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Communication

Effects of a Standardized Hydrogenated Extract of Curcumin (Curowhite™) on Melanogenesis: A Pilot Study

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Abstract: The stimulation of melanogenesis by novel natural products is desirable for cosmetic applications such as skin tanning, anti-greying, and clinical use for treating vitiligo and leukoderma disorders. Microphthalmia transcription factor (MITF) is a central transcription factor that controls the expression of tyrosinase, which is a key enzyme responsible for catalyzing the rate-limiting processes of melanin production. Tetrahydrocurcuminoids (THCr), which mostly consist of tetrahydrocurcumin (THC), are a colorless bioactive mixture derived from curcuminoids that are extracted from the *Curcuma longa* plant. THCr has been reported to exhibit superior properties, including antioxidant and anti-inflammatory effects. Our previous study reported the greater melanogenesis-stimulating effects of purified THC, compared to hexahydrocurcumin (HHC) or octahydrocurcumin (OHC). Curowhite™ (CW) is a proprietary extract that consists of 25% hydrogenated curcuminoids (mixture of THCr, hexahydrocurcuminoids, and octahydrocurcuminoids) encapsulated in a β-cyclodextrin (βCyD) excipient. The encapsulation of THCr in a suitable excipient, such as the widely popular cyclodextrins, helps to enhance the stability, solubility, and bioavailability of the THCr. CW is marketed as a nutraceutical with GRAS status and is safe when administered orally, as shown in vivo studies. However, the impact of CW on melanogenesis remains unexplored. Herein, the impact of CW on melanogenesis were investigated using B16F10 and MNT-1 cells. Our findings show that CW is markedly cytotoxic to B16F10 cells without affecting the cellular melanin content. However, in MNT-1 cells, CW significantly stimulated intracellular melanin content over the concentration range (20–60 μg/mL) with increased dendrite formation while being nontoxic to MNT-1 cells or HaCaT cells after a 5-day treatment. Examination of the effects of the excipient βCyD on cytotoxicity and melanogenesis confirmed that the excipient had no contribution to the biological impacts that were found to be exclusively attributable to the encapsulated mixture (THCr). The mechanisms of CW's promelanogenic effects in MNT-1 cells were found to be related, at least in part, to an increase in tyrosinase and MITF protein levels, as CW did not alter tyrosinase activity in MNT-1 cells. Moreover, CW exhibited antioxidant activity as obtained through DPPH radical scavenging assay. Together, the findings of this pilot study indicate that CW might hold an exciting avenue as a pro-pigmenting nutraceutical for treating hypopigmentation disorders, the detailed mechanisms of which warrant further exploration. Moreover, future investigations are necessary to examine CW's effects on melanogenesis in normal human melanocytes and in vivo studies.

Keywords: curowhite; tetrahydrocurcuminoids; antioxidant activity; B16F10 cells; MNT-1 cells; promelanogenic; tyrosinase

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1. Introduction

Melanocytes synthesize the pigment melanin in melanosomes which is the determining factor for the pigmentation of the hair and skin [1]. Melanin is recognized for its role in shielding against ultraviolet (UV) damage, chemicals, and free radicals [2,3]. Decreased melanin pigment in the skin causes hypopigmentation diseases such as leukoplakia, vitiligo, albinism, leukoderma, and postinflammatory hypopigmentation [4–6]. For people who

want a flawless complexion, these can be a major concern. About 0.5–2.0% of adults and children worldwide are estimated to have vitiligo [7], which is correlated with psychosocial morbidity and compromised life quality [8]. Decreased melanin production in hair leads to canities and premature greying [9]. Tyrosinase, a copper type-3 protein in the melanosomal membrane, is a central enzyme that catalyzes the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and its subsequent oxidation to DOPAquinone [10,11].

Several natural compounds have been explored for their potential utility in treating hypopigmentation disorders. However, the search for novel natural compounds continues to grow [12–14]. Natural/herbal products, functional food, and dietary supplements constitute the primary segments of the nutraceutical industry [15]. Curowhite™ (CW) is an edible nutraceutical formulation patented by Aurea Biolabs (Kerala, India) that is standardized to 25% hydrogenated curcuminoids root extract in a 75% β-cyclodextrin (βCyD) excipient [16]. The 25% hydrogenated curcuminoid root extract in the formulation is a mixture of 16–22% tetrahydrocurcuminoids (THCr), 1–6% hexahydrocurcuminoids (HHCr), and 0.5–2% octahydrocurcuminoids (OHCr). Compared to 95% curcuminoids, CW exhibits a 7-fold higher bioavailability [16]. The safety of CW for consumption as a food additive has been established, and it has been granted GRAS (Generally Regarded As Safe) regulatory status. CW was nontoxic up to high doses of 2000 mg/kg in rats when orally administered [17] and demonstrated no genotoxicity after oral administration in another follow-up study [18]. CW has also shown anti-inflammatory benefits in treating rheumatic arthritis in a clinical study in humans in the absence of side effects [19].

Food supplements for skin tanning or grey hair treatment without adverse effects are attractive to the cosmetic and clinical sectors. An apple-based nutraceutical formulation with apple extracts encapsulated in maltodextrin excipient showed promising promelanogenic activity [20]. Sabiwhite® (comprising 96% tetrahydrocurcumin; THC) and Tetrahydrocurcuminoids® CG (comprising 95% THCr) formulations are marketed by Sabinsa Corp. for skin whitening, and a 99% pure THC formulation (Tetrapure™) is sold for antifungal applications for the skin. CW differs from Sabiwhite through the presence of a mixture of the three hydrogenated curcuminoids, namely THCr, HHCr, and OHCr, instead of THC. Moreover, in contrast to Sabiwhite®, CW comprises THCr that is encapsulated in a βCyD excipient. CyDs are a popular excipient widely used to encapsulate bioactive compounds for cosmetic and pharmaceutical formulations [21,22], owing to their natural origin and GRAS status [23]. Accumulating evidence indicates that the complexation of curcumin or THC with CyDs enhances the solubility and stability of the compound [24–26]. Although the rationale for the selection of the percentage ratios of THCr (16–22%) and excipient βCyD (75%) mixture that comprise CW is not known due to its proprietary nature, accumulating evidence has shown that curcuminoids in mixture form have much greater stability as well as biological activity compared to curcuminoids in pure form [27,28]. Moreover, obtaining mixtures of curcuminoids is both low-cost and convenient as they eliminate the tedious steps of purification of compounds and provide benefits of greater potency of mixtures. Tetrahydrocurcuminoids ® CG, another commercial product is also a mixture of much greater amounts of THCr (95%) [29], and another study used a commercial cream based on THCr mixture for stimulating skin pigmentation [30]. The hydrogenation of a curcuminoid extract (that contains curcumin in addition to demethoxycurcumin, DMC; and bisdemethoxycurcumin, BDMC) typically results in a mixture of THC which will contain some amounts of THBDMC, as well as minor amounts of HHCr [consisting of a mixture of HHC, hexahydrodemethoxycurcumin (HHDMC), and hexahydrobisdemethoxycurcumin (HHBDMC)] and OHCr [consisting of a mixture of OHC and octahydrodemethoxycurcumin (OHDMC)] [31]. Several prior reports have used THCr (which contains other minor components of HHCr and OHCr) and not purified THC [32–34]. We recently showed that variation by a minor amount of one component (dihydrocurcumin derivative) could alter the efficacy of a THC derivative mixture's effects on melanogenesis [35]. In our previous study [36], we evaluated the effects of purified THC, HHC, and OHC on melanogenesis

using in vitro models of B16F10 and MNT-1 cells and showed that THC was the most potent stimulator of melanogenesis.

The novelty of this study is the evaluation of a mixture of hydrocurcuminoids (THCr, HHCr, and OHCr) encapsulated in a βCyD excipient as a commercial product that is more soluble, stable, and bioavailable compared to the parent compound, and would thus possess a unique biological activity. It is not yet known if a mixture of the hydrogenated curcuminoids might similarly stimulate melanogenesis and whether THC's promelanogenic activity is preserved after encapsulation in a CyD excipient. Hence, in this work, CW was examined for its effects on melanogenesis using B16F10 and MNT-1 cell models. As CW is safe as a nutraceutical food supplement, it may also be repurposed to treat skin hypopigmentation disorders. As a continuation of our ongoing search for new plant compounds with antioxidant properties for cosmetic use, we focus here on CW's ability to enhance melanogenesis and its potential as a promelanogenic candidate.

2. Materials and Methods

2.1. Materials

Curowhite™ sample (% purity of total white curcuminoids 25–27%; HPLC method), initially synthesized by Aurea Biolabs (Kerala, India), was provided by Glanbia Nutritionals (Carlsbad, CA, USA). The composition of the CW sample used in this study has been described in a previous report [18] and is schematically illustrated in Figure 1. β-cyclodextrin (βCyD) was from Cayman Chemicals (Ann Arbor, MI, USA). Kojic acid (KA) and L-3,4-dihydroxyphenylalanine (L-DOPA) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Pierce™ Bicinchoninic acid (BCA) assay kit was procured from Thermo Fisher Scientific (Waltham, MA, USA). MTS reagent was acquired from Promega Corporation (Madison, WI, USA). DPPH (2,2 diphenyl-1-picrylhydrazyl) reagent was procured from Molecular Probes (Invitrogen, Carlsbad, CA, USA). MITF and Tyrosinase cell-based ELISA kits were obtained from Lifespan Biosciences Inc. (Seattle, WA, USA).

