Applied Biochemistry and Biotechnology (2023) 195:369–385
https://doi.org/10.1007/s12010-022-04143-9

ORIGINAL ARTICLE

Chemical Composition and Tyrosinase Inhibitory Activities of Fatty Acids Obtained from Heterotrophic Microalgae, *S. limacinum* and *C. cohnii*

Ayse Kose[1]

Accepted: 28 August 2022 / Published online: 9 September 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
Tyrosinase is the rate-limiting enzyme for melanin production in plant and mammalian cells. Upregulation of this enzyme results in hyperpigmentation disorders. In order to treat pigmentation problems, novel skin whitening compounds are extremely screened. It is found that fatty acids based on their saturation levels either increase or decrease tyrosinase enzyme activity. Thus, fatty acids and their compositions are promising candidates for the treatment of hyperpigmentation or hypopigmentation disorders. Microalgae are rich in both saturated and unsaturated fatty acids, as well. In this study, *C. cohnii* and *S. limacinum* fatty acids were evaluated as tyrosinase inhibitor candidates. Mushroom tyrosinase activity studies displayed that both extracts increase tyrosinase enzyme activity dose-dependently. On the other hand, *S. limacinum* at 200 μg ml^{-1} concentration almost decreased half of tyrosinase enzyme activity in B16-F10 cells. Besides, it was 3 times more efficient for tyrosinase enzyme activity inhibition and 2 times more effective to decrease melanin synthesis compared to *C. cohnii*. Considering low toxicity to B16-F10 melanoma and healthy keratinocyte cells (HaCaT), *S. limacinum* fatty acids could be a suitable source for lipid-based tyrosinase inhibitory functional cosmetics products.

Keywords Tyrosinase · B16-F10 melanoma cells · Fatty acids · Microalgae · Melanogenesis

Introduction

Melanogenesis is the process involving the color development of skin by the production of a pigment called melanin [1]. Melanin pigments are produced in melanocytes which are embedded in specialized organelles called melanosomes [2]. Melanin has a major role in skin homeostasis via protecting the body from harmful UV light and toxic chemical stresses [3]. The imbalances in the melanin production may result in freckles, solar lentigo, vitiligo, and melanoma which are either hyper- or hypopigmenting disorders [4].

✉ Ayse Kose
aykoseay@gmail.com

[1] Department of Bioengineering, Faculty of Engineering, University of Ege, 35100, Bornova, Izmir, Turkey

Melanin is produced with complex cell signaling pathways and transcriptional regulations; however, the rate-limiting step is the activity of the tyrosinase enzyme [5].

Tyrosinase enzyme (EC 1.14.18.1) is not only essential for mammalians, but can be also found in bacteria and fungus. It is responsible for the enzymatic browning of fruits and vegetables [2]. Tyrosinase enzyme catalyzes the formation of 3,4-dihydroxyphenylalanine (L-DOPA) (monophenolase activity) by hydroxylation of L-tyrosine and oxidases L-DOPA to dopaquinone (diphenolase activity) [5].

Compounds that directly or indirectly regulate melanin synthesis and tyrosinase activity are emerging for the development of functional cosmetics agents for skin color corrections [6–8]. However, most of the compounds used in this field such as kojic acid, arbutin, azelaic acid, hydroquinone, resveratrol, and vitamin E either have a cytotoxic effect or low stability; thus, formulation of products containing these substances is a bit cumbersome [9]. However, natural compounds are good sources to mine possible promising novel substances to give a rise to develop tyrosinase inhibitory and skin pigmenting agents with fewer side effects.

Microalgae are emerging photosynthetic organisms for the development of aquatic biotechnology [10, 11]. The bioactive molecules in microalgae such as pigments (astaxanthin, canthaxanthin, lutein, beta-carotene, fucoxanthin) [12], mycosporine-like amino acids [13], and carbohydrates [14] are already utilized in cosmetics and functional cosmetics purposes. Their antioxidant, anticancer, antifungal, antibacterial, anti-tyrosinase, and immune regulatory effects have been shown by in vitro and in vivo studies [15–17]. Microalgae are also a rich source of polyunsaturated fatty acids (PUFAs): omega-3 and omega-6 fatty acids [18]. Among these species, *Schizochytrium limacinum* and *Crypthecodinium cohnii* are known as industrial producers of docosahexaenoic acid (DHA) [18, 19]. DHA has a great potential for human health; it lowers the risk of Alzheimer's disease and enhances the immune system, and it is related to autoimmune diseases and an important component to treat cardiovascular and nervous system disorders.

Fatty acids are essential components of the epidermis layer, and they are also found to be responsible for the regulation of melanogenesis through biosynthetic pathways. The reports indicate that the saturation level of fatty acids is critical for pigmentation. Saturated fatty acids (such as palmitic acid (C16:0) and stearic acid (C18:0)) increase pigmentation; on the other hand, unsaturated fatty acids (oleic acid (C18:1), linoleic acid (C18:2), and α-linolenic acid (C18:3)) down-regulate tyrosinase activity by proteasomal degradation [9, 20]. Another study revealed that DHA showed tyrosinase inhibitory activity on B16-F10 cells in a dose-dependent manner [21]. Even though several studies have been done to see the effect of plant-derived fatty acids on pigmentation and tyrosinase activity [22, 23], no study was done to evaluate the effect of microalgal fatty acids on pigmentation. However, microalgal fatty acids have superior advantages over plant-derived fatty acids due to their high growth rates, lipid content, and ease in the extraction processes.

In this study, *S. limacinum* and *C. cohnii* lipids were investigated for tyrosinase inhibition on spontaneous pigmentation with B16-F10 murine melanoma cells. Also, cytotoxic effects on normal keratinocytes (HaCaT) cells were done for further evaluation for skin treatment. As far as we know; this is the first report demonstrating tyrosinase inhibitory effects of algal lipids both in cell-free and cellular tyrosinase activity.

Materials and Methods

Chemicals and Reagents

Chloroform, methanol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Triton-X100, phenylmethylsulfonyl fluoride (PMSF), butylated hydroxytoluene (BHT), trypsin/EDTA, 3,4-dihydroxy-L-phenylalanine (L-DOPA), L-tyrosine, mushroom tyrosinase enzyme, synthetic melanin, dimethylsulfoxide (DMSO), and bovine serum albumin (BSA) were purchased from Sigma. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Gibco; gentamycin sulfate was purchased from Biological Industries (BI). *S. limacinum* was a kind gift from MarinBio, Turkey.

