Sains Malaysiana 55(8)(2026): 1382-1392
http://doi.org/10.17576/jsm-2026-5508-13

In vitro Anti-Melanogenic Potential and Preliminary Human Skin Biophysical Evaluation of *Schizophyllum commune* Water Extracts

(Potensi Anti-Melanogenik Secara *in vitro* dan Penilaian Awal Biofizik Kulit Manusia pada Ekstrak Air *Schizophyllum commune*)

NADIMA MOHAMAD[1], ROSNANI BINTI HASHAM[2], ANISAH JAMALUDDIN[3], DANG LELAMURNI ABD RAZAK[3] & NURUL YUZIANA MOHD YUSOF[1,*]

[1]*Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[2]*Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*
[3]*Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia*

Received: 30 May 2026/Accepted: 17 August 2026

ABSTRACT

This study evaluated the *in vitro* cytocompatibility and anti-melanogenic potential of *Schizophyllum commune* (cendawan kukur) extracts prepared at two temperatures, 4 °C (K4C) and 30 °C (K30C), followed by a human skin biophysical assessment of *S. commune*-based topical formulations. Cytotoxicity was assessed using MTT assays in CRL-2691 human dermal fibroblasts and MNT-1 human melanoma cells. Anti-melanogenic activity was evaluated in MNT-1 cells by measuring melanin content, tyrosinase (TYR) transcript abundance, and intracellular tyrosinase activity. Both extracts maintained high cell viability across the tested concentration range. K30C showed a more consistent response, with modest reductions in melanin content and TYR transcript abundance at 50 and 100 μg/mL, but did not significantly reduce intracellular tyrosinase activity, while K4C showed a less consistent response. A supportive human skin biophysical evaluation was subsequently conducted using topical creams containing the *S. commune* extract prepared at 30 °C. Fifteen participants used the formulations for 12 weeks. The exploratory evaluation indicated reductions in melanin and erythema indices. Overall, the *in vitro* findings and preliminary human skin observations support further investigation of *S. commune*-derived ingredients as potential natural cosmeceutical candidates.

Keywords: Anti-melanogenic activity; human skin assessment; *in vitro* assay; mushroom extract; *Schizophyllum commune*

ABSTRAK

Kajian ini menilai keserasian sel secara *in vitro* dan potensi anti-melanogenik ekstrak *Schizophyllum commune* (cendawan kukur) yang diekstrak pada dua suhu, iaitu 4 °C (K4C) dan 30 °C (K30C), diikuti dengan penilaian biofizik kulit manusia terhadap formulasi topikal berasaskan *S. commune*. Kesitotoksikan dinilai menggunakan ujian MTT dalam sel fibroblas dermal manusia CRL-2691 dan sel melanoma manusia MNT-1. Aktiviti anti-melanogenik dinilai dalam sel MNT-1 melalui pengukuran kandungan melanin, kelimpahan transkrip tirosinase (TYR) dan aktiviti tirosinase intrasel. Kedua-dua ekstrak mengekalkan keviabelan sel yang tinggi dalam julat kepekatan yang diuji. K30C menunjukkan tindak balas yang lebih tekal dengan pengurangan sederhana dalam kandungan melanin dan kelimpahan transkrip TYR pada 50 dan 100 μg/mL, tetapi tidak mengurangkan aktiviti tirosinase intrasel dengan ketara, manakala K4C menunjukkan tindak balas yang kurang tekal. Seterusnya, penilaian biofizik kulit manusia secara sokongan dijalankan menggunakan krim topikal yang mengandungi ekstrak *S. commune* yang disediakan pada 30 °C. Lima belas peserta menggunakan formulasi tersebut selama 12 minggu. Penilaian eksploratori menunjukkan pengurangan indeks melanin dan eritema. Secara keseluruhan, keputusan *in vitro* dan pemerhatian awal pada kulit manusia menyokong kajian lanjut terhadap bahan terbitan *S. commune* sebagai calon kosmeseutikal semula jadi.

Kata kunci: Aktiviti anti-melanogenik; ekstrak cendawan; penilaian kulit manusia; *Schizophyllum commune*; ujian *in vitro*

INTRODUCTION

Natural products have attracted interest as potential cosmeceutical ingredients because they contain antioxidant, anti-inflammatory, and enzyme-modulating compounds that may support skin health. Mushrooms are particularly relevant in this context because they contain polysaccharides, phenolic compounds, terpenoids, peptides, and other metabolites with reported cosmetic and cosmeceutical potential (Taofiq et al. 2016; Wu et al. 2022). *Schizophyllum commune*, locally known as cendawan kukur, is an edible basidiomycete valued in Southeast Asia and is known to produce bioactive constituents such as schizophyllan, a β-glucan with established biological relevance. Recent reviews have highlighted the nutritional, antioxidative and anti-inflammatory properties of *S. commune*, supporting its broader potential as a source of functional bioactive materials (Kumar & Xu 2025; Razak et al. 2024). Oxidative stress may contribute to melanogenesis by increasing reactive oxygen species and influencing melanogenesis-related signalling and tyrosinase activity. Previous work on non-fermented *S. commune* fruitbody extracts demonstrated antioxidant and cell-free anti-tyrosinase activities, suggesting that mushroom-derived antioxidant constituents may have relevance to pigmentation-related responses (Dang Lelamurni et al. 2020). However, its specific application as an anti-pigmentation cosmeceutical ingredient remains insufficiently explored.

Anti-melanogenic activity is commonly investigated using cell-based assays that measure cytotoxicity, melanin content, tyrosinase-related gene expression, and tyrosinase activity. These *in vitro* assays are useful for early-stage mechanistic screening because they allow controlled assessment of whether a test material affects melanogenesis-related cellular responses (Baber et al. 2023; Hassan, Shahzadi & Kloczkowski 2023; Kooltheat et al. 2023). However, cell-based assays alone cannot fully represent the complexity of topical cosmetic use, in which formulation properties, skin penetration, skin-barrier condition, baseline pigmentation, erythema, and participant-level variability can influence the observed outcome (Firooz et al. 2012; Moniz et al. 2020). Supportive human skin biophysical evaluation can therefore strengthen the translational relevance of *in vitro* findings, particularly when non-invasive parameters such as melanin and erythema indices are measured under controlled conditions (Fullerton et al. 1996; Takiwaki 1998). Combining *in vitro* and human assessments provides a more integrated evaluation. *In vitro* assays indicate possible cellular responses, whereas human skin measurements provide formulation-level evidence of whether a product influences pigmentation-related skin parameters after topical application.