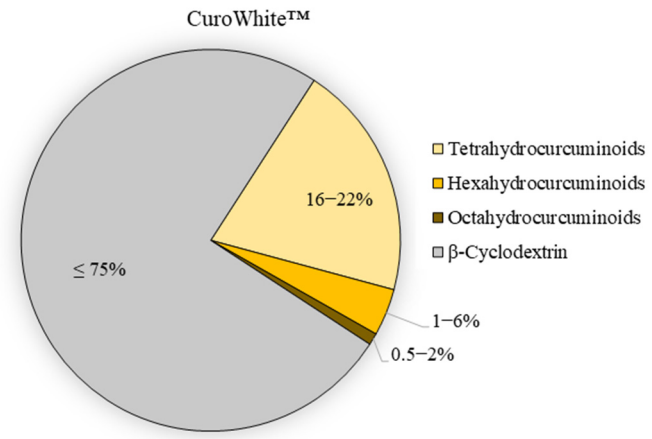

Figure 1. Schematic illustrating the composition of Curowhite, where total white curcuminoids comprise the mixture of tetrahydrocurcuminoids (THCr), hexahydrocurcuminoids (HHCr), and octahydrocurcuminoids (OHCr); all values shown in % *w*/*w*.

2.2. DPPH Antioxidant Assay

DPPH test is a commonly used method for evaluating the antioxidant activity of natural compounds [37] and is based on the principle of reduction of the purple-colored DPPH radical to yellow DPPH molecule by the hydrogen atom transfer mechanism of antioxidant compounds. The solution of DPPH was reconstituted in methanol and mixed in triplicate with varying concentrations of CW and βCyD in a 96-well plate; negative control was prepared with the combination of buffer and DPPH. The final concentration of DMSO in each well was 0.5%. The microplate was sealed and incubated for 30 min before recording the absorbance at the wavelength of 517 nm. The % DPPH radical scavenged

was obtained from the equation $(1 - (Abs_{sample}/Abs_{control})) \times 100\%)$, where Abs_{sample} and $Abs_{control}$ are the absorbances of samples and control, respectively.

2.3. Cell Culture

B16F10 melanoma cells (CRL-6475™) and HaCaT human keratinocytes were purchased from ATCC (Manassas, VA, USA) and AddexBio (San Diego, CA, USA), respectively. HaCaT and B16F10 cells were maintained in Dulbecco's modified Eagle's Medium (DMEM) with 10% heat-inactivated fetal bovine serum (HI-FBS; R&D Systems Inc., Minneapolis, MN, USA) and 1% antibiotic mixture of penicillin-streptomycin. MNT-1 human melanoma cells (graciously provided by Dr. Michael Marks, University of Pennsylvania) were cultured using DMEM supplemented with 18% HI-FBS, 1% minimum essential medium (MEM), 10% AIM-V medium (Gibco), and 1% antibiotics. All cells were cultured in an incubator with a humidified chamber of 95% air and 5% CO_2 at 37 °C.

2.4. MTS Cytotoxicity Assay

2.4.1. B16F10 Cells

B16F10 cells (5×10^3 cells /well) were inoculated in a 96-well tissue-culture plate for 24 h. The next day, CW was reconstituted using DMSO to make a 25 mg/mL stock solution; this was diluted using culture medium to various concentrations (20–100 μg/mL) and added to cells, and the cultures were maintained for 3 days. The final DMSO concentration for each group was 0.4%, and the control group treated with 0.4% DMSO served as solvent control. After 3 days, the culture medium was aspirated, followed by the addition of 100 μL of new media containing 20 μL MTS solution and incubation for 1 h at 37 °C. MTS is a tetrazolium-based salt that is converted to formazan dye by mitochondrial dehydrogenase in metabolically active cells and is widely used for assessing cell viability. The absorbance of formazan product was measured at 490 nm using a Versamax™ microplate reader. The viability values were determined from the absorbance measurements and shown as % of the control group. To exclude any contribution of the excipient βCyD to cytotoxicity, different concentrations of βCyD were also examined similarly using the MTS assay.

2.4.2. MNT-1 Human Melanoma Cells

The effects of CW on MNT-1 cell viability were next tested to identify a nontoxic range for further study on melanogenesis. To this end, 1.6×10^4 MNT-1 cells were inoculated in a 96-well plate, and after 48 h, different concentrations of CW were replaced. On the third day after the addition of CW, the culture medium was refreshed with a second dose of CW, and cultures were maintained for an additional 2 days. After the 5-day treatment period, the cell viability was estimated using the MTS assay using a similar method as B16F10 cells.

2.4.3. HaCaT Cells

To evaluate CW's safety for topical use, cytotoxicity to human keratinocytes was tested over 5 days. Briefly, 3.5×10^3 HaCaT cells were inoculated in a 96-well culture plate and grown for 48 h; varying concentrations of CW were then added, and cultures were continued for 5 days. MTS assay was carried out using the earlier method, except the incubation time of MTS dye with samples was 2 h.

2.5. Intracellular Melanin Assay

2.5.1. B16F10 Cells

A total of 5×10^4 B16F10 cells/well were plated in a 12-well plate and grown for 24 h. After this, CW was diluted in a culture medium at various concentrations, added to cultures, and incubated for 3 days. Subsequently, cells were trypsinized, washed in PBS, and aspirated. A total of 250 μL of 1N sodium hydroxide was added to pellets and heated to 70 °C in a water bath for 30 min to enable the solubilization of melanin. The absorbance of lysates was estimated at 475 nm, and the total protein content was determined by the

BCA assay. Relative melanin content was evaluated from the absorbance values that were determined at 475 nm and were adjusted to account for total protein content.

2.5.2. MNT-1 Human Melanoma Cells

MNT-1 cells were grown in 12-well plates for 48 h using 1.2×10^5 cells per well. Following 48 h, CW at nontoxic concentrations was added to the medium, and cultures continued for 5 days. After 5 days, the cultures were harvested and processed to estimate intracellular melanin, similar to the procedure described earlier for B16F10 cells.

2.6. Intracellular Tyrosinase Enzyme Activity

2.6.1. B16F10 Cells

B16F10 cells (2×10^4 cells/well) were cultured in 24-well plates for 24 h. The next day, the medium was replaced by CW at nontoxic concentrations, and the cultures were maintained for 3 days. After 3 days of treatments, cells were trypsinized, washed in PBS buffer, and lysed, followed by centrifugation to clarify the lysates. A total of 50 µL aliquots were then transferred to a 96-well microplate, after which 100 microliters of 3 mM freshly prepared L-DOPA solution (pH 6.5) was applied. The absorbance was recorded at 475 nm every 30 s for 30 min with a microplate reader. Tyrosinase activity for each sample relative to that of the control was calculated from the rate of dopachrome formation as determined using the linear range of the slope of absorbance increase after normalization to the total protein content.

2.6.2. MNT-1 Cells

MNT-1 cells (1.2×10^5 cells/well) were cultured in 12-well plates for 48 h, and then the medium was renewed with nontoxic CW concentrations. The cultures were maintained for a duration of 5 days. After treatment, cells were harvested, lysed, and assayed for tyrosinase activity as previously described for B16F10 cells.

2.7. Reactive Oxygen Species (ROS) Assay in MNT-1 Cells

MNT-1 cells were inoculated in a 24-well plate at 5×10^4 cells/well density and cultured for 24 h. CW at various concentrations prepared in a complete culture medium was then added, and cultures were kept for 5 days, with the compound replenished on the third day of culture. The method for determining cellular ROS levels was similar to the method we reported previously [38]. Briefly, after 5d, the cultures were washed with HBSS, and 200 µL of 50 µM DCFH-DA (2′,7′-Dichlorofluorescein diacetate) dye was added, and the plate was incubated at 37 °C for 30 min. Subsequently, cells were again washed in HBSS, lysed, and centrifuged. Fluorescence readings were read at a wavelength of 485/535 nm for excitation and emission in a fluorescence plate reader, normalized to total protein content, and reported as a percentage of the untreated control.

2.8. Tyrosinase and MITF Protein Levels in MNT-1 Cells

MNT-1 cells were seeded in a 96-well plate at 1×10^4 cells/well density. After 24 h of culture, varying CW concentrations were added, and cultures were kept for 5 days (with the compound renewed once). After the treatment duration, cells were fixed and processed, as per the instructions provided by the manufacturer in the assay kits, to determine the levels of tyrosinase protein. A similar set of experiments was conducted to determine MITF protein levels.

2.9. Statistical Analysis

All data as shown in the figures are reported as mean ± standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test. GraphPad Prism software 8.0 (La Jolla, San Diego, CA, USA) was used to conduct all analyses. At a threshold of $p < 0.05$, differences were considered to be

statistically significant, and the following symbols were used to denote significance levels: * $p < 0.05$, ** $p < 0.01$, and # $p < 0.0001$.

3. Results

3.1. CW Exhibits Antioxidant Activity

CW scavenged the DPPH radical with increasing concentration; a suppression of 18.48%, 24.09%, 32.27%, 36.06%, and 44.39% was achieved at concentrations of 20, 40, 60, 75, and 100 µg/mL, respectively (Figure 2A). The excipient βCyD was also evaluated to exclude any contributions from the excipient to the antioxidant activity. The results validated that the excipient βCyD showed no scavenging at any concentration as there was no change in absorbances of DPPH radical (Figure 2B). Collectively, these results highlight that the antioxidant activity of THCr is retained after encapsulation in the βCyD excipient.

