the Skin. 2006;33:35-41.
3. Xu X, Yan H, Chen J, Zhang X. Bioactive proteins from mushrooms. Biotechnology advances. 2011;29(6):667-74.

4. Wong KH, Cheung PC. Nutritional evaluation of some subtropical red and green seaweeds Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. Food chemistry. 2001;72(1):11-7.
5. Harrysson H, Konasani VR, Toth GB, Pavia H, Albers E, Undeland I. Strategies for improving the protein yield in ph-shift processing of Ulva lactuca linnaeus: Effects of ulvan lyases, ph-exposure time, and temperature. ACS Sustain. Chem. Eng. 2019;7(15):12688-91.
6. Juul L, Steinhagen S, Bruhn A, Jensen S, Undeland I, Dalsgaard T. Combining pressing and alkaline extraction to increase protein yield from *Ulva fenestrata* biomass. Food Bioprod. Process. 2022;134:80-5.
7. Liu S, Li Z, Yu B, Wang S, Shen Y, Cong H. Recent advances on protein separation and purification methods. Adv. Colloid Interface Sci. 2020;284:102254.
8. Antunes F, Marçal S, Taofiq O, MMB Morais A, Freitas AC, CFR Ferreira I, et al. Valorization of mushroom by-products as a source of value-added compounds and potential applications. Molecules. 2020;25(11):2672.
9. Deng Y, Huang L, Zhang C, Xie P, Cheng J, Wang X, et al. Skin-care functions of peptides prepared from Chinese quince seed protein: Sequences analysis, tyrosinase inhibition and molecular docking study. Ind. Crops Prod. 2020;148:112331.
10. Bollag D, Edelstein S. Protein method Wiley-Liss publication. 1991.
11. Jovanovic S, Barac M, Macej O, Vucic T, Lacnjevac C. SDS-PAGE analysis of soluble proteins in reconstituted milk exposed to different heat treatments. Sensors. 2007;7(3):371-83.
12. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965;16(3):144-58.
13. Granato D, Karnopp AR, van Ruth SM. Characterization and comparison of phenolic composition, antioxidant capacity and instrumental taste profile of juices from different botanical origins. J Sci Food Agric. 2015;95(10):1997-2006.
14. Chen H, Dai F, Chen C, Fan S, Zheng J, Huang Y, et al. Evaluating the antioxidants, whitening and antiaging properties of rice protein hydrolysates. Molecules. 2021;26(12):3605.
15. Song W, Liu L, Ren Y, Wei S, Yang H. Inhibitory effects and molecular mechanism on mushroom tyrosinase by condensed tannins isolation from the fruit of *Ziziphus jujuba* Mill. var. spinosa (Bunge) Hu ex HF Chow. Int. J. Biol. Macromol. 2020;165:1813-21.
16. Gaweł-Bęben K, Strzępek-Gomółka M, Czop M, Sakipova Z, Głowniak K, Kukula-Koch W. Achillea millefolium L. and Achillea biebersteinii Afan. hydroglycolic extracts–bioactive ingredients for cosmetic use. Molecules. 2020;25(15):3368.
17. Arezki NR, Williams AC, Cobb AJ, Brown MB. Design, synthesis and characterization of linear unnatural amino acids for skin moisturization. Int. J. Cosmet. Sci. 2017;39(1):72-82.
18. Badami JV, Bui HS. Quantification of the Color Transfer from Long-Wear Face Foundation Products: The Relevance of Wettability. Surface Science and Adhesion in Cosmetics, Wiley, 2021. 2021:379-99.
19. Yang W, Hao X, Zhang X, Zhang G, Li X, Liu L, et al. Identification of antioxidant peptides from cheddar cheese made with *Lactobacillus helveticus*. LWT - Food Sci. Technol. 2021;141:110866.
20. You L, Zhao M, Regenstein JM, Ren J. Purification and identification of antioxidative peptides from loach (*Misgurnus anguillicaudatus*) protein hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry. Food Res. Int. 2010;43(4):1167-73.
21. Hearing VJ, Tsukamoto K. Enzymatic control of pigmentation in mammals. FASEB J. 1991;5(14):2902-9.
22. Meng T, Zhang C, Miyamoto T, Ishikawa H, Shimizu K, Ohga S, et al. The melanin biosynthesis stimulating compounds isolated from the fruiting bodies of *Pleurotus citrinopileatus*. J. Cosmet. Dermatol. Sci. Appl. 2012;2(03):151.
23. Alam N, Yoon KN, Lee KR, Shin PG, Cheong JC, Yoo YB, et al. Antioxidant activities and tyrosinase inhibitory effects of different extracts from *Pleurotus ostreatus* fruiting bodies. Mycobiology. 2010;38(4):295-301.
24. Feng Y, Wang Z, Chen J, Li H, Wang Y, Ren D, et al. Separation, identification, and molecular docking of tyrosinase inhibitory peptides from the hydrolysates of defatted walnut (*Juglans regia* L.) meal. Food chem. 2021;353:129471.
25. Addar L, Bensouici C, Zennia SSA, Haroun SB, Mati A. Antioxidant, tyrosinase and urease inhibitory activities of camel αS-casein and its hydrolysate fractions. Small Rumin. Res. 2019;173:30-5.
26. Grancieri M, Martino HSD, de Mejia EG. Digested total protein and protein fractions from chia seed (*Salvia hispanica* L.) had high scavenging capacity and inhibited 5-LOX, COX-1-2, and iNOS enzymes. Food chem. 2019;289:204-14.
27. Zhong H, Zhang Y, Deng L, Zhao M, Tang J, Zhang H, et al. Exploring the potential of novel xanthine oxidase inhibitory peptide (ACECD) derived from Skipjack tuna hydrolysates using affinity-ultrafiltration coupled with HPLC–MALDI-TOF/TOF-MS. Food Chem. 2021;347:129068.
28. Duan X, Wu G, Jiang Y. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules. 2007;12(4):759-71.
29. Pan Y, Wang K, Huang S, Wang H, Mu X, He C, et al. Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus Longan* Lour.) peel. Food chem. 2008;106(3):1264-70.
30. Zhao M, Yang B, Wang J, Li B, Jiang Y. Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chem. 2006;98(3):539-44.
31. Baek HS, Rho HS, Yoo JW, Ahn SM, Lee JY, Jeonga-Lee J-L, et al. The inhibitory effect of new hydroxamic acid derivatives on melanogenesis. ChemInform. 2008;39(23):i.
32. Kim YJ, Kang KS, Yokozawa T. The anti-melanogenic effect of pycnogenol by its anti-oxidative actions. Food Chem. Toxicol. 2008;46(7):2466-71.
33. Choi J, Song E, Gwon H, Song E, Kalaras MD, Beelman RB, et al. Aqueous extracts of *Pleurotus ostreatus* and hericium erinaceus protect against ultraviolet A-induced damage in human dermal fibroblasts. Int. J. Med. Mushrooms. 2022;24(2).
34. Ścibisz M, Arct J, Pytkowska K. Protein hydrolysates in cosmetics production, part II. SÖFW J Wydanie Polskie. 2008;4:12-9.
35. Wang H, Lai M, Li H, Jiang L, Wei Y, Yu Z, et al. Moisturizing and aroma-enhancing effects of low molecular weight fenugreek polysaccharides in cigarettes. Int. J. Biol. Macromol. 2024;259:129320.
36. Mokrejs P, Hutta M, Pavlackova J, Egner P, Benicek L. The cosmetic and dermatological potential of keratin hydrolysate. J. Cosmet. Dermatol. 2017;16(4):1021-7.