Previous work evaluated hot- and cold-water extracts of *S. commune* fruitbodies using chemical-content, antioxidant, anti-hyaluronidase, and cell-free mushroom tyrosinase assays (Dang Lelamurni et al. 2020). The present study differs by examining *S. commune* water extracts prepared at 4 °C and 30 °C and evaluating their potential anti-melanogenic effects using *in vitro* assays. It also incorporates supportive human skin biophysical observations from a formulated topical product. Thus, the present work extends the earlier cell-free screening to cellular and formulation-level assessments, although it remains a preliminary investigation of crude extracts.

MATERIALS AND METHODS

In vitro ANTI-MELANOGENIC EXPERIMENTS

Cell Culture

Two human skin-derived cell lines were used: (i) CRL-2691 cells, also referred to as CCD-1135Sk human dermal fibroblasts (ATCC, USA), and (ii) MNT-1 human melanoma cells (a gift from Prof. Michael Marks, University of Pennsylvania). CRL-2691 fibroblasts were maintained in IMDM supplemented with 10% FBS and 1% amino acids, whereas MNT-1 melanoma cells were maintained in DMEM supplemented with 20% FBS, 10% AIM-V medium, 1% non-essential amino acids, and 1% sodium pyruvate. Both cell lines were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

PREPARATION OF *Schizophyllum commune* EXTRACTS

Two *S. commune* (cendawan kukur) extracts prepared by researchers at MARDI were used; K4C (extracted at 4 °C) and K30C (extracted at 30 °C), as described by Dang Lelamurni et al. (2020). For each extract, the stock solution was diluted in cell-culture medium containing <0.1% dimethyl sulfoxide (DMSO) to obtain final concentrations of 1, 5, 10, 50, 100, and 500 µg/mL. The full concentration range was used for cell-viability testing; 50 and 100 µg/mL were selected for the subsequent melanin-content, TYR transcript, and intracellular tyrosinase-activity assays. Growth medium without extract was used as the negative control.

CELL VIABILITY ASSAY

The effects of K4C and K30C on cell viability were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. CRL-2691 fibroblasts and MNT-1 melanoma cells were seeded in 96-well plates (~5×10^4 – 1×10^5 cells/mL) and incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. The growth medium was replaced with treatment medium containing K4C or K30C at final concentrations of 1, 5, 10, 50, 100 or 500 µg/mL, and the cells were incubated for 24 h. After treatment, the cells were washed twice with 200 µL of DPBS, and 100 µL of MTT solution (1 mg/mL) was added to each well, followed by

incubation for 3 h. The MTT solution was then removed, and 100 µL of isopropanol was added to solubilise the formazan. After shaking for 2 h at room temperature, the solutions were transferred to a new 96-well plate, and optical density was measured at 570 nm using a plate spectrophotometer.

MELANOMA CELL TREATMENT

MNT-1 cells were selected because they produce high and consistent levels of melanin without requiring α-melanocyte-stimulating hormone stimulation. The cells were seeded in 12-well culture plates (1.5×10^5 cells/well in 1 mL) in triplicate and treated with K4C or K30C at concentrations of 50 and 100 µg/mL. Medium containing kojic acid (100 µg/mL) was used as a positive control for the anti-melanogenic assays. Treatment was conducted for 48 h.

MELANIN CONTENT ANALYSIS

Melanin content was measured using a protocol adapted from Martínez-Gutiérrez et al. (2024). After treatment, the cells were harvested and counted, and an equal number of viable cells from each treatment group was used for melanin extraction. The cells were washed twice with DPBS and lysed in 2% SDS at 65 °C for 1 h. To solubilise melanin, the cell lysates were treated with 1 M NaOH in 10% DMSO (total volume, 200 µL) and incubated at 80 °C for 1 h. A 100 µL aliquot of each extract was transferred to a 96-well plate, and absorbance was measured at 340 nm using a plate spectrophotometer. Melanin content was expressed relative to the untreated control. Because equal numbers of viable cells were analysed, additional normalisation to total protein was not performed.

TYR TRANSCRIPT ABUNDANCE ANALYSIS

RNA was extracted from treated cells using GENEzol reagent according to the manufacturer's protocol (Geneaid, Germany). Purified RNA (10 ng/µL) was converted to cDNA using Thunderbird ReverTra Ace RT Master Mix (Toyobo, Japan). Quantitative PCR (qPCR) was performed using a CFX96 real-time PCR system (Bio-Rad, USA), Thunderbird SYBR qPCR Mix (Toyobo, Japan), and tyrosinase-specific primers (forward: 5′-AGATTCAGACGCAGACTCTTTTCA-3′; reverse: 5′-GACACAGCAAGCTCACAAGC-3′). A standard curve was generated using pJET-cbl plasmid DNA containing a 134-bp insert at copy numbers ranging from 10^1 to 10^8. Forty qPCR cycles were performed at 95 °C for 30 s (denaturation), 55 °C for 15 s (annealing), and 72 °C for 30 s (extension). Fluorescence signals detected during the annealing step were used to determine Cq values, which were plotted against the standard curve [Slope = -3.5869; R^2=0.9897; 90.02%]. Data were expressed as $\log_{10}$ copy number in 10 ng of cDNA.