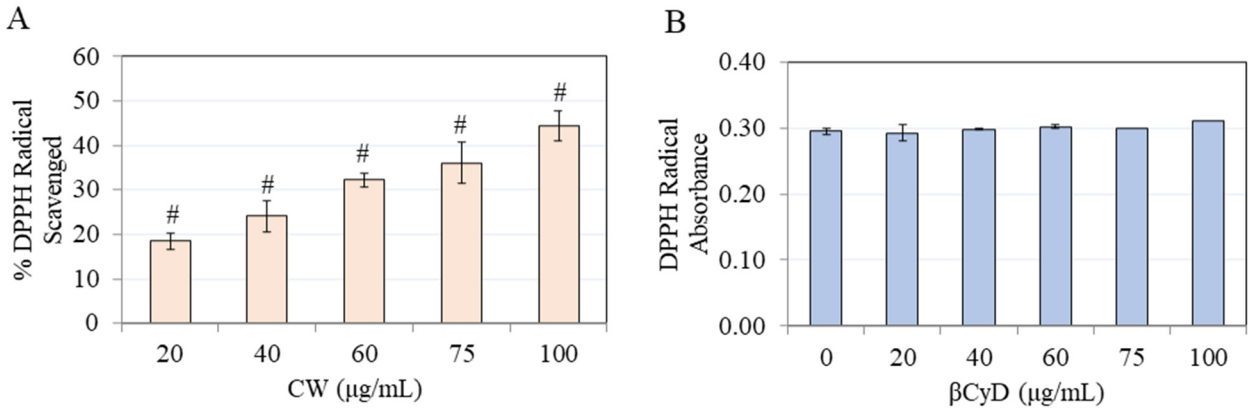

Figure 2. (**A**) DPPH radical scavenging activity of CW (20–100 µg/mL); (**B**) DPPH radical absorbance of excipient βCyD (20–100 µg/mL); all data are average of triplicate determinations.

3.2. CW Did Not Affect B16F10 Mouse Melanoma Cells Melanogenesis at Nontoxic Concentrations

CW was evaluated for cytotoxicity in B16F10 cells in the concentration range of 20–100 µg/mL. The examination of cellular morphology from the photomicrographs revealed a decline in the density of cells incubated with CW at the 40 µg/mL concentration and onwards (Figure 3A). Quantitation of cytotoxicity by MTS assay confirmed that CW induced significant cytotoxicity in cells that were dependent on CW concentration, as cell viability was significantly lowered by 25.83%, 37.06%, 68.52%, and 78.24% at CW concentrations of 40, 60, 75, and 100 µg/mL, respectively (Figure 3B). To rule out any contribution of cytotoxicity from the excipient βCyD itself, we also examined B16F10 cell viability after treatment with similar concentrations of βCyD. Results from cell photomicrographs (Figure 3C) and cell viability measurement (Figure 3D) confirmed that cytotoxicity was exclusively attributable to the bioactive mixture encapsulated in the βCyD excipient and not the excipient per se.

CW at ≤20 µg/mL concentrations were nontoxic and thus selected for further experiments. Next, the results of intracellular melanin content revealed that CW did not alter melanin content in cells over the concentration range of 5–20 µg/mL (Figure 3E). In addition, the tyrosinase activity of B16F10 cells treated with CW was also unchanged at the tested concentrations (Figure 3F). These results highlight that CW does not affect melanin production in B16F10 cells.

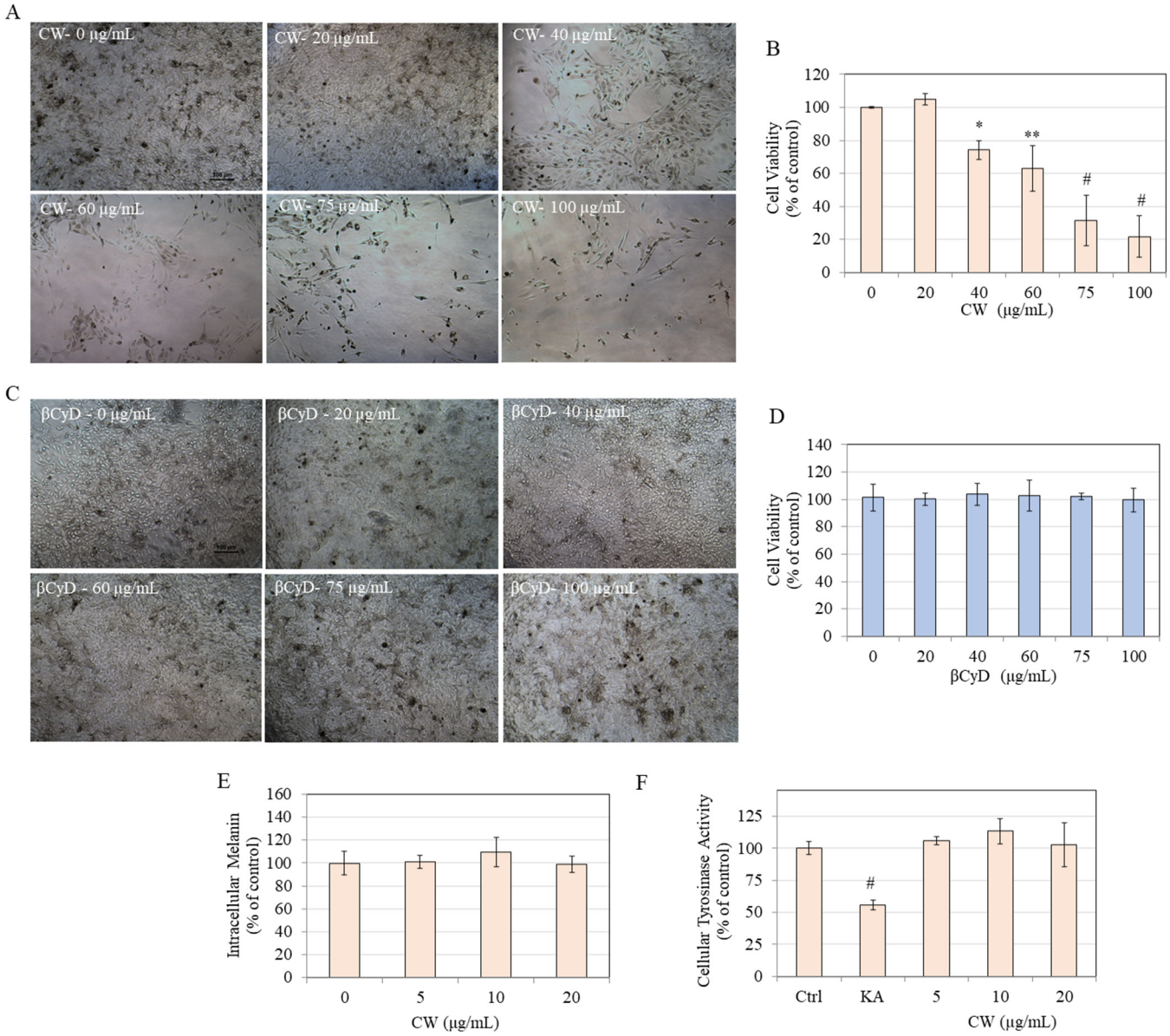

Figure 3. (**A**) Representative bright-field micrographs (×20 objective magnification) and (**B**) viability of B16F10 cells treated for 3 days with CW at a concentration range 0–100 µg/mL; (**C**) representative bright-field micrographs at ×20 objective magnification and (**D**) viability of B16F10 cells treated with βCyD excipient at a concentration range 0–100 µg/mL; (**E**) intracellular melanin levels and (**F**) cellular tyrosinase activity of B16F10 cells treated for 3 days with CW; kojic acid (KA; 0.5 mM) refers to the positive control; data for (**B**) are average of three independent experiments, and all other data are average of values combined from two independent experiments (n = 4).

3.3. CW Stimulated Melanogenesis in MNT-1 Cells at Nontoxic Concentrations

CW was next tested for any cytotoxicity to MNT-1 cells; results showed that CW was nontoxic at all concentration ranges 20–100 µg/mL (Figure 4A). The excipient βCyD was also examined for effects on MNT-1 cell viability over 5 days and showed no change (Figure 4B).

Next, the examination of cell photomicrographs revealed elongated dendrites in CW-treated groups in contrast to control group that had short dendrites (Figure 5A). Quantitation of melanin content showed that CW stimulated melanogenesis; a significant increase of 24.19%, 27.11%, 28.85%, and 24.88% was obtained at concentrations of 20, 40, 60, and 75 µg/mL, respectively (Figure 5B). The effect of the excipient βCyD was also

examined; results showed that βCyD did not have any effect on cellular morphology (Figure 5C) or melanin contents within cells at any concentration (Figure 5D).

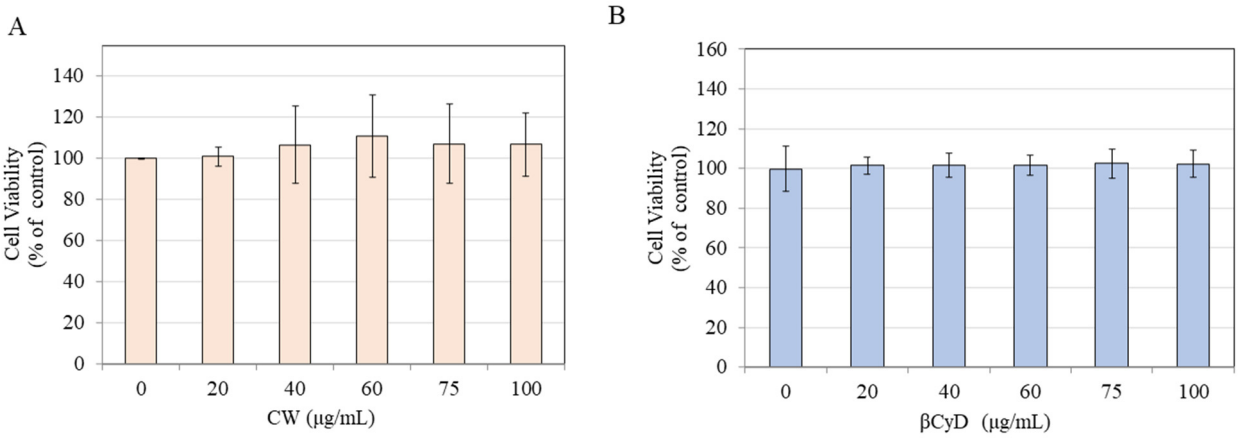

Figure 4. MNT-1 human melanoma cell viability after a 5-day treatment with (**A**) CW; or (**B**) excipient βCyD across a concentration range of 0–100 µg/mL; data for (**A**) are average of at least three separate experiments, and for (**B**) are average of triplicates from one representative experiment of two separate experiments; no significance was found between groups.