Preparation of Crude Lipid Extracts

Ten grams of *C. cohnii* (CCMP 316) and *S. limacinum* dry biomasses was mixed with 30 ml of 2:1 (v/v) chloroform:methanol containing 250 μg ml^{-1} BHT. The mixture was sonicated for 3 min at 9 cycles at 50% power with a probe-type sonicator (Bandelin, Sonopuls, UK). The sonication process was done in an ice bath to prevent excessive heating. The samples were sealed and incubated overnight in a rotary shaker with a mixing frequency of 120 rpm. Then, the samples were centrifuged at 4000 rpm for 5 min (Nüve, Turkey). The supernatants were filtered with a syringe-type filter (0.22-μm diameter, PTFE, Sartorious Stedim, Germany) to remove the remaining particles. The supernatants were evaporated at 45 °C using a rotary evaporator (Stuart, RE300, UK). The samples were kept at − 20 °C for further analysis. Fatty acid methyl esters (FAME) analyses were done according to a previous study [24]. Fatty acids were trans-methylated with hydrochloric acid/methanol prior to gas chromatography analysis. After this step, FAMEs were extracted with hexane and analyzed by Agilent 7890 GC equipped with a flame ionization detector and a Supelco sp-2380 A capillary column (60 m × 250 μm × 0.2 μm) using helium as the carrier gas. The detector and inlet temperatures were set to 260 °C. The oven temperature was started at 100 °C, then increased at 10 °C min^{-1} intervals to 250 °C, and held there for 3 min. One microliter sample was injected, and FAMEs were identified by chromatographic comparison with authentic standards.

Cell Culture

The B16-F10 murine melanoma cells were a kind gift of Prof. Dr. Azmi Yerlikaya of Kütahya Dumlupınar University. Cells were cultured in DMEM supplemented with 10% FBS, 0.1% penicillin/streptomycin, and 0.1% of gentamycin sulfate (50 mg ml^{-1}), and maintained at 37 °C in a CO_2 incubator humidified atmosphere containing 5% CO_2. The cells were sub-cultured every 2 days to maintain the logarithmic growth phase and harvested with 0.25% trypsin/EDTA when they reach approximately 80% confluency.

Mushroom Tyrosinase Inhibition Assay

Monophenolase and diphenolase activities of mushroom tyrosinase with various concentrations (100, 200, 400, 800 µg ml^{-1}) of *C. cohnii* and *S. limacinum* lipids were done with some minor modifications according to the previous report [22]. Forty microliters of 5 mM L-tyrosine (monophenolase activity) or 2 mM L-DOPA (diphenolase activity) (prepared in 0.1 M sodium phosphate buffer pH 6.8) was mixed with 80 µl of 0.1 M phosphate buffer (pH 6.8) in a 96-well plate. Forty microliters of various concentrations of samples and 40 µl of mushroom tyrosinase (50 U ml^{-1} in phosphate buffer) were added to the reaction mixture. The plate was incubated at 37 °C and the absorbance of the samples was measured at 490 nm using a microplate reader (BioTek Industries, USA). PBS was used as a blank control. The tyrosinase inhibition was calculated as Eq. 1. Kojic acid (200 µg ml^{-1}) is used as reference.

$$\text{Inhibiton}\% = \left(1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}}\right)\right) \times 100 \tag{1}$$

Cell Viability Assay

The effect of *C. cohnii* and *S. limacinum* lipids on cell viability was assessed with the MTT assay. For this study, cells were seeded to 96-well plates with a density of 2.5×10^3 cells $well^{-1}$ (100 µl $well^{-1}$). The plates were incubated overnight for cells to adhere. After 24 h of incubation, various concentrations (25, 50, 100, 200, 400, and 800 µg ml^{-1}) of samples were added to each well. The plates were incubated in a CO_2 incubator humidified with 5% CO_2 at 37 °C for 48 and 72 h. Cells were incubated with 100 µl MTT solution (90% (v/v) medium and 10% (v/v) 5 mg ml^{-1} MTT) was added to each well and incubated for 3 h at 37 °C and 5% CO_2 in the dark. After 3 h, the MTT solution was replaced with 100 µl DMSO and dissolved formazan crystals absorbance was measured at 570 nm using a microplate reader (BioTek Instruments, USA). The experiments were done as triplicates. Cells without sample were used as positive control. The viability of the cells was determined as percentage viability using Eq. 2.

$$\text{Viability}\% = \frac{(A_{\text{Sample}} - A_{\text{Blank}})}{(A_{\text{Control}} - A_{\text{Blank}})} \times 100 \tag{2}$$

Cellular Tyrosinase Assay

Cellular tyrosinase activity was measured according to a previous report with slight modifications [25]. B16-F10 cells were seeded with a density of 2×10^5 cells $well^{-1}$ (2 ml $well^{-1}$) to 6-well plates (SPL, Korea). Plates were incubated overnight in a humidified CO_2 incubator at 37 °C and 5% CO_2 for cells to adhere to the plate surface. After 24 h, the medium was replaced with fresh DMEM, containing various concentrations (12.5, 25, 50, 100, and 200 µg ml^{-1}) of lipids for 72 h. Cells were washed twice with ice-cold PBS (0.1 M, pH 6.8). Three hundred microliters of cell lysis buffer (1% Triton-×100, 0.1 M PMSF in 0.1 M sodium phosphate buffer, pH 6.8) was added to each well and incubated for 3–5 min. Cells were detached from the well surface with cell scrappers (SPL, Korea) and transferred to 1.5 ml tubes. The cells were sonicated for 5 s

(Bandelin, Sonapuls, UK) at 9 cycles and 50% power in an ice bath and kept in ice for 15 min. The lysates were then clarified with a centrifuge at 13,000 rpm, 15 min, + 4 °C. The protein concentration was determined according to the Bradford method. BSA was used as the protein standard. One hundred micrograms of protein from each cell lysates in 100 µl 0.1 M phosphate buffer (pH 6.8) and 100 µl 5 mM L-DOPA (prepared to 0.1 M phosphate buffer, pH 6.8) was added to each well of a 96-well plate. The reaction mixture was incubated for 1 h at 37 °C in dark. The absorbance was measured at 490 nm using a microplate reader (BioTek Instruments, USA). The tyrosinase activity was determined by Eq. 3. Cells cultivated only in DMEM were used as a control. The experiments were done in triplicates.

$$\text{Tyrosinaseactivity}(\%) = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100 \tag{3}$$

Intracellular Melanin Content Assay

Intracellular melanin content was measured as described in a previous report with slight modifications [26]. B16-F10 cells were seeded with a density of 2×10^5 cells well^{-1} (2 ml well^{-1}) to 6-well plates (SPL, Korea). Plates were incubated overnight in a humidified CO_2 incubator at 37 °C and 5% CO_2 for cells to adhere to the plate surface. After 24 h, cells were treated with various concentrations (12.5, 25, 50, 100, and 200 µg ml^{-1}) of lipids for 72 h. After cell lysis, the cell debris was washed twice with ice-cold PBS (0.1 M, pH 6.8). The intracellular melanin was solubilized in 500 µl 1 M NaOH containing 10% DMSO for 1 h at 80 °C. One hundred-microliter melanin samples were transferred to each well of a 96-well plate and the absorbance was measured at 405 nm. The melanin content was determined from a standard curve prepared from a standard of synthetic melanin.