INTRACELLULAR TYROSINASE ACTIVITY ASSAY

Protein concentrations in the cell lysates were estimated using a microplate bicinchoninic acid (BCA) assay, with bovine serum albumin (BSA) as the standard, before the tyrosinase activity assay. The BCA working reagent was prepared by mixing reagent A (BCA solution) with reagent B (4% copper (II) sulphate pentahydrate solution) at a ratio of 50:1. Ten microlitres of each sample or standard was added to a 96-well microplate, followed by 200 µL of freshly prepared BCA working reagent. The microplate was gently shaken and incubated at 37 °C for 30 min or at 60 °C for 15 min. Absorbance was then measured at 551 nm using a microplate reader (BioTek®), and protein concentrations were estimated from the BSA standard curve. After the 48-h treatment, the six-well plate containing melanoma cells was placed on ice, and the attached cells were washed with 1 mL of ice-cold PBS to remove residual growth medium. The PBS was aspirated, and 500 µL of ice-cold lysis buffer consisting of 0.1 M sodium phosphate buffer (pH 6.8), 1% Triton X-100, and a protease-inhibitor cocktail was added to each well. The cell suspensions were transferred to pre-cooled microcentrifuge tubes and centrifuged at 16,099 × g for 20 min at 4 °C. The supernatants were recovered, and their protein concentrations were determined using the BSA standard curve. After the protein concentrations had been quantified and adjusted with lysis buffer, 50 µL of each lysate was transferred to a 96-well plate, followed by 125 µL of 0.1 M sodium phosphate buffer (pH 6.8) and 25 µL of freshly prepared 10 mM L-DOPA in 0.1 M sodium phosphate buffer (pH 6.8). The reaction mixture was incubated for 30 min at 37 °C, and tyrosinase activity was measured at 475 nm using a microplate reader. The percentage of tyrosinase activity was calculated relative to the untreated control using the following equation, where A represents the absorbance of a treated sample and B represents the absorbance of the untreated control: intracellular tyrosinase activity (%) = (A/B) × 100.

HUMAN SKIN BIOPHYSICAL EVALUATION

STUDY DESIGN AND PARTICIPANTS

A supportive human skin biophysical evaluation was incorporated using data from a controlled topical-formulation study of *S. commune*-based creams. Eligible participants were healthy Malaysian women aged 25-55 years with no active facial dermatological disorders. Participants were excluded if they were pregnant or breastfeeding; had known hypersensitivity to cosmetic ingredients; had undergone dermatological or cosmetic procedures within the previous three months; were using topical depigmenting or anti-ageing products; or had a medical condition or were taking medication known to influence skin pigmentation or wound healing.

The test formulation was applied to one side of the face, and a placebo base cream without *S. commune* extract was applied to the contralateral side. Treatment allocation was known only to the research assistant, whereas the participants, investigators, and sponsor were blinded. The human study was approved by the Research Ethics Committee of Universiti Kebangsaan Malaysia (reference UKM PPI/111/8/JEP-2020-525; approval dated 17 October 2020) under the title 'Anti-Aging and Whitening Effect of *Schizophyllum commune*-Based Topical Cream on Human Skin'. The approved protocol included several cosmetic outcomes, including anti-ageing and whitening (pigmentation-related) endpoints. This paper reports only melanin and erythema indices, which are directly relevant to the study objective. Written informed consent was obtained from all participants before enrolment, and the study was conducted in accordance with the relevant institutional ethical requirements.

SAMPLE SIZE DETERMINATION

The sample size was estimated using Sample Size Calculation Software (version 3.0.14), with a two-tailed significance level of 0.05 and 80% statistical power. Estimates were informed by variability reported in a previous exploratory split-site cosmetic study (Snatchfold & Targett 2019). Given the pilot and exploratory nature of the present evaluation, 15 participants were recruited.

TEST PRODUCTS AND APPLICATION PROCEDURE

The investigated products comprised a placebo cream and an active formulation containing *S. commune* extract. The placebo comprised the identical cream base without the extract. The cream base contained purified water, emulsifying agents, emollients, humectants, and cosmetic preservatives. The active formulation contained 1% (w/w) *S. commune* extract incorporated into the cream base. Both formulations were prepared under hygienic laboratory conditions and immediately transferred to identical sterile, opaque polypropylene containers to minimise contamination and preserve blinding. The products were stored at room temperature (25 ± 2 °C) and protected from direct light throughout the study. Packaging integrity was inspected before the products were dispensed to participants. Microbiological quality testing confirmed compliance with cosmetic microbiological specifications before participant use. The human skin biophysical evaluation used a randomised, participant- and assessor-blinded, split-face design. An independent research assistant managed treatment allocation and product coding, whereas participants, assessors, and data analysts remained blinded throughout the study. Participants were instructed to apply the assigned formulations twice daily, in the morning and evening, to the designated areas throughout the 12-week study. They were allowed to continue their routine skincare and make-up but were instructed to avoid other anti-ageing, firming, or anti-pigmentation products during the study.

SKIN BIOPHYSICAL MEASUREMENTS

Skin biophysical parameters were measured at baseline and during follow-up visits over the 12-week study. Measurements were obtained using the DermaLab Combo® (Cortex Technology, Denmark) according to the manufacturer's operating procedures. The instrument was calibrated before each measurement session. Melanin and erythema indices were measured in triplicate at the same predetermined facial location by the same trained assessor throughout the study. Before each assessment, participants underwent a 20-min acclimatisation period in a controlled environment (22 ± 2 °C; relative humidity, 45-60%). Participants were instructed to refrain from applying skincare products, cosmetics, or sunscreen for at least 12 h before each visit.

STATISTICAL ANALYSIS

Data were analysed using SPSS. All *in vitro* experiments were performed in triplicate, and the data are presented as the mean ± standard error of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Values of $p < 0.05$ were considered statistically significant. For the human skin biophysical analyses, changes in melanin and erythema indices between baseline and follow-up measurements were analysed using paired-samples t-tests. The test and placebo sides were also compared at each time point using paired-samples t-tests because the measurements were obtained from the same participants. To account for multiple comparisons across the three follow-up visits (Weeks 4, 8, and 12), a Bonferroni correction was applied, resulting in a significance threshold of $p < 0.033$.

RESULTS AND DISCUSSION

EFFECT OF EXTRACTS ON CELL VIABILITY

The MTT assay showed that CRL-2691 fibroblasts and MNT-1 melanoma cells maintained high viability after treatment with K4C or K30C across the tested concentration range of 1-500 μg/mL (Figure 1). In MNT-1 cells, viability remained close to or above 95%, whereas CRL-2691 fibroblasts also showed no evidence of extract-induced cytotoxicity under the tested conditions. These findings indicate that neither *S. commune* extract caused marked cytotoxicity in the cell models used and support the selection of 50 and 100 μg/mL for the subsequent anti-melanogenic assays.