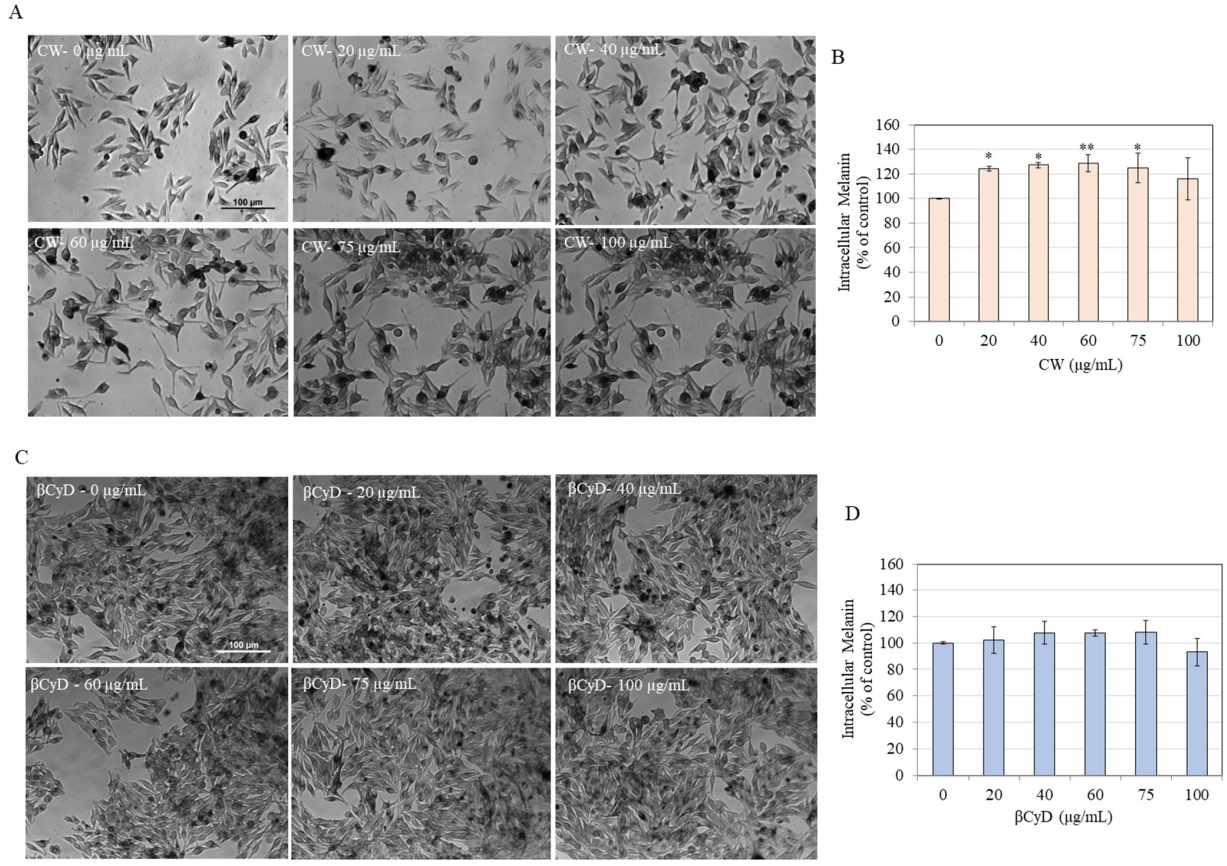

Figure 5. (**A**) Representative bright-field micrographs (×20 objective magnification) of MNT-1 cells after treatment with CW (0–100 µg/mL); note the elongation of cell dendrites with increasing concentrations of CW in all groups as compared to control; (**B**) MNT-1 cell melanin levels after treatment with CW for 5 days; data are average of three independent experiments; (**C**) representative bright-field micrographs (×20 objective magnification) of MNT-1 cells treated βCyD at a concentration range 0–100 µg/mL; (**D**) intracellular melanin in βCyD-treated MNT-1 cells for 5 days; data are average of values combined from two separate experiments (n = 4).

Overall, our results demonstrate that CW exhibited the capacity to stimulate melanogenesis at the lowest concentration of 20 µg/mL. Increased concentrations had equal increments, with no concentration-dependent enhancement.

3.4. CW Effects on Tyrosinase Activity and ROS Production in MNT-1 Cells

Next, MNT-1 cells' tyrosinase activity was evaluated to assess if the enhanced melanogenesis by CW could be partially attributable to an increase in the tyrosinase enzymatic activity. Surprisingly, results revealed no alteration in the tyrosinase activity over the 20–75 µg/mL concentration range. Instead, significant suppression of tyrosinase activity (27.22%) was noted unexpectedly at the greatest concentration of 100 µg/mL (Figure 6A).

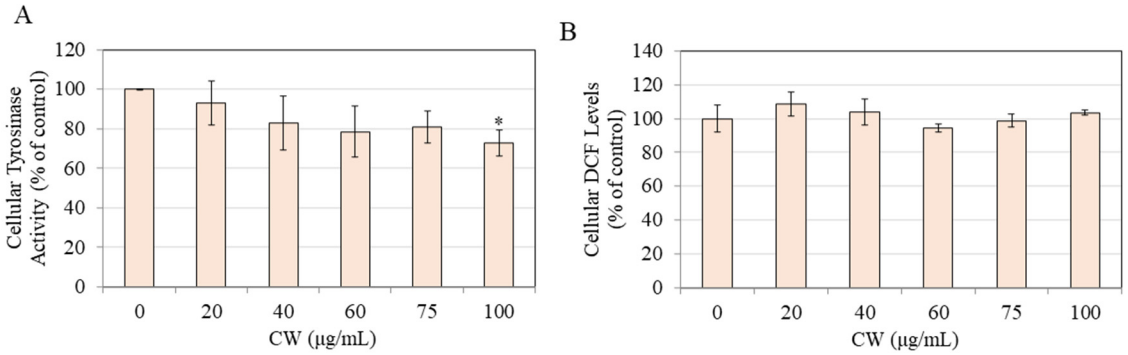

Figure 6. (**A**) Tyrosinase activity and (**B**) cellular ROS levels of MNT-1 cells treated with CW (0–100 µg/mL) for 5 days; data for (**A**) are average of three independent experiments, and data for (**B**) are average of values combined from two separate experiments (n = 4).

CW's effects on intracellular ROS levels were also analyzed, as it was of interest to ascertain that the increased cellular melanin content within MNT-1 cells by CW did not arise because of an increase in ROS production. Encouragingly, treatment with CW did not alter ROS levels within cells at any concentration (Figure 6B).

Analysis of protein levels of tyrosinase and MITF showed a significant increase at a CW concentration of 40 µg/mL (Table 1). These results indicate that CW increases melanin contents without altering tyrosinase activity or ROS levels. These results demonstrate that the mechanism of stimulation of melanin production by CW is not correlated to changes in tyrosinase activity but might involve effects on tyrosinase protein synthesis.

Table 1. Relative levels of Tyrosinase and MITF proteins in MNT-1 cells after incubation with CW (0—60 µg/mL) for 5 days, determined using cell-based ELISA assays.

CW (µg/mL)	Tyrosinase Protein Levels (%)	MITF Protein Levels (%)
0	99.97 ± 5.32	100.20 ± 4.17
20	106.44 ± 2.93	115.32 ± 12.52
40	123.04 ± 3.40 [a]	116.62 ± 4.85 [c]
60	152.24 ± 10.52 [b]	117.56 ± 16.39

Values are mean ± SD (n = 3 per group); values indicated with the lowercase letter a and b show significant differences (p = 0.0131 and p = 0.0046) compared to CW (0 µg/mL), using Student's t-test; values indicated with lowercase letter c show a significant difference (p = 0.0112) compared to CW (0 µg/mL), using Student's t-test.

3.5. Effects of CW on Cytotoxicity in Human Keratinocytes

To confirm safety for topical use, CW was evaluated for any cytotoxicity to HaCaT cells; results showed that CW significantly suppressed keratinocyte viability by 27.63%, 46.3%, and 73.47% at 60, 75, and 100 µg/mL, respectively (Figure 7). These results reveal that CW can be used safely without any cytotoxicity to keratinocytes at concentrations < 60 µg/mL in skin-care cosmetics.

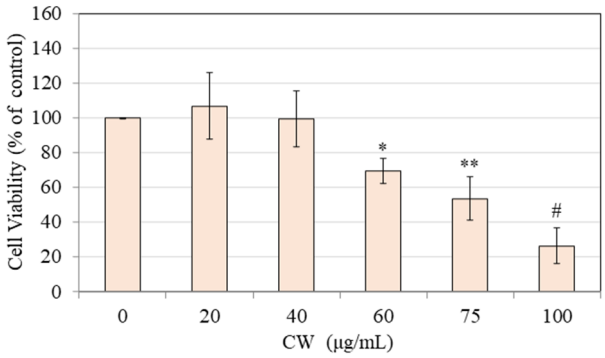

Figure 7. Viability of HaCaT keratinocytes after treatment with varying CW concentrations; data are average of three independent experiments.