Cytotoxicity Measurements on HaCaT Cells

HaCaT (human keratinocytes cells) cells were grown in DMEM supplemented with 10% FBS, 0.1% penicillin/streptomycin, and 0.1% of gentamycin sulfate (50 mg ml^{-1}) in culture flasks, and maintained at 37 °C in a CO_2 incubator humidified atmosphere containing 5% CO_2 in the air. The cytotoxic effects of *C. cohnii* and *S. limacinum* lipids on HaCaT cells were measured using MTT cell viability assay. Cells were seeded with a density of 1×10^5 cells well^{-1} (100 µl well^{-1}). Cells were incubated overnight for cells to adhere to the surface. After 24 h, cells were incubated with 25, 50, 100, and 200 µg ml^{-1} concentration of crude lipids for 48 and 72 h in a CO_2 incubator humidified with 5% CO_2 at 37 °C for 48 and 72 h. Cells were incubated with 100 µl MTT solution (90% (v/v) medium and 10% (v/v) 5 mg ml^{-1} MTT), added to each well, and incubated for 3 h at 37 °C and 5% CO_2 in the dark. After 3 h, the MTT solution was replaced with 100 µl DMSO and dissolved formazan crystals absorbance was measured at 570 nm using a microplate reader (BioTek Instruments, USA). The experiments were done as triplicates. Cells without samples were used as a positive control. The viability of the cells was determined as percentage viability using Eq. 2.

Statistical Analysis

All the analysis was done in triplicates and presented with the average value. Data were expressed as means ± standard deviation. The experimental data were analyzed using a one-way analysis of variance (ANOVA). A probability value of $p < 0.05$ was considered to define a statistically significant difference.

Results and Discussion

Skin pigmentation disorders, either hypopigmentation or hyperpigmentation, cause serious psychological and physiological problems [27]. Topical active metabolites from synthetic, semi-synthetic, and natural sources are commonly utilized for skin color-correcting cosmeceutical products [28]. However, there is emerging research ongoing for finding natural sources to be novel skin whitening agents. Fatty acids are vital molecules in the regulation of cellular signaling [20]. They are the foundation of cell structure and are responsible for important metabolism in mammalian and plant organization. Fatty acids have anticancer, UV-protective, antioxidant, and pro-inflammatory effects [29]. Some studies also evaluated the effect of saturated and unsaturated fatty acids including PUFAs on melanin production and melanoma cell viability [21, 30]. Some fatty acids such as DHA and linoleic acid significantly decreased melanin production and down-regulated tyrosinase enzyme expression by proteasomal degradation [20]. However, fatty acids such as palmitic acid, lauric acid, and stearic acid increased tyrosinase enzyme expression and melanin production [31–33]. As it could be seen here, fatty acids create a differing response with respect to their saturation level.

C. cohnii and *S. limacinum* species are promising heterotrophic microalgae for rapid and mass production of omega-3 fatty acids, EPA, and DHA [18, 19]. In this study, lipids from *C. cohnii* and *S. limacinum* were evaluated as novel natural sources for skin pigmentation disorders, considering the relation of fatty acids with tyrosinase enzyme activity and melanin synthesis. The previous reports suggest that fatty acids, as also one of the major components in the epidermis, have a strong relation with tyrosinase enzyme activity which regulates skin pigmentation directly or indirectly [20, 22, 34]. Thus, this study aimed to display that microalgal lipids can be sustainable sources for the formulation of skin pigmentation products.

Fatty Acid Composition

The extraction yield was 25–30% (w/w) lipid to biomass and no significant difference was seen for both species ($p > 0.05$). Fatty acid distribution was checked using GC-FID. Of the total composition, 99.51% and 99.92% were determined from *C. cohnii* and *S. limacinum*, respectively (Table 1). Total unsaturated fatty acids were 20.86% and 32.95%; total saturated fatty acids were 77.69% and 66.55% in *C. cohnii* and *S. limacinum*, respectively. The majority of polyunsaturated fatty acids (PUFAs) were DHA for both species. DHA content in *S. limacinum* was almost 2 times higher than *C. cohnii* (*S. limacinum*, 27.63% and *C. cohnii*, 13.92%). Linolenic acid was not detected in *C. cohnii*. However, *S. limacinum* had trace amounts of linolenic acid which could be neglected. Followed by linolenic acid, EPA was also found in trace

Table 1 Fatty acid composition (%) according to gas chromatography (GC)-FID analysis of *C. cohnii* and *S. limacinum* crude lipid extracts

Fatty acids	*C. cohnii*	*S. limacinum*
Omega-3		
Docosahexaenoic acid (C22:6 n-3)	13.93	27.63
Eicosapentaenoic acid (C20:5 n-3)	0.02	0.82
Linolenic acid (C18:3 n-3)	-	0.10
Omega-6		
Oleic acid (C18:1n9c)	6.73	1.11
Linoleic acid (C18:2n6c)	0.18	2.99
Others		
Caproic acid (C6:0)	0.15	-
Caprylic acid (C8:0)	0.74	-
Capric acid (C10:0)	5.48	0.01
Lauric acid (C12:0)	19.15	0.26
Myristic acid (C14:0)	28.23	5.92
Pentadecanoic acid (C15:0)	0.07	4.12
Palmitic acid (C16:0)	22.28	53.86
Palmitoleic acid (C16:1)	0.56	0.16
Heptadecanoic acid (C17:0)	-	1.16
Stearic acid (C18:0)	1.32	1.22
Arachidic acid (C20:0)	0.25	-
Total saturated	77.69	66.55
Total unsaturated	20.86	32.95
Trace quantities total	0.96	0.42
Total	99.51	99.92

amounts. The results were also in accordance with previous studies [18, 19]. Oleic acid was 6 times higher in *C. cohnii*; however, linoleic acid, a major skin whitening fatty acid for hyperpigmentation disorders, was at trace amounts. On the other hand, it was 3% in *S. limacinum*.