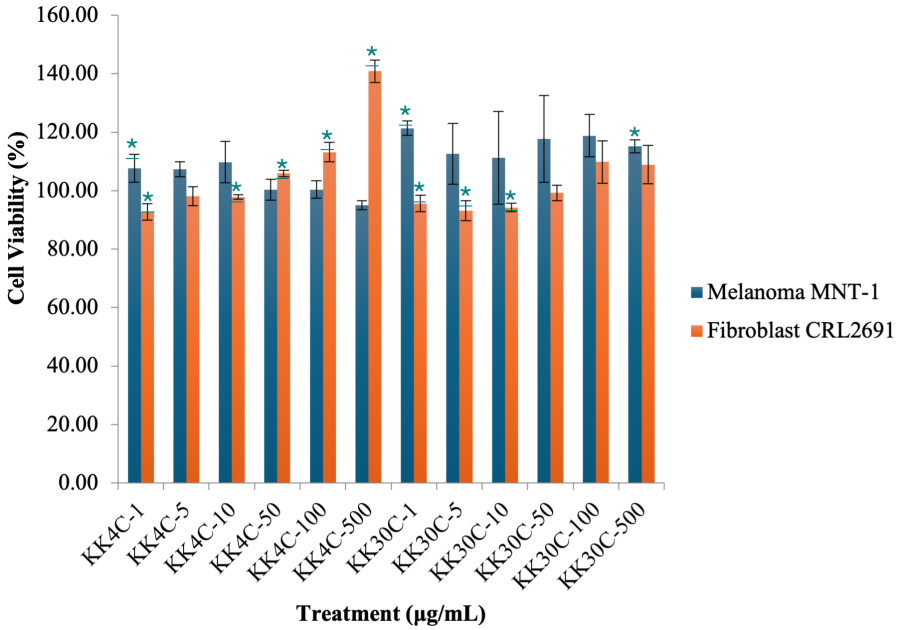
The absence of marked cytotoxicity is important because anti-melanogenic effects must be distinguished from non-specific reductions in cell number or metabolic activity. In melanogenesis studies, a reduction in melanin content may be misleading if the test material is cytotoxic or suppresses cell viability. The MTT results therefore provide essential cytocompatibility context for interpreting the subsequent melanin, TYR transcript, and tyrosinase-activity assays. However, these findings indicate *in vitro* cytocompatibility only and do not replace formulation-level safety assessments, such as irritation, sensitisation, or phototoxicity testing.

EFFECTS OF EXTRACTS ON MELANIN CONTENT

Treatment with K30C was associated with a modest reduction in melanin content in MNT-1 cells at both 50 and 100 µg/mL, whereas K4C reduced melanin content at 50 µg/mL but not at 100 µg/mL (Figure 2). These findings indicate a measurable response under the conditions tested, with K30C showing a more consistent pattern across the two concentrations. However, the limited number of concentrations and absence of a clear dose–response relationship preclude conclusions regarding relative potency or efficacy.

The reduction in melanin content suggests that selected *S. commune* extracts may contain bioactive constituents capable of modulating melanogenesis. Natural products continue to be investigated as sources of tyrosinase inhibitors and melanogenesis-modulating compounds, although their effects frequently depend on concentration, extract composition, cell model, and experimental endpoint (Baber et al. 2023; Hassan, Shahzadi & Kloczkowski 2023). In the present study, K30C reduced melanin content at both 50 and 100 µg/mL, whereas K4C produced a reduction at 50 µg/mL but not at 100 µg/mL. These findings do not demonstrate a clear concentration-dependent response, and the K4C pattern was non-monotonic under the conditions tested. Melanin content is a downstream endpoint influenced by several processes, including TYR protein abundance, maturation and turnover, tyrosinase activity, melanosome biogenesis and trafficking, and the timing of cellular responses. Therefore, the observed changes in melanin content cannot be causally attributed to the measured changes in TYR transcript abundance. Instead, the results should be interpreted as preliminary, associated responses at the individual concentrations tested. Kojic acid was included as a positive control to confirm assay responsiveness; direct comparisons of potency or efficacy are inappropriate because the treatments differed in concentration and may act through different mechanisms.

Kojic acid is a well-established tyrosinase inhibitor and reference compound in melanogenesis studies. Nevertheless, its use in cosmetic products is subject to safety assessment and concentration limits. The Scientific Committee on Consumer Safety concluded that kojic acid is safe as a skin-lightening agent in cosmetic products at concentrations up to 1% (SCCS 2022). Therefore, the absence of marked cytotoxicity for



Values are mean ± SEM. *p < 0.05 versus the untreated control, based on Tukey's HSD test

FIGURE 1. Effects of K4C (KK4C-1, 5, 10, 50, 100 & 500) and K30C (KK30C-1, 5, 10, 50, 100 & 500) extracts on the viability of CRL-2691 fibroblasts and MNT-1 melanoma cells, as determined using the MTT assay. Cell viability is expressed relative to the untreated control

K4C and K30C in the present *in vitro* assays supports further evaluation but does not establish cosmetic safety. Additional studies of irritation, sensitisation, phototoxicity, formulation stability, skin penetration, and controlled human use are needed before these extracts can be proposed as cosmetic active ingredients.