4. Discussion

Our results of marked cytotoxicity with no changes in melanogenesis in B16F10 cells somewhat contrast with the findings of our earlier report [36] on unencapsulated purified THC, which in B16F10 cells was nontoxic up to 40 µM and exhibited increased melanin production dose-dependently. Our results in this study suggest that upon encapsulation, THC might become cytotoxic and ineffective at melanogenesis in B16F10 melanoma cells. We speculate that it may be due to the THC-CyD inclusion complex formed by the interaction of compounds of the mixture with an inner hydrophobic pocket of CyD; this complex may have a higher antiproliferation potential than THC. This is particularly feasible as a prior study showed that in cancer cells, the curcumin-cyclodextrin complex exhibited a greater anticancer effect and cell death than curcumin alone, partly attributable to the greater intracellular uptake of the complex [39].

In our study, CW exhibited the capacity to stimulate melanin production at the lowest concentration of 20 µg/mL, but no concentration-dependent enhancement was seen. The precise explanation for this occurrence is unknown, but it might be due to a saturation effect, wherein after a specific degree of enhancement of intracellular melanin, further addition of a higher concentration of the compound might not have any further additive effect. The effects of CW were examined at different treatment durations of 3 days and 5 days on B16F10 and MNT-1 cells, respectively. This was chosen because we have employed different treatment durations (3 days for B16F10 and 5 days for MNT-1 cells) when examining melanogenic responses of different compounds in our previous studies [36,40]. Moreover, another report also examined the impact of a compound citric acid on B16F10 and HMV-II human melanoma cells for 4 days and 6 days treatment durations, respectively [41]. This difference may be attributed, in part, to the varying proliferation rates of mouse and human melanoma cells. Specifically, B16F10 cells have a higher proliferation rate in contrast to MNT-1 cells. B16F10 mouse and MNT-1 human melanoma cells have intrinsic molecular differences in cellular metabolism, genetic differences, and stress responses. Moreover, melanoma cells can have different redox states that can dictate cytotoxic responses differently. The effects of antioxidants on cytotoxicity can be complex and depend on varying endogenous glutathione (GSH) levels of different cells and specific conditions which may cause them to become pro-oxidant. We cannot confirm whether the marked cytotoxicity of CW obtained to B16F10 cells might be ascribed to ROS-mediated cell death, as cellular ROS was not measured in B16F10 cells. Nevertheless, our findings of selective high cytotoxicity of CW in B16F10 cells with no toxicity in MNT-1 cells are similar to the results of our earlier study [35], that examined the cytotoxicity of hydrogenated chemically modified curcumin (CMC2.24), which lacks antioxidant activity and comprises higher amounts of tetrahydro- with lower amounts of hexahydro- and octahydro- derivatives of CMC2.24, similar to the antioxidant CW that is also a mixture comprising a higher percentage of tetrahydro- with lower percentages of hexahydro- and octahydro- derivatives of curcumin. Hence, it can be speculated that a mixture of the three hydrogenated compounds might become selectively

cytotoxic to mouse B16F10 cells than human MNT-1 cells due to interaction effects in the mixture, which are independent of antioxidant activity. This is plausible as we previously showed that purified compounds THC, HHC, and OHC were not cytotoxic up to 40 μM in both B16F10 and MNT-1 cells [36]. Our data show that CW is most cytotoxic to B16F10 cells followed by HaCaT cells, and nontoxic in MNT-1 cells, which suggests that CW has a cell-type specific cytotoxicity. Notably, all these three cells are immortalized, and the order of intrinsic melanin pigmentation in them is MNT-1 >> B16 > HaCaT, with HaCaT cells lacking pigmentation altogether. We also conducted a preliminary MTS cell viability experiment in primary human melanocytes from darkly pigmented skin (HEMn-DP cells) which had intrinsic melanin levels that were lower than B16F10 and MNT-1 cells. We found the cytotoxic response of this pigmented primary cell line to be similar to that of the immortalized unpigmented HaCaT cells (Figure S1). Thus, the revised cytotoxicity order is B16F10 >> HEMn-DP = HaCaT > MNT-1. Based on this, it can be speculated that the presence of melanin pigment protects against cytotoxicity by CW, yet the species-effect and cell immortalization state also play a role. We did not elucidate on CW's cytotoxicity and its mechanisms per se in this study as it was not the focus of this study.

Earlier reports have also shown the opposite effects or differential efficacies of compounds on melanogenesis in B16F10 and MNT-1 melanoma cells. For example, citric acid stimulated melanogenesis in B16F10 cells but suppressed it in MNT-1 cells [41]. Elsewhere, gomisin N suppressed melanogenesis in B16F10 cells but was ineffective in MNT-1 cells [42], while we have shown previously that OHC stimulated melanogenesis in B16F10 cells but had no effect in MNT-1 cells, while HHC promoted melanogenesis to a greater extent in MNT-1 cells than B16F10 cells, thus pointing the cell-type dependent differential efficacies [36]. A prior study showed that an extract of the plant Cymbopogon schoenanthus (CYM) enhanced melanogenesis, although its individual components suppressed melanogenesis [43]. Elsewhere, rosmarinic acid diminished melanin synthesis at a lower concentration (0.01 mM) but stimulated melanin synthesis at a 100-fold higher concentration (1 mM) in the same cell model [44]. Based on these studies, it can be observed that opposite or differential effects of compounds on melanogenesis are dependent on cell type (mouse vs. human), the presence of the compound as a pure isolate or in a mixture, and the concentration of the compound (contrasting effects at higher concentrations of the same compound).

Furthermore, accumulating evidence has shown that antioxidant natural products exert a complex effect on melanogenesis, where the effects (promelanogenic or antimelanogenic) are dependent on the activation state of melanocytes. For instance, the antioxidant retinoic acid enhanced melanogenesis in melanoma cells under basal conditions but suppressed melanogenesis in αMSH hormone activated [45] or UV-irradiated melanoma cells [46]. In addition, antioxidant resveratrol (RV) suppressed melanin production [47], but when present in an extract form (grape extract containing 6% RV), it did not affect melanogenesis; however, upon hormone stimulation, it showed the opposite effect and promoted melanogenesis [48]. A similar finding was obtained with rosmarinic acid (RA), an antioxidant that showed no appreciable effects on basal melanin production in B16F10 cells; however, upon stimulation with L-tyrosine (a substrate of melanin synthesis), it enhanced melanogenesis at lower concentrations while suppressing melanogenesis at higher concentrations, again demonstrating a biphasic effect that is concentration-dependent [44]. Interestingly, in the same study, two extracts that were standardized to RA at different contents showed differential effects that did not align with the effect shown by the purified component (RA), thus revealing the disparate responses of isolated compounds vs. standardized extracts of the same compound. Paeoniflorin, a natural antioxidant, was shown to promote melanogenesis under basal conditions in human melanocytes [49], while it suppressed melanogenesis in B16F10 cells under αMSH stimulated conditions in another study [50], further indicating the disparate responses that were dependent on melanocytic activation state. Additionally, it is worth noting that previous studies showed that THC suppressed melanin production under hormone

stimulated conditions in B16F10 cells [51,52]. Although a prior study [30] described that a THCr cream in conjunction with UVB irradiation promoted melanogenesis in a vitiligo model, future studies that examine the effects of CW under hormone stimulation or UV irradiation will be necessary to identify the impact of CW on melanogenesis fully.

To date, only our earlier study [36] reported on the effects of pure THC, HHC, and OHC on in vitro melanogenesis under basal conditions in cell models. To our knowledge, there is no published report yet wherein the effects of THCr, HHCr, or OHCr separately were explored on melanin production under basal conditions of in vitro melanin production in cell models. Notably, THCr has shown conflicting results on melanogenesis as commercial products such as Tetrahydrocurcuminoids® CG (containing 95% THCr) and Sabiwhite® (containing 96% THC), by Sabinsa Corporation, are marketed as skin whiteners that suppress melanogenesis [29,34,53], while another study showed that a commercial THCr cream (composition not provided) increased melanogenesis under UVB radiation in vivo [30]. We previously showed that purified THC enhanced melanogenesis to a greater extent than purified HHC, while purified OHC had no effect [36]. The determination of which constituent in CW was the driver of the promelanogenic effect was not the goal of this study, particularly because the use of individual components to recreate the unencapsulated mixture of hydrogenated curcuminoids with THCr, HHCr, and OHCr (that could have served as a control in the experiments) was not possible as, except for THCr, HHCr and OHCr are not available commercially. However, as CW is a proprietary product, it might be possible to obtain the unencapsulated control mixture from the manufacturer directly in future studies in order to identify whether the mixture is contributing to promelanogenic activity or if it is the contribution of THCr, HHCr, or OHCr. In this study, we cannot conclusively attest that the encapsulation of THCr in βCyD enhanced or diminished the antioxidant or melanogenesis-stimulating activities of THCr since there is no study on the effects of the THCr mixture per se. In our previous study [36], we tested purified THC, HHC, and OHC, which can be used as a baseline to discuss the effects of each component. We reported that in B16F10 cells, purified THC enhanced melanin levels by greater amounts compared to HHC or OHC [36]. Hence, the mixture used in CW (THCr) might be less promelanogenic than pure THC, which can occur due to masking effects in a mixture [54]. CW concentrations of 20, 40, and 60 μg/mL contain THCr in concentration ranges of 3.2–4.4, 6.4–8.8, and 9.6–13.2 μg/mL, respectively (Table S1). Accordingly, the maximum concentration ranges of THC in them will correspond to ranges of 8.59–11.8 μM, 17.18–23.63 μM, and 25.77–35.44 μM, respectively, assuming if only THC was present in THCr, which is not the case (Table S1). In our earlier report [36], we established that THC increased the melanin contents in MNT-1 cells by 20% and 35.71% at 20 and 40 μM, respectively. Thus, it is likely that the increases we obtained at CW concentrations of 40 μg/mL (increase by 27.11%) and 60 μg/mL (increase by 28.85%) are in a close range. Moreover, the actual concentrations of HHC and OHC in CW which are <10 μM and <5 μM, respectively (Table S1), might not be contributing to increased melanogenesis. Encouragingly, these results validate that the melanotropic activity of THC is preserved after encapsulation in a CyD excipient, confirming our previously reported findings.