Myristic acid (28.23) followed by palmitic acid (22.28%) and lauric acid (19.15%) was abundant in *C. cohnii* fatty acid composition. Especially, palmitic acid, myristic acid, stearic acid, and lauric acid are known to be tyrosinase activator fatty acids in mushroom and mammalian enzymes. These 4 fatty acids contributed to 70.98% of *C. cohnii* and 61.26% of *S. limacinum*. Palmitic acid in *S. limacinum* was more than half of the fatty acids (53.86%). Stearic acid was 1.32 and 1.22% for *C. cohnii* and *S. limacinum*. A trace amount of lauric acid was also found in *S. limacinum*. In general, the fatty acid distribution of both species was valuable for tyrosinase enzyme activity regulation.

Mushroom Tyrosinase Inhibition

Prior to animal cell culture experiments, the effect of microalgal lipids on mushroom (*Agaricus bisporus*) tyrosinase enzyme activity was determined with increasing lipid concentrations. As a universal tyrosinase enzyme inhibitor, kojic acid (200 μg ml^{-1}) was used as a positive control. At this concentration, mushroom tyrosinase activity was

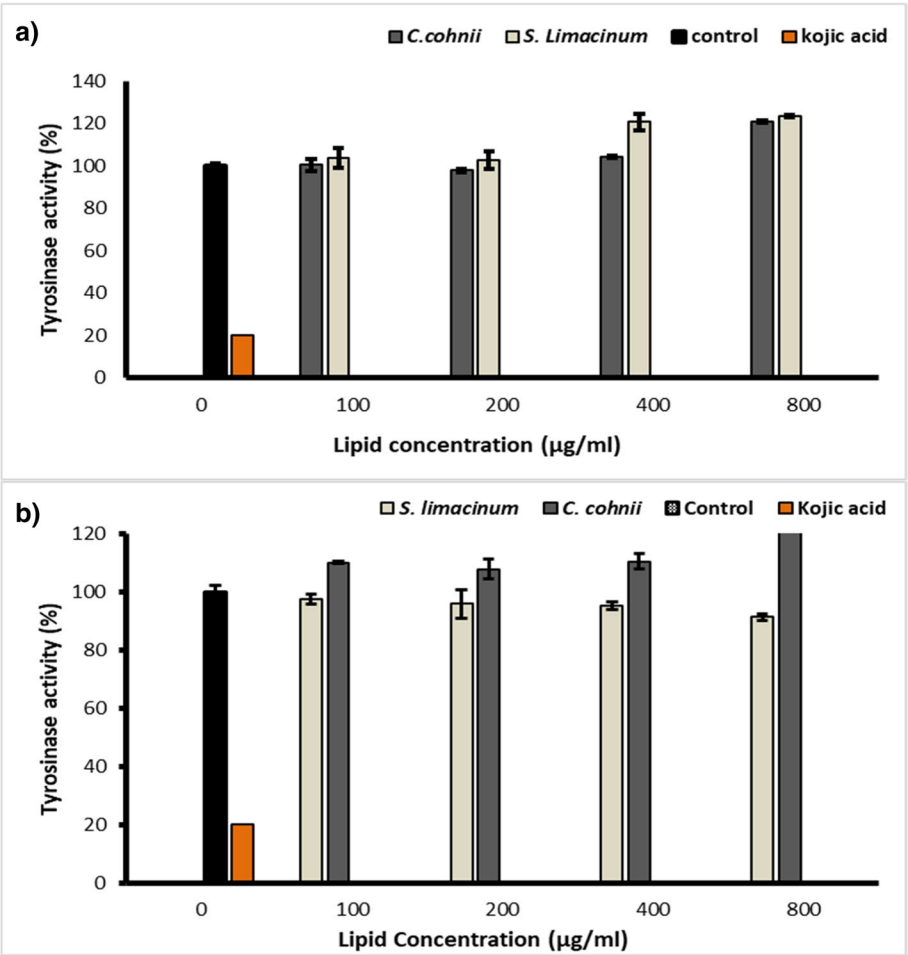

Fig. 1 Mushroom tyrosinase activity of *C. cohnii* and *S. limacinum* lipids; **a** monophenolase activity of mushroom tyrosinase (L-tyrosine), **b** diphenolase activity of mushroom tyrosinase (L-DOPA). Each column represents the mean ± SEM of three independent experiments. The x-axis represents the various concentrations of *C. cohnii* and *S. limacinum* lipids

20.17 ± 1.25%. The concentration of microalgal lipids increased mushroom tyrosinase activity after 100 µg ml^{-1}, significantly ($p < 0.05$), when L-tyrosine was used as substrate (Fig. 1a, monophenolase activity). Mushroom tyrosinase enzyme activity was 121.04 ± 0.48% and 123.4 ± 0.47% for *C. cohnii* and *S. limacinum*, respectively, for 800 µg ml^{-1}. However, there was no significant difference between *C. cohnii* and *S. limacinum*. When L-DOPA was used as substrate (Fig. 1b, diphenolase activity), *S. limacinum* did not significantly affect enzyme activity ($p > 0.05$). The enzyme activity was around 95 ± 2%. But a significant increase in enzyme activity with respect to increasing concentration was observed when the enzyme was treated with *C. cohnii* lipids ($p < 0.001$). The increase in the enzyme activity could be attributed to the higher amount of saturated fatty acids in both microalgae lipids. *C. cohnii* lipids were almost 10% higher in saturated lipid concentration, but in mushroom tyrosinase enzyme, these

differences in the concentration did not affect enzyme activity in between samples considering monophenolase activity. However, diphenolase activity results clearly displayed the difference in saturated lipid values for *C. cohnii*.

The results in mushroom tyrosinase enzyme indicated that lipid extracts from *C. cohnii* increased tyrosinase enzyme activity by monophenolase and diphenolase activity in a dose-dependent way. On the other hand, significant activation occurred for *S. limacinum* only on monophenolase activity. The increased effect has resulted from the high amount of saturated fatty acids. Even though *S. limacinum* has 53.86% of its lipid composition consisting of palmitic acid, *C. cohnii*'s overall saturated lipid levels are 10% higher than *S. limacinum*. The previous report showed that DHA has no significant activity on mushroom tyrosinase activity [21]. It is considered that the activation effect is based on palmitic acid in *S. limacinum* and a synergistic effect of myristic acid, palmitic acid, and lauric acid for *C. cohnii*. There is no data on the effect of capric acid and tyrosinase enzyme activity relations. However, *C. cohnii* also contained almost 6% of capric acid too. This fatty acid is also considered to have an activation effect on mushroom tyrosinase enzyme, which requires further investigation.