EFFECT OF EXTRACTS ON TYR TRANSCRIPT ABUNDANCE

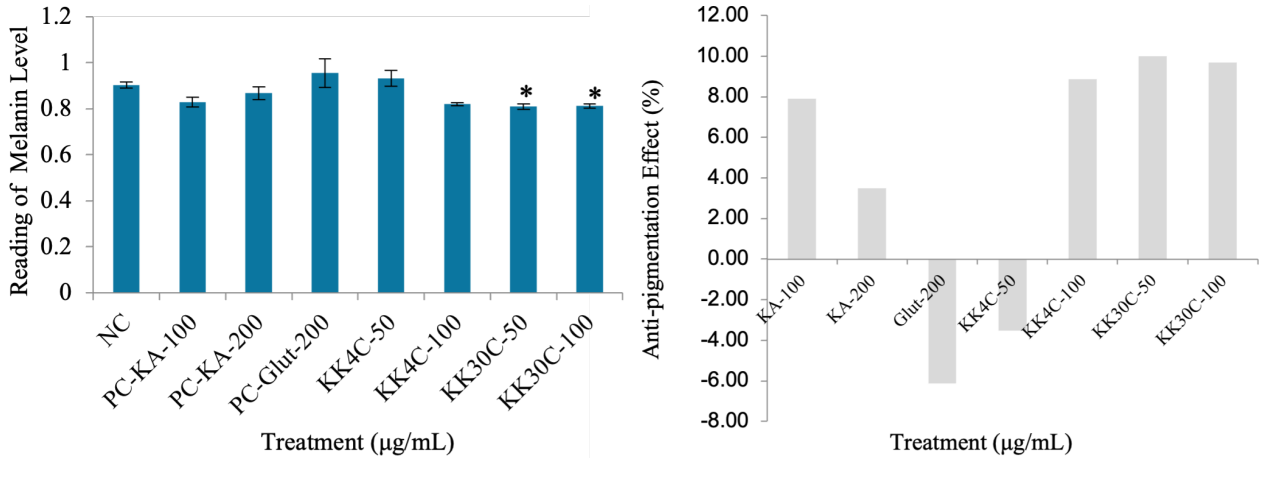
K30C treatment at 50 and 100 µg/mL was associated with lower TYR transcript abundance than the negative control. K4C produced a concentration-dependent pattern, with a reduction observed at 100 µg/mL (Figure 3). These findings describe changes in TYR transcript abundance and do not establish the underlying regulatory mechanism. K30C produced a more consistent pattern of reduced TYR transcript abundance than K4C. The partial mismatch between melanin content and TYR transcript abundance, particularly for K4C, should not be considered contradictory. Melanin accumulation is a downstream phenotype influenced not only by TYR transcription but also by tyrosinase maturation, protein stability, melanosome biology, substrate availability, oxidative state, and the timing of the cellular response. Previous studies and reviews have shown that melanogenesis is controlled at multiple regulatory levels, with tyrosinase functioning as a key enzyme but not the sole determinant of melanin production (D'Mello et al. 2016; Iozumi et al. 1993; Videira, Moura & Magina 2013; Wang et al. 2024).

Melanogenesis involves a coordinated regulatory network that extends beyond tyrosinase catalytic activity. MITF is widely recognised as a central transcriptional regulator of melanocyte development and melanogenesis and regulates melanogenesis-related genes such as TYR, TYRP1, and DCT/TYRP2. Upstream pathways, including cAMP/PKA/CREB, MAPK/ERK, PI3K/AKT, and Wnt/β-catenin signalling, can influence MITF expression, tyrosinase expression, melanogenic-enzyme stability, and melanosome maturation (D'Mello et al. 2016; Wang et al. 2024). The reduced TYR transcript abundance observed particularly in K30C-treated cells was associated with lower melanin content, but TYR transcript measurements alone do not establish a causal mechanism. Future studies should examine MITF, TYRP1, DCT/TYRP2, and selected signalling proteins, such as phosphorylated CREB, ERK, and AKT, to investigate whether the extracts affect upstream melanogenic regulation.

EFFECT OF EXTRACTS ON INTRACELLULAR TYROSINASE ACTIVITY

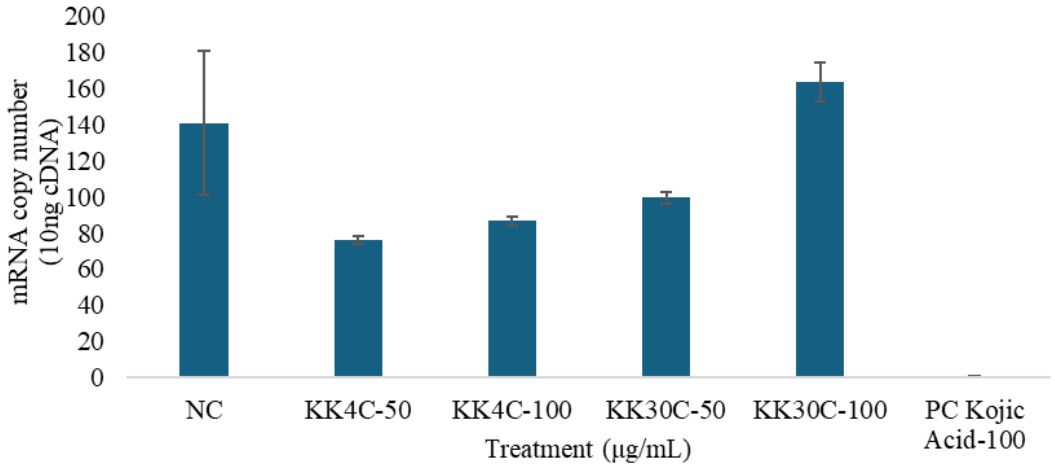
Neither K4C nor K30C significantly reduced intracellular tyrosinase activity at the tested concentrations, whereas kojic acid showed the expected inhibitory effect (Figure 4). Thus, the reductions in melanin content observed with K30C, and to a lesser extent K4C, were not accompanied by a detectable reduction in intracellular tyrosinase activity under the conditions tested. The mechanism underlying the observed melanin response remains unresolved.

This distinction is important because some anti-melanogenic studies use the term 'tyrosinase inhibition' broadly, although reduced melanin content can occur through several mechanisms. Anti-melanogenic compounds may act by reducing TYR transcript abundance, modulating MITF, altering oxidative stress,



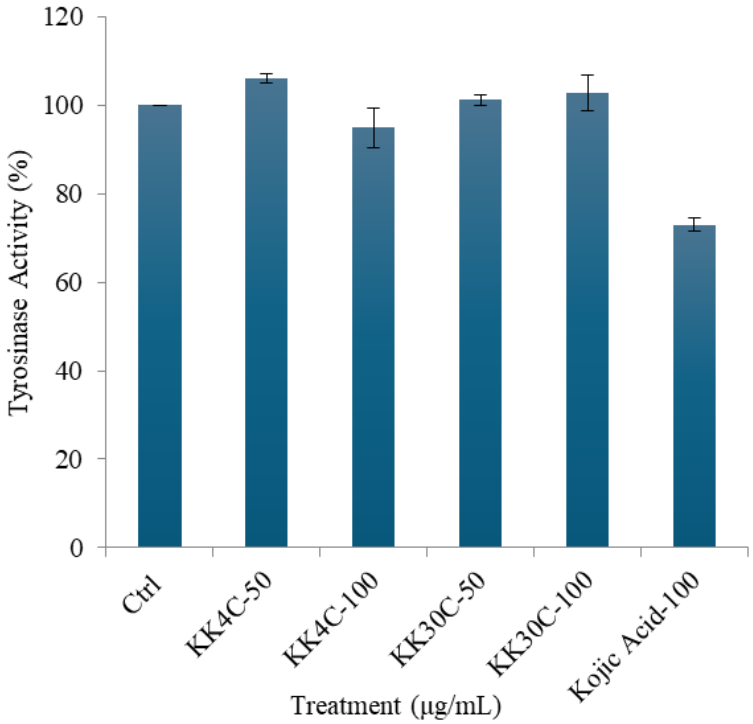
Values are mean ± SEM from three replicates. *p < 0.05 versus the negative control (NC), based on Tukey's HSD test