MITF is the main regulator of the proliferation, survival, and differentiation of melanocytes [55,56]. Our results of elongated dendrites and upregulated MITF protein levels by CW are similar to the effects induced by the natural flavonoid kaempferol [57] and flavonoid cirsimaritin [58] in B16F10 mouse melanoma cells, where both compounds stimulated melanin content and induced morphological changes of elongated dendrites, that correlated to increased levels of MITF protein. Interestingly, cirsimaritin also increased tyrosinase protein levels as well as the tyrosinase enzyme activity [58], which partly agrees with our results in MNT-1 cells where we obtained increased levels of tyrosinase protein by CW, although tyrosinase activity was unaffected. Our results of diminished tyrosinase activity but enhanced melanin production in MNT-1 cells by CW at the highest concentration of 100 μg/mL bear similarity to the results of another study [44], which showed that the fluid extract (FE) of sage containing the antioxidant rosmarinic acid stimulated

melanin production but also lowered cellular tyrosinase activity at 10 μM. Because CW also showed potent antioxidant activity, it is possible that at higher concentrations, it might reduce the formation of oxidative intermediates (dopaquinone), leading to polymerized melanin, thus suppressing the tyrosinase activity, although the direct effects of CW on the activity of tyrosinase (derived from mushrooms) showed no change (Figure S2).

Besides tyrosinase, tyrosinase-related protein-1 (TRP-1) and TRP-2 are two additional melanogenic enzymes that regulate melanogenesis downstream of tyrosinase [59]. Specifically, TRP-2, also referred to as dopachrome tautomerase, catalyzes the rearrangement of DOPAchrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) [60], which is then oxidized to carboxylated indole-quinone by the action of TRP-1 [61]. MITF regulates the expression levels of the three key melanogenic enzymes: tyrosinase, TRP-1, and TRP-2 [62]. A previous study showed that a natural compound syringetin enhanced melanogenesis, in part, due to increased protein levels of TRP-2 and TRP-1 in addition to MITF and tyrosinase proteins [63], while another study showed that sarsasapogenin enhanced melanogenesis due to increased protein levels of MITF and tyrosinase proteins, with no change in TRP-2 or TRP-1 protein levels [64]. Nevertheless, whether the promelanogenic effect of CW might also involve the upregulation of levels of TRP-2 and TRP-1 proteins was not examined in this study and warrants future investigation. The upregulation of MITF that leads to increased melanogenesis is mediated by the activation of the p38 mitogen-activated protein kinase (MAPK) signal pathway [65,66]. Examination of the involvement of these pathways was not the objective of this study and warrants future investigations. In addition, whether CW affects levels of other proteins involved in regulating melanosome export, such as Rab27A, Cdc42, and RhoA [67], needs to be determined in future studies.

CW is a colorless powder with a mild taste and smell attributable to βCyD. Natural compounds that stimulate melanogenesis, provide antioxidant benefits, and aesthetic appeal by virtue of their lack of color are desirable for cosmeceutical applications. As THC has demonstrated superior antioxidant activity compared to the parent compound curcumin [68–70], we also assessed if encapsulated THC in CW may retain its antioxidant activity, and our results demonstrate the antioxidant potential of CW. The therapeutic use of plant-based natural products for the treatment of vitiligo has been established [71]. Earlier studies have shown that compounds that increase pigmentation while also being able to protect melanocytes from oxidative stressors, such as H_2O_2, are attracting interest. For example, prior studies evaluated the protective effects of natural compounds from oxidative stress using PIG3V cells that are human immortalized vitiligo melanocytes [72–74]. Hence, further studies that use vitiligo melanocytes to corroborate the therapeutic efficacy of CW for vitiligo will be worthy of future investigations.

The findings of this study need to be viewed in light of some limitations. CW's effects on melanogenesis were not examined in primary human melanocytes due to some constraints, hence, it is still premature to conclude that CW is a promelanogenic candidate. Future studies to examine the impact of CW on primary human melanocytes as well as 3D skin tissue equivalents (derived from Asian/Caucasian donors) are essential to validate the findings of promelanogenic capacity of CW. Furthermore, the examination of the underlying molecular mechanisms and the involvement of MAPK and PI3K/Akt signaling pathways in the effects of CW on melanogenesis was not undertaken in the current study, and future studies are needed to explore them. Despite the increased bioavailability of the nutraceutical CW, whether it can achieve the concentrations necessary to affect epidermal melanogenesis after oral administration and the study of the mechanisms of ingested CW reaching skin cells were not explored as it was beyond the scope of this study. Nonetheless, a previous study showed that orally administered turmeric extract of Curcuma longa (at 1000 mg/kg, twice daily) suppressed melanin production in UVB irradiated hairless mice model [75]. Because CW as a nutraceutical has a much greater bioavailability and solubility compared to curcuminoids [16], we speculate the concentrations after ingestion will be sufficient to affect melanogenesis, although future studies are necessary to validate this.

5. Conclusions

We conclude by demonstrating a proof-of-concept study for the capacity of CW to stimulate melanogenesis in MNT-1 human melanoma cells at a dose range (20–40 μg/mL) without toxicity to human keratinocytes in that narrow range. Although the results of this pilot study suggest the suitability of CW for treating hypopigmentation, further studies to evaluate CW in a 3D skin-tissue equivalent consisting of primary human melanocytes are essential to validate the promelanogenic capacity of CW. In addition, the molecular mechanisms and signaling pathways underlying the stimulation of melanogenesis by CW were not elucidated in this study and warrant future investigation. Lastly, clinical studies that demonstrate the capacity of the nutraceutical CW to affect epidermal melanogenesis after oral ingestion will be worthy of future investigations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nutraceuticals3030031/s1, Reference [76] are cited in the supplementary materials. Figure S1: MTS cell viability data of HEMn-DP cells after a 5-day treatment with varying concentrations of CW; (n = 3 per group); Table S1: Estimates of concentration ranges (in μg/mL) of THCr, HHCr, and OHCr in CW concentrations of 20, 40, and 60 μg/mL (based on THCr (16–22%), HHCr (1–6%), and OHCr (0.5–2%), with corresponding concentration of THC, HHC, and OHC (based on the assumption if all THCr contained 100% THC, HHCr contained 100% HHC, and OHCr contained 100% OHC); Figure S2: Activity of tyrosinase enzyme measured with different concentrations of CW; Data are mean ± SD of two independent experiments, each with triplicate determinations.

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References

1. Raposo, G.; Marks, M.S. The dark side of lysosome-related organelles: Specialization of the endocytic pathway for melanosome biogenesis. *Traffic* **2002**, *3*, 237–248. [CrossRef] [PubMed]
2. Jablonski, N.G.; Chaplin, G. Epidermal pigmentation in the human lineage is an adaptation to ultraviolet radiation. *J. Hum. Evol.* **2013**, *65*, 671–675. [CrossRef] [PubMed]
3. Lin, J.Y.; Fisher, D.E. Melanocyte biology and skin pigmentation. *Nature* **2007**, *445*, 843–850. [CrossRef] [PubMed]
4. Yamaguchi, Y.; Brenner, M.; Hearing, V.J. The regulation of skin pigmentation. *J. Biol. Chem.* **2007**, *282*, 27557–27561. [CrossRef]
5. Nicolaidou, E.; Katsambas, A.D. Pigmentation disorders: Hyperpigmentation and hypopigmentation. *Clin. Dermatol.* **2014**, *32*, 66–72. [CrossRef]
6. Madu, P.N.; Syder, N.; Elbuluk, N. Postinflammatory hypopigmentation: A comprehensive review of treatments. *J. Dermatol. Treat.* **2022**, *33*, 704–708. [CrossRef]
7. Patel, R.; Pandya, A.G.; Sikirica, V.; Gandhi, K.; Daniel, S.R.; Anastassopoulos, K.P.; Yamaguchi, Y.; Napatalung, L.; Baik, R.; Ezzedine, K. Prevalence of Vitiligo among Children and Adolescents in the United States. *Dermatology* **2023**, *239*, 227–234. [CrossRef]
8. Mahama, A.N.; Haller, C.N.; Ahmed, A.M. Psychosocial considerations in the management of vitiligo. *Clin. Dermatol.* **2023**, *41*, 82–88. [CrossRef]
9. Tobin, D.J. Biology of hair follicle pigmentation. In *Hair Growth and Disorders*; Springer: Berlin/Heidelberg, Germany, 2008; pp. 51–74.
10. Sánchez-Ferrer, Á.; Rodríguez-López, J.N.; García-Cánovas, F.; García-Carmona, F. Tyrosinase: A comprehensive review of its mechanism. *Biochim. Biophys. Acta (BBA)-Protein Struct. Mol. Enzymol.* **1995**, *1247*, 1–11. [CrossRef]