Effects of Microalgal Lipids on B16-F10 Cell Viability

Cytotoxic effect on murine melanoma, B16-F10 cells, at various concentrations ranging from 25 to 800 μg ml^{-1} (Fig. 2) was evaluated for 48 and 72 h. The lipid extracts showed a significant level of cytotoxicity which was well observed at dose after 200 μg ml^{-1}. The cytotoxic effect of *C. cohnii* extracts was significantly different than *S. limacinum* ($p < 0.05$). At 72 h, almost more than 90% of cells were dead. *C. cohnii* showed a more toxic effect than *S. limacinum*. However, both microalgae lipid extracts were significantly cytotoxic with respect to concentration ($p < 0.001$). Based on toxicity, IC_{50} values were 121.99 ± 0.61 μg ml^{-1} and 151.23 ± 4.65 μg ml^{-1} for *S. limacinum* and *C. cohnii*. Considering the toxic effect of the lipid extracts, tyrosinase activity, and melanin content was evaluated up to 200 μg ml^{-1} extract concentration.

The cell viability experiments were done on B16-F10 murine melanoma cells prior to understanding the effect of crude lipid extracts on melanin production and tyrosinase enzyme activity. Both extracts showed a high cytotoxic effect with respect to increasing concentration. However, the lethal effect of *C. cohnii* was slightly higher than *S. limacinum*. At 800 μg ml^{-1}, only 3% of viable cells were detected. It is known that fatty acids could be highly lethal to cancer cell lines [35]. A study done with pure DHA on B16-F10 showed that up to 25 μg ml^{-1} concentration cell viability was not affected [21]. DHA content was almost 2 times higher in *S. limacinum* extracts, so the higher lethal effect of *C. cohnii* is thought to be not only related to DHA content. Along with DHA, EPA, and arachidonic acid, linoleic acid is also known to be a great tumor-suppressing fatty acid based on in vitro, in vivo, and clinical studies. Dietary intake of EPA and DHA is known as a great suppressor of melanoma as well. One other study revealed algal oils rich in PUFAs (DHA and EPA) decreased the metastatic effect of B16-F10 cells in the lungs [36]. When melanoma cells were injected with algal oils through an intravenous application on mice, melanoma cells showed metastatic events in the lungs but not in the liver or kidney. After several weeks, algal oil-treated samples showed great recovery from pulmonary metastasis [36]. Fatty acids derived from algal extracts generally show a cytotoxic effect on cancerous cells but not on healthy cells. A great example is the selective toxicity of palmitic acid to leukemic cells and normal human dermal fibroblasts (Harada et al., 2002). Almond oil rich in oleic acid showed a cytotoxic effect in human colon cancer cell line models [37]. *C.*

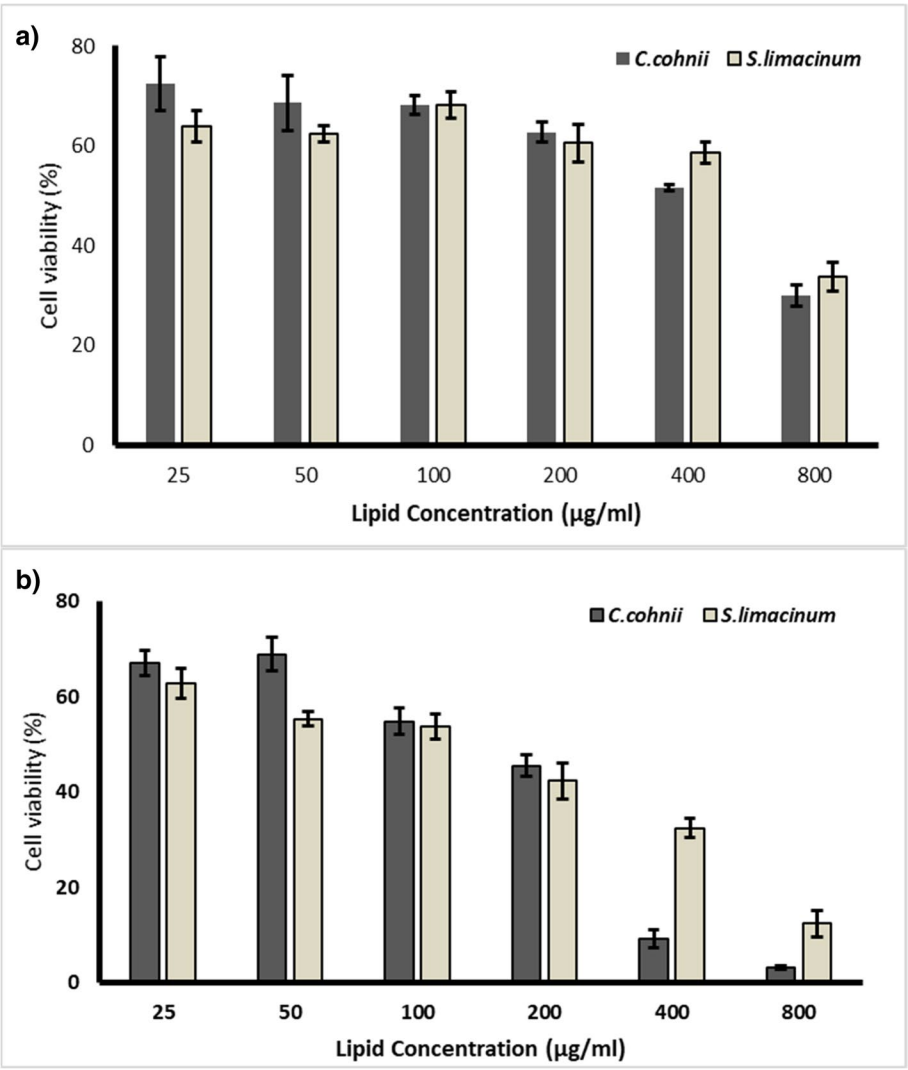

Fig. 2 Effect of *C. cohnii* and *S. limacinum* lipids on cell viability (%) to B16-F10 cells, **a** 48 h, **b** 72 h. Each column represents the mean ± SEM of three independent experiments. The x-axis represents the various concentrations of *C. cohnii* and *S. limacinum* lipids

cohnii was rich in oleic acid compared to S. *limacinum* and this could reveal that a part of the cytotoxic effect is based on the synergistic activity of lipid extracts.