FIGURE 2. Effects of K4C (KK4C-50 & 100) and K30C (KK30C-50 & 100) extracts on melanin content in MNT-1 melanoma cells with kojic acid as positive control (PC-KA-100). (a) Melanin content after treatment; (b) melanin content relative to the negative control (NC).



Data are expressed as copy number in 10 ng of cDNA. Values are mean ± SEM from three replicates. *p < 0.05 versus the negative control (NC), based on Tukey's HSD test

FIGURE 3. Effects of K4C (KK4C-50 & 100) and K30C (KK30C-50 & 100) extracts on TYR transcript abundance in MNT-1 melanoma cells with kojic acid as positive control (PC-KA-100)



Values are mean ± SEM from three replicates. *p < 0.05 versus the negative control (NC), based on Tukey's HSD test

FIGURE 4. Effects of K4C (KK4C-50 & 100) and K30C (KK30C-50 & 100) extracts on intracellular tyrosinase activity in MNT-1 melanoma cells with kojic acid as positive control (PC-KA-100)

affecting melanosome maturation, or influencing intracellular trafficking and enzyme stability (D'Mello et al. 2016; Wang et al. 2024; Zolghadri et al. 2019). Based on the present dataset, K4C and K30C should therefore be described as extracts with anti-melanogenic potential rather than as direct tyrosinase inhibitors. This cautious interpretation is consistent with the absence of a significant reduction in intracellular tyrosinase activity.

Overall, K30C was associated with reduced melanin content and TYR transcript abundance in MNT-1 cells, whereas neither extract significantly reduced intracellular tyrosinase activity. The mechanism underlying these observations remains unresolved. Additional analyses of MITF, TYRP1, DCT/TYRP2, oxidative-stress markers, and metabolite composition are needed to investigate the relevant regulatory pathways and identify the active constituents.

INTEGRATED INTERPRETATION OF *in vitro* ANTI-MELANOGENIC EFFECTS

When the *in vitro* findings are considered together, K30C showed a more consistent pattern across the two tested concentrations, although it did not significantly reduce intracellular tyrosinase activity (Table 1). K4C showed a less consistent pattern: K4C at 50 μg/mL reduced melanin content without a measurable reduction in TYR transcript abundance, whereas K4C at 100 μg/mL reduced TYR transcript abundance without reducing melanin content. These findings suggest that extraction temperature may influence the bioactivity of *S. commune* extracts; however, further chemical profiling is required before the differences can be attributed to specific metabolites.

The different response patterns of K4C and K30C may reflect differences in chemical composition resulting from the different extraction temperatures. The constituents responsible for the observed responses were not identified in the present study. Previous analysis of *S. commune* fruitbody extracts detected phenolic, polysaccharide, and glucan components and suggested that their biological activities may reflect the combined contribution of multiple constituent classes rather than a single compound (Dang Lelamurni et al. 2020). Phenolics and polysaccharides may therefore be possible contributors to the activity observed in the present extracts. However, extraction or processing conditions may influence extract composition, and the observed effects cannot be attributed to any particular constituent without direct chemical profiling, marker-based standardisation, and bioassay-guided fractionation.

SUPPORTIVE HUMAN SKIN BIOPHYSICAL EVALUATION OF AN *S. commune*-BASED ANTI-PIGMENTATION CREAM

The supportive human skin biophysical evaluation provides formulation-level context for the *in vitro* findings. In the human study, an *S. commune*-based anti-pigmentation cream was applied using a controlled split-face design, with the product applied to one side of the face and a placebo cream applied to the other. The study protocol included non-invasive assessments of pigmentation-related skin parameters, particularly melanin and erythema indices, at baseline and throughout the 12-week application period. This design is relevant because human skin assessment captures formulation-related factors that cannot be represented in cell culture, including product delivery, skin-barrier interactions, baseline skin variability, and local skin responses.

The *S. commune*-based anti-pigmentation cream was associated with reductions in melanin index during the 12-week application period relative to baseline and placebo-treated sites (Figure 5). This observation is directionally consistent with the *in vitro* reduction in melanin content observed for *S. commune* extracts, particularly K30C. Objective measurements of skin colour, pigmentation, and erythema using non-invasive instruments are widely used in dermatology and cosmetic science; however, results can be influenced by anatomical site, baseline skin tone, environmental conditions, and inter-individual variability (Fullerton et al. 1996; Takiwaki 1998).

Absolute erythema index values and percentage changes from baseline are presented in Figure 6. Mean erythema decreased on both sides at Week 4 and subsequently approached baseline. At Week 12, the

TABLE 1. Summary of the *in vitro* anti-melanogenic responses to K4C and K30C

Treatment	Melanin level reduced	TYR transcript abundance reduced	Intracellular tyrosinase activity reduced
K4C-50	+	-	-
K4C-100	-	+	-
K30C-50	+	+	-
K30C-100	+	+	-

Note: (+) indicates an anti-melanogenic effect observed in the assay; (−) indicates no significant effect under the tested conditions