11. Bae-Harboe, Y.-S.C.; Park, H.-Y. Tyrosinase: A central regulatory protein for cutaneous pigmentation. *J. Investig. Dermatol.* **2012**, *132*, 2678–2680. [CrossRef]
12. Niu, C.; Aisa, H.A. Upregulation of melanogenesis and tyrosinase activity: Potential agents for vitiligo. *Molecules* **2017**, *22*, 1303. [CrossRef] [PubMed]
13. Rachmin, I.; Ostrowski, S.M.; Weng, Q.Y.; Fisher, D.E. Topical treatment strategies to manipulate human skin pigmentation. *Adv. Drug Deliv. Rev.* **2020**, *153*, 65–71. [CrossRef] [PubMed]
14. A Ali, S.; Naaz, I.; Uddin Zaidi, K.; S Ali, A. Recent updates in melanocyte function: The use of promising bioactive compounds for the treatment of hypopigmentary disorders. *Mini Rev. Med. Chem.* **2017**, *17*, 785–798. [CrossRef] [PubMed]
15. Das, L.; Bhaumik, E.; Raychaudhuri, U.; Chakraborty, R. Role of nutraceuticals in human health. *J. Food Sci. Technol.* **2012**, *49*, 173–183. [CrossRef]
16. Sreeraj, G.; Jacob, J.; George, R.; Sreeraj, T. A unique formulation of hydrogenated curcuminoids with higher bio availability and the application in food matrices. *J. Nutr. Food Sci.* **2016**, *6*, 1.
17. Gopi, S.; Jacob, J.; Mathur, K.Y. Acute and subchronic oral toxicity studies of hydrogenated curcuminoid formulation 'CuroWhite' in rats. *Toxicol. Rep.* **2016**, *3*, 817–825. [CrossRef]
18. Ravikumar, A.N.; Jacob, J.; Gopi, S.; Jagannath, T.S. A Toxicological Evaluation of a Standardized Hydrogenated Extract of Curcumin (CuroWhite). *J. Toxicol.* **2018**, *2018*, 5243617. [CrossRef]
19. Jacob, J.; Amalraj, A.; Raj, K.K.J.; Divya, C.; Kunnumakkara, A.B.; Gopi, S. A novel bioavailable hydrogenated curcuminoids formulation (CuroWhite) improves symptoms and diagnostic indicators in rheumatoid arthritis patients—A randomized, double blind and placebo controlled study. *J. Tradit. Complement. Med.* **2019**, *9*, 346–352. [CrossRef]
20. Ferraro, M.G.; Piccolo, M.; Pezzella, A.; Guerra, F.; Maione, F.; Tenore, G.C.; Santamaria, R.; Irace, C.; Novellino, E. Promelanogenic Effects by an Annurca Apple-Based Natural Formulation in Human Primary Melanocytes. *Clin. Cosmet. Investig. Dermatol.* **2021**, *14*, 291. [CrossRef]
21. Jambhekar, S.S.; Breen, P. Cyclodextrins in pharmaceutical formulations II: Solubilization, binding constant, and complexation efficiency. *Drug Discov. Today* **2016**, *21*, 363–368. [CrossRef]
22. Loftsson, T.; Hreinsdóttir, D.; Másson, M. Evaluation of cyclodextrin solubilization of drugs. *Int. J. Pharm.* **2005**, *302*, 18–28. [CrossRef] [PubMed]
23. Braga, S.S. Cyclodextrins: Emerging medicines of the new millennium. *Biomolecules* **2019**, *9*, 801. [CrossRef] [PubMed]
24. Tønnesen, H.H. Solubility, chemical and photochemical stability of curcumin in surfactant solutions. Studies of curcumin and curcuminoids, XXVIII. *Die Pharm.* **2002**, *57*, 820–824.
25. Tomren, M.; Másson, M.; Loftsson, T.; Tønnesen, H.H. Studies on curcumin and curcuminoids: XXXI. Symmetric and asymmetric curcuminoids: Stability, activity and complexation with cyclodextrin. *Int. J. Pharm.* **2007**, *338*, 27–34. [CrossRef] [PubMed]
26. Loron, A.; Gardrat, C.; Tabary, N.; Martel, B.; Coma, V. Tetrahydrocurcumin encapsulation in cyclodextrins for water solubility improvement: Synthesis, characterization and antifungal activity as a new biofungicide. *Carbohydr. Polym. Technol. Appl.* **2021**, *2*, 100113. [CrossRef]
27. Peram, M.R.; Jalalpure, S.S.; Palkar, M.B.; Diwan, P.V. Stability studies of pure and mixture form of curcuminoids by reverse phase-HPLC method under various experimental stress conditions. *Food Sci. Biotechnol.* **2017**, *26*, 591–602. [CrossRef]
28. Kiuchi, F.; Goto, Y.; Sugimoto, N.; Akao, N.; Kondo, K.; Tsuda, Y. Nematocidal activity of turmeric: Synergistic action of curcuminoids. *Chem. Pharm. Bull.* **1993**, *41*, 1640–1643. [CrossRef]
29. Natural Actives Product List, Sabinsa Cosmetics. Available online: https://sabinsacosmetics.com/product-list (accessed on 1 July 2023).
30. Asawanonda, P.; Klahan, S.-O. Tetrahydrocurcuminoid cream plus targeted narrowband UVB phototherapy for vitiligo: A preliminary randomized controlled study. *Photomed. Laser Surg.* **2010**, *28*, 679–684. [CrossRef]
31. Luz-Veiga, M.; Amorim, M.; Pinto-Ribeiro, I.; Oliveira, A.L.; Silva, S.; Pimentel, L.L.; Rodríguez-Alcalá, L.M.; Madureira, R.; Pintado, M.; Azevedo-Silva, J. Cannabidiol and cannabigerol exert antimicrobial activity without compromising skin microbiota. *Int. J. Mol. Sci.* **2023**, *24*, 2389. [CrossRef]
32. Trivedi, M.K.; Panda, P.; Sethi, K.K.; Gangwar, M.; Mondal, S.C.; Jana, S. Solid and liquid state characterization of tetrahydrocurcumin using XRPD, FT-IR, DSC, TGA, LC-MS, GC-MS, and NMR and its biological activities. *J. Pharm. Anal.* **2020**, *10*, 334–345. [CrossRef]
33. Majeed, M.; Badmaev, V. Use of Tetrahydrocurcuminoids to Regulate Physiological and Pathological Events in the Skin and Mucosa. AU Patent 2,006,235,807, 11 September 2008.
34. Majeed, M.; Badmaev, V. Cross-Regulin Composition of Tumeric-Derived Tetrahydrocurcuminoids for Skin Lightening and Protection against UVB Rays. U.S. Patent 6,653,327, 25 November 2003.
35. Goenka, S. Novel Hydrogenated Derivatives of Chemically Modified Curcumin CMC2. 24 Are Potent Inhibitors of Melanogenesis in an In Vitro Model: Influence of Degree of Hydrogenation. *Life* **2023**, *13*, 1373. [CrossRef] [PubMed]
36. Goenka, S.; Simon, S.R. Comparative Study of Curcumin and Its Hydrogenated Metabolites, Tetrahydrocurcumin, Hexahydrocurcumin, and Octahydrocurcumin, on Melanogenesis in B16F10 and MNT-1 Cells. *Cosmetics* **2021**, *8*, 4. [CrossRef]
37. Musa, K.H.; Abdullah, A.; Kuswandi, B.; Hidayat, M.A. A novel high throughput method based on the DPPH dry reagent array for determination of antioxidant activity. *Food Chem.* **2013**, *141*, 4102–4106. [CrossRef] [PubMed]