Melanin Synthesis and Tyrosinase Enzyme Activity

Tyrosinase enzyme activity results (Fig. 3a) showed that *C. cohnii* lipids could decrease enzyme activity at concentrations over 100 µg ml^{-1}. However, the effect of concentration for *C. cohnii* lipids was statistically insignificant ($p > 0.05$). The tyrosinase activity was

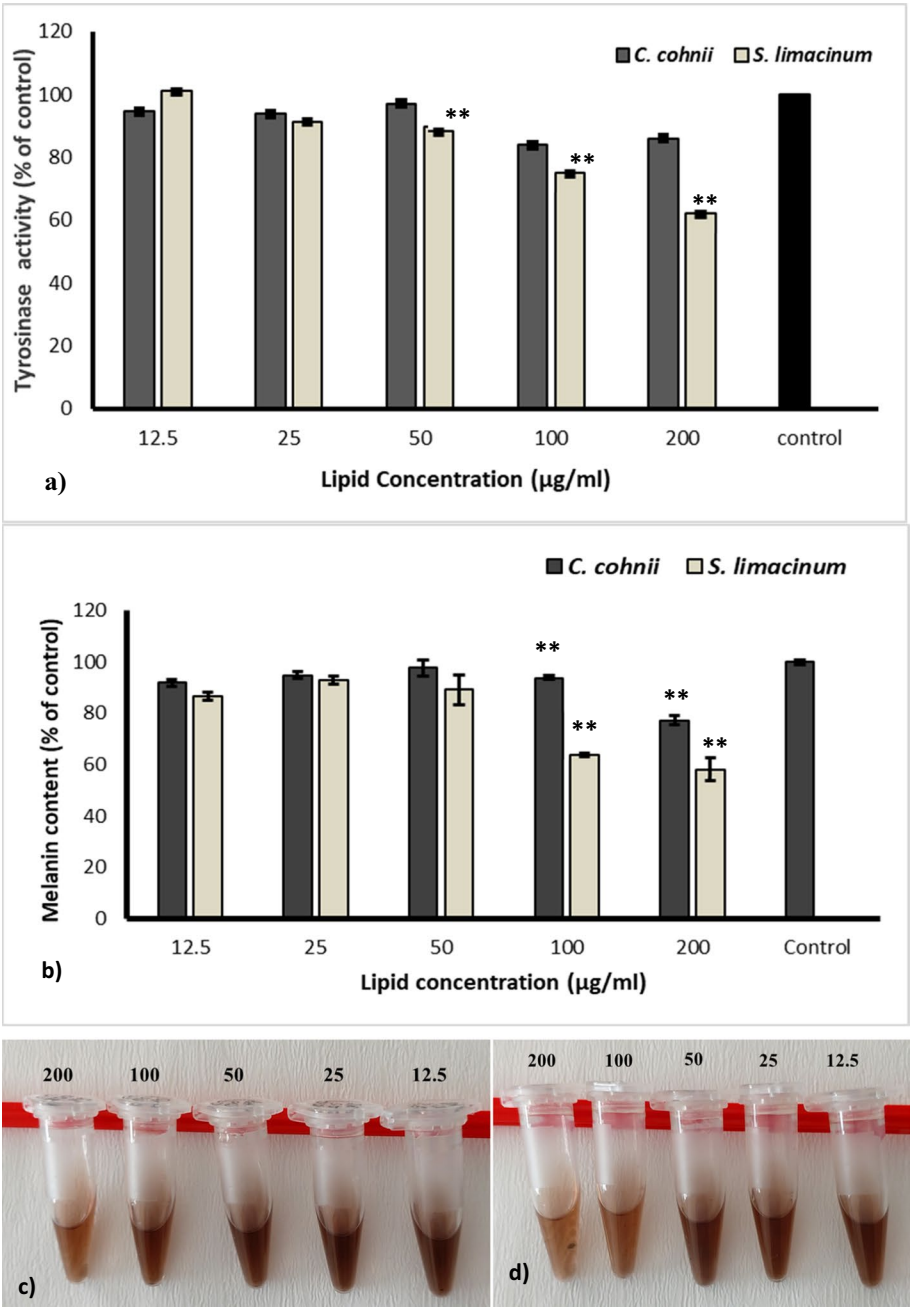

Fig. 3 Tyrosinase activity and melanin content of B16-F10 cells, cells were incubated with 12.5, 25, 50, 100, and 200 µg mL^{-1} *C. cohnii* and *S. limacinum* crude lipid extracts for 72 h. Values represent the mean ± SD of three independent experiments performed in triplicates. $^{**}p < 0.05$. **a** Tyrosinase activity (% of control), **b** melanin content (% of control), **c** dose-dependent melanin concentration with *C. cohnii* lipid extracts, **d** dose-dependent melanin concentration with *S. limacinum* lipids

decreased to 86.14 ± 0.02% at 200 µg ml^{-1} concentration. On the other hand, *S. limacinum* lipids showed a good dose-dependent enzyme inhibition ($p < 0.05$). The activity at 200 µg ml^{-1} was 63.27 ± 0.25%. Almost 40% of enzyme activity inhibition was observed. IC_{50} values were 260 ± 2.38 µg ml^{-1} and 797.92 ± 3.61 µg ml^{-1} for *S. limacinum* and *C. cohnii*, respectively. Kojic acid had 70 ± 2.3% enzyme inhibition at 200 µg ml^{-1} concentration and the IC_{50} value was 123.48 ± 3.84 µg ml^{-1}.

Along with tyrosinase enzyme activity, melanin synthesis was also checked to see the extract's effects on melanin synthesis (Fig. 3b). Even though *C. cohnii* extracts did not significantly down-regulate enzyme activity, and a dose-dependent melanin inhibition was observed ($p < 0.05$), *S. limacinum* showed a higher potential to decrease melanin synthesis. At 200 µg ml^{-1} concentration, *C. cohnii* had 77.93 ± 1.85% but *S. limacinum* had 58.53 ± 4.64% compared to the control sample. IC_{50} values were 222.34 ± 1.38 µg ml^{-1} and 477.48 ± 2.69 µg ml^{-1} for *S. limacinum* and *C. cohnii*, respectively. As is observed from IC_{50} values, *S. limacinum* was 2 times more effective to decrease melanin synthesis. As for tyrosinase activity, it was 3 times more potential to inhibit the enzyme's catalytic reactions.

After 72 h of treatment, the microscopic images were taken from each sample (Fig. 4). A visible decrease in melanin deposits was monitored with the increasing concentration of each sample. The control sample (Fig. 4f) had higher numbers of melanin deposits. Especially at 200 µg ml^{-1}, *S. limacinum* had fewer deposits compared to *C. cohnii* as melanin content results also corresponded to similar results (Fig. 3c, d). As a result, *S. limacinum* had a better relation to decreasing tyrosinase enzyme activity and melanin synthesis.