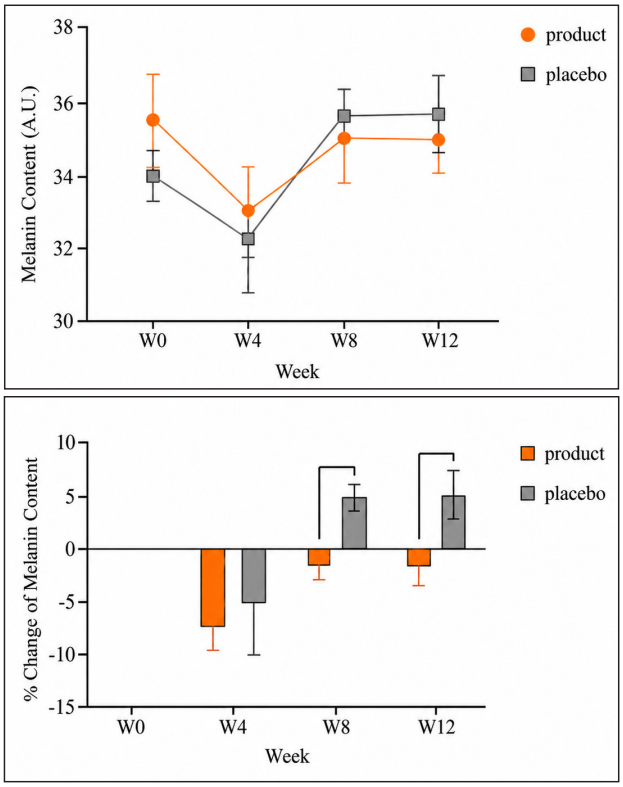
product-treated side remained below baseline, whereas the placebo-treated side showed a modest, variable increase; however, the between-side difference was not statistically significant. The cause of this variation cannot be determined from the available data. These exploratory measurements should be interpreted cautiously and do not establish product safety or exclude irritation, particularly given the limited adverse-event documentation and absence of clinical dermatological assessment.

Overall, the human skin evaluation provides exploratory formulation-level observations that are directionally consistent with the *in vitro* melanin findings. However, the human study should be regarded as supportive and exploratory because participant-level numerical data and adverse-event documentation were incomplete. Fifteen participants were enrolled, and some withdrew after reporting skin-related complaints. Detailed information on onset, duration, severity, affected side, management, and causality was not prospectively collected and was unavailable for analysis. Consequently, no definitive conclusions regarding product safety can be drawn. Larger controlled studies with complete participant accounting, clear inclusion and exclusion reporting, adverse-event documentation, standardised dermatological assessment, and formulation characterisation are required before definitive anti-pigmentation or clinical efficacy claims can be made.

LIMITATIONS AND FUTURE WORK

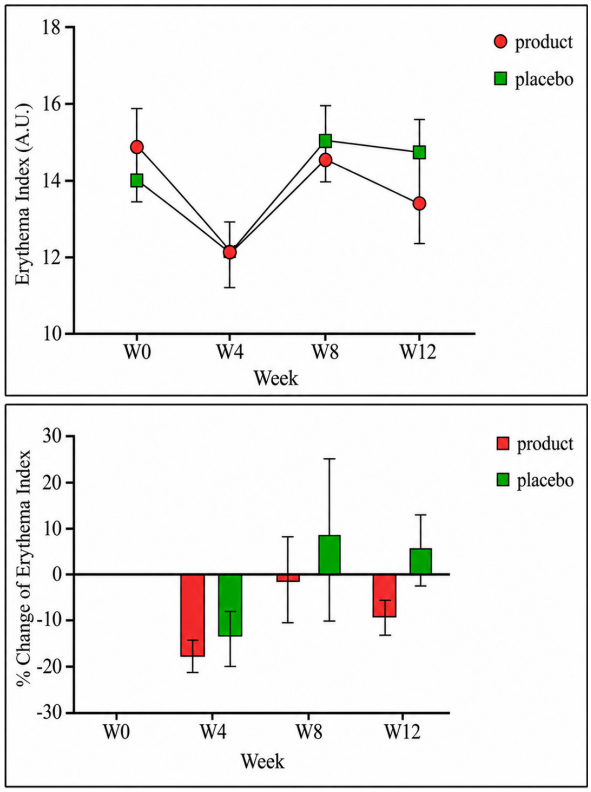
Several limitations constrain the interpretation of this study. Only two concentrations were evaluated, and no clear dose–response relationship was established; notably, K4C showed a non-monotonic response. The mechanistic assessment was limited to TYR transcript abundance and intracellular tyrosinase activity. Because melanin content is a downstream endpoint influenced by TYR protein turnover, melanosome biology, response timing, and other regulatory processes, the findings demonstrate an association rather than a causal mechanism.

The use of highly pigmented MNT-1 melanoma cells may also limit extrapolation to normal human melanocytes. In addition, the crude extracts were not comprehensively characterised or standardised, leaving the active constituents and potential batch variability unresolved. The human evaluation was exploratory and limited by the small sample, use of formulated products, and incomplete participant-level and adverse-event data. Future studies should examine a broader concentration range, additional melanogenesis markers, and physiologically relevant skin models, together with extract standardisation and adequately powered human studies with complete efficacy and safety reporting.



Values are mean ± SEM (n = 15). *p < 0.033 versus placebo, based on a paired-samples t-test

FIGURE 5. Absolute melanin index and percentage change from baseline after 12 weeks of application



Values are mean ± SD (n = 15). *p < 0.033 versus placebo, based on a paired-samples t-test

FIGURE 6. Absolute erythema index and percentage change from baseline after 12 weeks of application

TABLE 2. Summary of supportive human skin biophysical observations after 12 weeks of topical application

Parameter	Main observation	Interpretation
Melanin index	Anti-pigmentation cream decreased melanin index at weeks 4, 8 and 12 compared with baseline and placebo	Directionally supports anti-pigmentation potential observed *in vitro*
Erythema index	Erythema decreased after *S. commune* cream application; placebo showed higher erythema at week 12	Exploratory erythema measurement; does not establish safety or exclude irritation; adverse events require fuller reporting

CONCLUSION

The research questions were addressed at a preliminary level. The *S. commune* extracts were non-cytotoxic at the concentrations tested under the present *in vitro* conditions. K30C treatment was associated with modest reductions in melanin content and TYR transcript abundance at 50 and 100 µg/mL, whereas K4C produced a less consistent response. Neither extract significantly reduced intracellular tyrosinase activity under the conditions tested; therefore, the underlying mechanism remains unresolved. The human skin biophysical observations were exploratory and do not establish clinical efficacy or product safety. Interpretation is limited by the restricted concentration range, absence of a clear dose–response relationship, use of MNT-1 melanoma cells, limited mechanistic and chemical characterisation, small human sample, and incomplete participant-level and adverse-event documentation. Further standardised mechanistic, formulation, efficacy, and safety studies are required before definitive cosmeceutical claims can be made.