38. Goenka, S. Comparative Study of Δ9-Tetrahydrocannabinol and Cannabidiol on Melanogenesis in Human Epidermal Melanocytes from Different Pigmentation Phototypes: A Pilot Study. *J. Xenobiot.* **2022**, *12*, 131–144. [CrossRef] [PubMed]
39. Zhang, L.; Man, S.; Qiu, H.; Liu, Z.; Zhang, M.; Ma, L.; Gao, W. Curcumin-cyclodextrin complexes enhanced the anti-cancer effects of curcumin. *Environ. Toxicol. Pharmacol.* **2016**, *48*, 31–38. [CrossRef]
40. Goenka, S.; Simon, S.R. Organogold drug Auranofin exhibits anti-melanogenic activity in B16F10 and MNT-1 melanoma cells. *Arch. Dermatol. Res.* **2020**, *312*, 213–221. [CrossRef]
41. Zhou, S.; Sakamoto, K. Citric acid promoted melanin synthesis in B16F10 mouse melanoma cells, but inhibited it in human epidermal melanocytes and HMV-II melanoma cells via the GSK3β/β-catenin signaling pathway. *PLoS ONE* **2020**, *15*, e0243565. [CrossRef]
42. Chae, J.K.; Subedi, L.; Jeong, M.; Park, Y.U.; Kim, C.Y.; Kim, H.; Kim, S.Y. Gomisin N inhibits melanogenesis through regulating the PI3K/Akt and MAPK/ERK signaling pathways in melanocytes. *Int. J. Mol. Sci.* **2017**, *18*, 471. [CrossRef]
43. Villareal, M.O.; Kume, S.; Neffati, M.; Isoda, H. Upregulation of Mitf by phenolic compounds-rich cymbopogon schoenanthus treatment promotes melanogenesis in b16 melanoma cells and human epidermal melanocytes. *BioMed Res. Int.* **2017**, *2017*, 8303671. [CrossRef]
44. Oliveira, K.B.; Palú, É.; Weffort-Santos, A.M.; Oliveira, B.H. Influence of rosmarinic acid and Salvia officinalis extracts on melanogenesis of B16F10 cells. *Rev. Bras. Farmacogn.* **2013**, *23*, 249–258. [CrossRef]
45. Sato, K.; Morita, M.; Ichikawa, C.; Takahashi, H.; Toriyama, M. Depigmenting mechanisms of all-trans retinoic acid and retinol on B16 melanoma cells. *Biosci. Biotechnol. Biochem.* **2008**, *72*, 2589–2597. [CrossRef] [PubMed]
46. Roméro, C.; Aberdam, E.; Larnier, C.; Ortonne, J.-P. Retinoic acid as modulator of UVB-induced melanocyte differentiation. Involvement of the melanogenic enzymes expression. *J. Cell Sci.* **1994**, *107*, 1095–1103. [CrossRef] [PubMed]
47. Na, J.-I.; Shin, J.-W.; Choi, H.-R.; Kwon, S.-H.; Park, K.-C. Resveratrol as a multifunctional topical hypopigmenting agent. *Int. J. Mol. Sci.* **2019**, *20*, 956. [CrossRef]
48. Zhou, S.; Riadh, D.; Sakamoto, K. Grape extract promoted α-msh-induced melanogenesis in b16f10 melanoma cells, which was inverse to resveratrol. *Molecules* **2021**, *26*, 5959. [CrossRef]
49. Hu, M.; Chen, C.; Liu, J.; Cai, L.; Shao, J.; Chen, Z.; Lin, L.; Zheng, T.; Ding, X.; Li, Z. The melanogenic effects and underlying mechanism of paeoniflorin in human melanocytes and vitiligo mice. *Fitoterapia* **2020**, *140*, 104416. [CrossRef]
50. Wen, S.Y.; Wu, Y.S.; Liu, H.; Ng, S.C.; Padma, V.V.; Huang, C.Y.; Kuo, W.W. Paeoniflorin found in Paeonia lactiflora root extract inhibits melanogenesis by regulating melanin-related signal transduction in B16F10 cells. *J. Cosmet. Dermatol.* 2023, *online ahead of print*. [CrossRef]
51. Ku, B.; Kim, D.; Choi, E.-M. Tetrahydrocurcumin Inhibits α-MSH-induced Melanogenesis via GSK3β Activation in B16F10 Melanoma Cells. *Toxicol. Environ. Health Sci.* **2019**, *11*, 210–218. [CrossRef]
52. Trivedi, M.K.; Gangwar, M.; Mondal, S.C.; Jana, S. Protective effects of tetrahydrocurcumin (THC) on fibroblast and melanoma cell lines in vitro: It's implication for wound healing. *J. Food Sci. Technol.* **2017**, *54*, 1137–1145. [CrossRef]
53. Majeed, M.; Pineda, R.T.V.; Chan, G.; Gabriel, T.; Dayrit, J.; Pelayo, C.A.; Prakash, L. A randomized, double-blind, placebo-controlled, comparative study, The safety and efficacy of 0.25% tetrahydrocurcumin (tumeric) cream as depigment agent against 4% hydroquinone cream. *HPC Today* **2010**, *3*, 44–46.
54. Benediktsdottir, B.E.; Baldursson, O.; Gudjonsson, T.; Tønnesen, H.H.; Masson, M. Curcumin, bisdemethoxycurcumin and dimethoxycurcumin complexed with cyclodextrins have structure specific effect on the paracellular integrity of lung epithelia in vitro. *Biochem. Biophys. Rep.* **2015**, *4*, 405–410. [CrossRef]
55. Kawakami, A.; Fisher, D.E. The master role of microphthalmia-associated transcription factor in melanocyte and melanoma biology. *Lab. Investig.* **2017**, *97*, 649–656. [CrossRef]
56. Levy, C.; Khaled, M.; Fisher, D.E. MITF: Master regulator of melanocyte development and melanoma oncogene. *Trends Mol. Med.* **2006**, *12*, 406–414. [CrossRef] [PubMed]
57. Tang, H.; Yang, L.; Wu, L.; Wang, H.; Chen, K.; Wu, H.; Li, Y. Kaempferol, the melanogenic component of Sanguisorba officinalis, enhances dendricity and melanosome maturation/transport in melanocytes. *J. Pharmacol. Sci.* **2021**, *147*, 348–357. [CrossRef] [PubMed]
58. Kim, H.J.; Kim, I.S.; Dong, Y.; Lee, I.-S.; Kim, J.S.; Kim, J.-S.; Woo, J.-T.; Cha, B.-Y. Melanogenesis-inducing effect of cirsimaritin through increases in microphthalmia-associated transcription factor and tyrosinase expression. *Int. J. Mol. Sci.* **2015**, *16*, 8772–8788. [CrossRef] [PubMed]
59. Del Marmol, V.; Beermann, F. Tyrosinase and related proteins in mammalian pigmentation. *FEBS Lett.* **1996**, *381*, 165–168. [CrossRef]
60. Yokoyama, K.; Yasumoto, K.-i.; Suzuki, H.; Shibahara, S. Cloning of the human DOPAchrome tautomerase/tyrosinase-related protein 2 gene and identification of two regulatory regions required for its pigment cell-specific expression. *J. Biol. Chem.* **1994**, *269*, 27080–27087. [CrossRef]
61. Kobayashi, T.; Urabe, K.; Winder, A.; Jiménez-Cervantes, C.; Imokawa, G.; Brewington, T.; Solano, F.; García-Borrón, J.; Hearing, V. Tyrosinase related protein 1 (TRP1) functions as a DHICA oxidase in melanin biosynthesis. *EMBO J.* **1994**, *13*, 5818–5825. [CrossRef]
62. Aoki, H.; Moro, O. Involvement of microphthalmia-associated transcription factor (MITF) in expression of human melanocortin-1 receptor (MC1R). *Life Sci.* **2002**, *71*, 2171–2179. [CrossRef]

63. Han, H.; Hyun, C.-G. Syringetin Promotes Melanogenesis in B16F10 Cells. *Int. J. Mol. Sci.* **2023**, *24*, 9960. [CrossRef]
64. Moon, E.; Kim, A.-J.; Kim, S.Y. Sarsasapogenin increases melanin synthesis via induction of tyrosinase and microphthalmia-associated transcription factor expression in melan-a cells. *Biomol. Ther.* **2012**, *20*, 340. [CrossRef]
65. Jung, E.; Kim, J.H.; Kim, M.O.; Jang, S.; Kang, M.; Oh, S.W.; Nho, Y.H.; Kang, S.H.; Kim, M.H.; Park, S.-H. Afzelin positively regulates melanogenesis through the p38 MAPK pathway. *Chem.-Biol. Interact.* **2016**, *254*, 167–172. [CrossRef]
66. Zhou, J.; Song, J.; Ping, F.; Shang, J. Enhancement of the p38 MAPK and PKA signaling pathways is associated with the pro-melanogenic activity of Interleukin 33 in primary melanocytes. *J. Dermatol. Sci.* **2014**, *73*, 110–116. [CrossRef] [PubMed]
67. Scott, G.; Leopardi, S.; Printup, S.; Madden, B.C. Filopodia are conduits for melanosome transfer to keratinocytes. *J. Cell Sci.* **2002**, *115*, 1441–1451. [CrossRef] [PubMed]
68. Aggarwal, B.B.; Deb, L.; Prasad, S. Curcumin differs from tetrahydrocurcumin for molecular targets, signaling pathways and cellular responses. *Molecules* **2014**, *20*, 185–205. [CrossRef] [PubMed]
69. Sugiyama, Y.; Kawakishi, S.; Osawa, T. Involvement of the β-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem. Pharmacol.* **1996**, *52*, 519–525. [CrossRef]
70. Somparn, P.; Phisalaphong, C.; Nakornchai, S.; Unchern, S.; Morales, N.P. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. *Biol. Pharm. Bull.* **2007**, *30*, 74–78. [CrossRef]
71. Pang, Y.; Wu, S.; He, Y.; Nian, Q.; Lei, J.; Yao, Y.; Guo, J.; Zeng, J. Plant-derived compounds as promising therapeutics for vitiligo. *Front. Pharmacol.* **2021**, *12*, 685116. [CrossRef]
72. Jian, Z.; Li, K.; Song, P.; Zhu, G.; Zhu, L.; Cui, T.; Liu, B.; Tang, L.; Wang, X.; Wang, G. Impaired activation of the Nrf2-ARE signaling pathway undermines H2O2-induced oxidative stress response: A possible mechanism for melanocyte degeneration in vitiligo. *J. Investig. Dermatol.* **2014**, *134*, 2221–2230. [CrossRef]
73. Fang, W.; Tang, L.; Wang, G.; Lin, J.; Liao, W.; Pan, W.; Xu, J. Molecular hydrogen protects human melanocytes from oxidative stress by activating Nrf2 signaling. *J. Investig. Dermatol.* **2020**, *140*, 2230–2241.e9. [CrossRef]
74. Tang, L.; Fang, W.; Lin, J.; Li, J.; Wu, W.; Xu, J. Vitamin D protects human melanocytes against oxidative damage by activation of Wnt/β-catenin signaling. *Lab. Investig.* **2018**, *98*, 1527–1537. [CrossRef]
75. Sumiyoshi, M.; Kimura, Y. Effects of a turmeric extract (*Curcuma longa*) on chronic ultraviolet B irradiation-induced skin damage in melanin-possessing hairless mice. *Phytomedicine* **2009**, *16*, 1137–1143. [CrossRef]
76. Goenka, S.; Ceccoli, J.; Simon, S.R. Anti-melanogenic activity of ellagitannin casuarictin in B16F10 mouse melanoma cells. *Nat. Prod. Res.* **2021**, *35*, 1830–1835. [CrossRef] [PubMed]