Contrary to the results obtained from mushroom tyrosinase activity, lipid extracts showed an inhibitory effect on B16-F10 tyrosinase enzyme activity and melanin production. Results from both enzymes concluded differently which could be attributed to the protein structure differences between mammalian and mushroom tyrosinase. In the case of DHA, this fatty acid did not significantly affect mushroom tyrosinase activity however decrease mammalian tyrosinase enzyme activity based on cellular signaling metabolism [21]. Mushroom tyrosinase-based experiments are a good screening tool for the primary introduction of possible tyrosinase enzyme inhibitory candidates in cell-free systems. However, when candidates are treated with B16-F10 melanoma cells, various signaling pathways, transcriptional, and post-transcriptional controls could be observed to down-regulate or up-regulate tyrosinase enzyme activity [2].

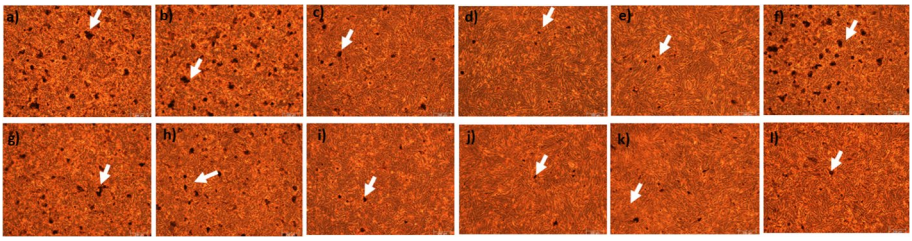

Fig. 4 The microscopic images of B16-F10 cells under various concentrations of *C. cohnii* and *S. limacinum* lipids. The scale bars represent 200 µm, the magnification of the microscope is 10×(microscope code marka), and the white arrows represent the melanin deposits in melanıma cells. **a** *C. cohnii* 12.5 µg ml^{-1}, **b** *C. cohnii* 25 µg ml^{-1}, **c** *C. cohnii* 50 µg ml^{-1}, **d** *C. cohnii* 100 µg ml^{-1}, **e** *C. cohnii* 200 µg ml^{-1}, **f** control (without any sample), **g** *S. limacinum* 12.5 µg ml^{-1}, **h** *S. limacinum* 25 µg ml^{-1}, **i** S. *limacinum* 50 µg ml^{-1}, **j** *S. limacinum* 100 µg ml^{-1}, **k** *S. limacinum* 200 µg ml^{-1}, **l** Kojic acid 200 µg $ml.^{-1}$

S. limacinum extracts were 3 times more efficient for tyrosinase inhibition and 2 times more effective to decrease melanin deposition. These results could be attributed to higher DHA levels and lower saturated fatty acid content of *S. limacinum* (DHA was found 2 times higher). Even though the overall contribution to linoleic acid was low (3%) in *S. limacinum*, there was only a trace amount (0.18%) of it in *C. cohnii*. Linoleic acid is a well-documented agent to reverse hyperpigmentation and it shows inhibitory activity through proteasomal degradation. Palmitic acid was dominant in *S. limacinum* (53.86%) and 2.4 times higher than *C. cohnii*. However, the results displayed that even though 1:3 of total lipids were unsaturated, probably synergistic effect of linoleic acid and DHA was dominant. *S. limacinum* IC_{50} values were almost 2 times lower than kojic acid but the results were promising to develop algae-based tyrosinase inhibitor cosmetics for skin whitening purposes.

Lotus flower oils rich in saturated fatty acids, especially methyl esters of palmitic acid, showed increased melanogenesis in human melanoma cells [32]. However, due to the relatively high concentration of palmitic acid and related methyl esters, the essential oil extracts significantly increased melanogenesis via microphthalmia-associated transcription factor-M and tyrosinase-related protein-2. Besides, these fatty acids increased the phosphorylation of ERK and cAMP response element-binding protein. Another study using Amazonian forest flora plant oils found that the degree of melanogenesis is highly affected by the fatty acid composition [38]. Acai plant fatty acids had a significant effect on mushroom tyrosinase inhibition. The composition analysis revealed a high amount of (> 50%) oleic acid followed by palmitic acid and linoleic acid. Another study revealed *Camellia oleifera* seed oil rich in oleic acid resulted in the efficient decrease of melanin production in the B16 melanoma cell line [33]. *Schinus terebinthifolius Raddi* extract and linoleic acid from *Passiflora edulis* synergistically decreased melanin formation in B16 cells. *Schinus terebinthifolius Raddi* extract was able to down-regulate tyrosinase activity but adding linoleic acid increased the turnover rate of melanin production [39]. As it was

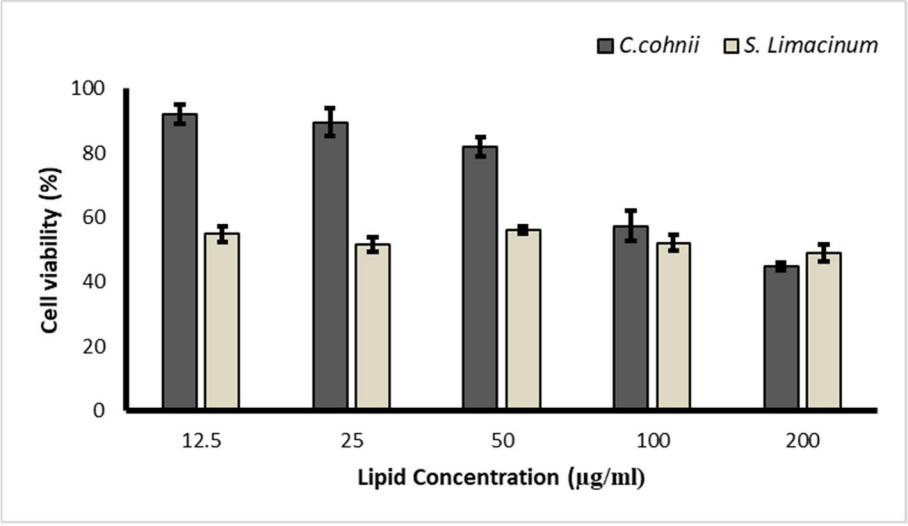

Fig. 5 Effect of *C. cohnii* and *S. limacinum* lipids on cell viability (%) to HaCaT cells after 72 h. Each column represents the mean ± SEM of three independent experiments. x-axis represents the various concentrations of *C. cohnii* and *S. limacinum* lipids

displayed by various oil-rich plants and seeds, fatty acid concentration and distribution critically affect melanin production and tyrosinase enzyme activity.