ACKNOWLEDGEMENTS

This study was funded by the UKM-MARDI Industrial Grant (ST-2017-004) and the RMK-11 Developmental Project research grant (P21003004170001) from the Malaysian Agricultural Research and Development Institute (MARDI). We would like to thank all individuals who provided technical assistance and support throughout the research project.

REFERENCES

Baber, M.A., Crist, C.M., Devolve, N.L. & Patrone, J.D. 2023. Tyrosinase inhibitors: A perspective. *Molecules* 28(15): 5762. DOI: 10.3390/molecules28155762

Dang Lelamurni Abd Razak, Anisah Jamaluddin, Nur Yuhasliza Abd Rashid, Nor Ajila Sani & Musaalbakri Abdul Manan. 2020. Assessment of cosmeceutical potentials of selected mushroom fruitbody extracts through evaluation of antioxidant, anti-hyaluronidase and anti-tyrosinase activity. *J* 3(3): 329-342. https://doi.org/10.3390/j3030026

D'Mello, S.A.N., Finlay, G.J., Baguley, B.C. & Askarian-Amiri, M.E. 2016. Signaling pathways in melanogenesis. *International Journal of Molecular Sciences* 17(7): 1144. https://doi.org/10.3390/ijms17071144

Firooz, A., Sadr, B., Babakoohi, S., Sarraf-Yazdy, M., Fanian, F., Kazerouni-Timsar, A., Nassiri-Kashani, M., Naghizadeh, M.M. & Dowlati, Y. 2012. Variation of biophysical parameters of the skin with age, gender, and body region. *The Scientific World Journal* 2012: 386936. DOI: 10.1100/2012/386936

Fullerton, A., Fischer, T., Lahti, A., Wilhelm, K.P., Takiwaki, H. & Serup, J. 1996. Guidelines for measurement of skin colour and erythema. *Contact Dermatitis* 35(1): 1-10.

Hassan, M., Shahzadi, S. & Kloczkowski, A. 2023. Tyrosinase inhibitors naturally present in plants and synthetic modifications of these natural products as anti-melanogenic agents: A review. *Molecules* 28(1): 378. DOI: 10.3390/molecules28010378

Iozumi, K., Hoganson, G.E., Pennella, R., Everett, M.A. & Fuller, B.B. 1993. Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. *Journal of Investigative Dermatology* 100(6): 806-811. https://doi.org/10.1111/1523-1747.ep12476630

Kooltheat, N., Tedasen, A., Yamasaki, K. & Chatatikun, M. 2023. Melanogenesis inhibitory activity, chemical composition and molecular docking of Thai medicinal plant extracts. *Molecules* 28(3): 1269. DOI: 10.3390/molecules28031269

Kumar, Y. & Xu, B. 2025. Bioactive compounds, health benefits, and practical applications of edible mushroom *Schizophyllum commune* Fr.: A decade-long critical review. *Food Chemistry* 492: 145583.

Martínez-Gutiérrez, A., Bertran, A., Noya, T., Pena-Rodríguez, E., Gómez-Escalante, S., Pascual, S., Luis, L.S. & González, M.C. 2024. New chalcone-derived molecule for the topical regulation of hyperpigmentation and skin aging. *Pharmaceutics* 16(11): 1405. doi: 10.3390/pharmaceutics16111405

Moniz, T., Costa Lima, S.A., Reis, S. & Bonifácio, M.J. 2020. Human skin models: From healthy to disease-mimetic systems. *International Journal of Molecular Sciences* 21(17): 6091. DOI: 10.3390/ijms21176091

Razak, D.L.A., Abd Ghani, A., Mohd Lazim, M.I., Khulidin, K.A., Shahidi, F. & Ismail, A. 2024. *Schizophyllum commune* (Fries) mushroom: A review on its nutritional components, antioxidative, and anti-inflammatory properties. *Current Opinion in Food Science* 56: 101129. DOI: 10.1016/j.cofs.2024.101129

Scientific Committee on Consumer Safety (SCCS). 2022. *Opinion on Kojic Acid, SCCS/1637/21.* European Commission.

Snatchfold, J. & Targett, D. 2019. Exploratory study to evaluate two clinical methods for assessing moisturizing effect on skin barrier repair. *Skin Research and Technology* 25(3): 251-257. https://doi.org/10.1111/srt.12632

Takiwaki, H. 1998. Measurement of skin color: Practical application and theoretical considerations. *Journal of Medical Investigation* 44(3-4): 121-126.

Taofiq, O., González-Paramás, A.M., Martins, A., Barreiro, M.F. & Ferreira, I.C.F.R. 2016. Mushrooms extracts and compounds in cosmetics, cosmeceuticals and nutricosmetics-A review. *Industrial Crops and Products* 90: 38-48. https://doi.org/10.1016/j.indcrop.2016.06.012

Videira, I.F.S., Moura, D.F.L. & Magina, S. 2013. Mechanisms regulating melanogenesis. *Anais Brasileiros de Dermatologia* 88(1): 76-83. https://doi.org/10.1590/S0365-05962013000100009

Wang, M., Zhang, Y., Zhang, T. & Zhang, J. 2024. The biochemistry of melanogenesis: An insight into the metabolic pathways and gene regulation. *International Journal of Molecular Sciences* 25(15): 8407. DOI: 10.3390/ijms25158407

Wu, D.T., Li, W.X., Wan, J.J., Hu, Y.C., Gan, R.Y. & Zou, L. 2022. A comprehensive review of edible mushroom polysaccharides: Extraction, purification, structural characteristics, bioactivities, and applications. *Critical Reviews in Food Science and Nutrition* 63(27): 8263-8293. https://doi.org/10.1080/10408398.2022.2053050

Zolghadri, S., Bahrami, A., Khan, M.T.H., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F. & Saboury, A.A. 2019. A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry* 34(1): 279-309. DOI: 10.1080/14756366.2018.1545767

*Corresponding author; email: yuziana@ukm.edu.my