HaCaT Cell Viability with Exposure to Microalgal Lipids

Due to the high toxicity of microalgal extracts on B16-F10 mouse melanoma cells, normal healthy keratinocyte cells (HaCaT) were used to evaluate the toxicity of lipid extracts. In skin layers, melanocyte cells are highly communicating with keratinocyte cells. Melanocytes are surrounded by keratinocytes and it is important to see the effect of a potential skin whitening compound on healthy skin cells. As it can be seen from Fig. 5, the lower concentration of *S. limacinum* extracts (12.5–50 μg ml^{-1}) was not toxic to HaCaT cells. On the other hand, *C. cohnii* showed a dramatic decrease in cell viability from 90.17 ± 2.21 to $58.92 \pm 1.78\%$ when the concentration is doubled from 12.5 to 25 μg ml^{-1}. Cell viability at 200 μg ml^{-1} concentration was 60.73 ± 1.80 and $28.07 \pm 1.34\%$ for *S. limacinum* and *C. cohnii*, respectively. *S. limacinum*-treated HaCaT cells were almost 2.5 times more viable than *C. cohnii*-treated samples ($p < 0.05$). IC_{50} values were 253.67 ± 0.95 μg ml^{-1} and 81.28 ± 1.47 μg ml^{-1} for *S. limacinum* and *C. cohnii*. These results indicate that for melanoma and healthy keratinocytes, *C. cohnii* showed a higher cytotoxic effect than *S. limacinum*.

The toxic effect of candidate compounds on healthy skin cells is important. It is known that fatty acids show a toxic effect on cancer cell lines with various pathways including autophagy induction; however, toxicity to healthy skin cells is not desired from a possible cosmeceutical active ingredient. In this study, lower doses of microalgal lipid extracts showed no significant toxicity in *S. limacinum*-treated HaCaT cells; however, the toxic effect of *C. cohnii* was visible even though in low concentrations (25 μg ml^{-1}).

Microalgae possess a great potential to replace vegetable oils for cosmeceutical purposes. For years, the food vs. fuel debate was argued through the scientific evidence to whether to use plant-based oils for biofuel purposes. During that time, microalgal fatty acids were introduced as a novel ingredient for fuel applications to decrease the tension on using vegetable and arable lands for fuel purposes. Since that, microalgal fatty acids contribute to various industries for animal and aquaculture feed, nutraceutical extracts, omega-3 fatty acid supplements, baby food formulations, and so on [40]. The volumetric production capacity and composition of fatty acids are competing with plant-based resources; thus, microalgal fatty acids are considered a sustainable and efficient replacement for plant oils as the means of value-added molecules. Fatty acids are one of the essential components of the skin layer and are highly used in the formulation of topical cosmetic applications such as lotion, hair conditioners, shampoos, lipsticks, moisturizing creams, and cleaning products in the form of bars, sticks, or liquid [33, 41]. Also, fatty acids are formulated to help recover from dermal conditions primarily psoriasis and eczema. The interaction of fatty acids for hyper- or hypopigmentation disorders gains new aspects for the formulation of efficient skin tone correcting products with possibly high transdermal ability. The formulations based on microalgal lipids could give a new approach to tyrosinase inhibitory and activator functional cosmetics approach. Microalgal fatty acids could be formulated either as the only active ingredient for skin pigmentation regulators or added to the formulations to affect synergistically. Another aspect could be the utilization of algal fatty acids for liposome formulations of certain candidate skin whitening compounds to increase the stability and use, (i) skin whitening efficiency and (ii) increasing dermal transportation ability of the bioactive molecule [42].

Conclusion

Pigmentation disorders are common problems affecting individuals psychologically and physiologically. There is a high motivation to discover novel, sustainable tyrosinase inhibitory compounds from natural resources with less toxicity and high efficiency. Fatty acids are catching attention as tyrosinase enzyme activity enhancers of inhibitors based on the saturation level. In this study, for the first time, oil-rich heterotrophic microalgae species, *C. cohnii* and *S. limacinum*, was utilized for cell-free and cellular tyrosinase enzyme activity determination. The results showed that microalgal lipids rich in PUFAs and saturated fatty acids increase mushroom tyrosinase activity. Since previous reports mention that PUFAs do not affect mushroom tyrosinase activity, the increase can be attributed to saturated lipids. However, the B16-F10 murine melanoma cell tyrosinase enzyme activity was inhibited. The inhibitory effect of *S. limacinum* was 2–3 times more efficient compared to *C. cohnii* with less toxicity effect. This study is promising for novel skin whitening cosmeceutical formulation from microalgal fatty acids as a sustainable replacement for vegetable oils.

Acknowledgements This research study was done at Ege University Bioengineering Department, Microalgal Bioprocess lab. The author would like to thank Assoc. Prof. Dr. Suphi S. Oncel and Prof. Dr. Murat Elibol for their valuable contribution.

Author Contribution Ayse Kose (AK) designed the experiments, conducted the analysis, and wrote the manuscript.

Data Availability Not applicable.

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Chang, T. S. (2012). Natural melanogenesis inhibitors acting through the down-regulation of tyrosinase activity. *Materials, 5*(9), 1661–1685. https://doi.org/10.3390/ma5091661
2. 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), 1–18. https://doi.org/10.3390/ijms17071144
3. Heo, S. J., Ko, S. C., Cha, S. H., Kang, D. H., Park, H. S., Choi, Y. U., & Jeon, Y. J. (2009). Effect of phlorotannins isolated from Ecklonia cava on melanogenesis and their protective effect against photooxidative stress induced by UV-B radiation. *Toxicology in Vitro*. https://doi.org/10.1016/j.tiv.2009.05.013
4. Pillaiyar, T., Namasivayam, V., Manickam, M., & Jung, S. H. (2018). Inhibitors of melanogenesis: An updated review. *Journal of Medicinal Chemistry, 61*(17), 7395–7418. https://doi.org/10.1021/acs.jmedchem.7b00967
5. Oh, T. I., Yun, J. M., Park, E. J., Kim, Y. S., Lee, Y. M., & Lim, J. H. (2017). Plumbagin suppresses α-MSH-induced melanogenesis in B16F10 mouse melanoma cells by inhibiting tyrosinase activity. *International Journal of Molecular Sciences*, *18*(2). https://doi.org/10.3390/ijms18020320

6. Chan, C. F., Huang, C. C., Lee, M. Y., & Lin, Y. S. (2014). Fermented broth in tyrosinase- and melanogenesis inhibition. *Molecules, 19*(9), 13122–13135. https://doi.org/10.3390/molecules190913122
7. Kubglomsong, S., Theerakulkait, C., Reed, R. L., Yang, L., Maier, C. S., & Stevens, J. F. (2018). Isolation and identification of tyrosinase-inhibitory and copper-chelating peptides from hydrolyzed rice-bran-derived albumin. *Journal of Agricultural and Food Chemistry*, *66*(31), 8346–8354. research-article. https://doi.org/10.1021/acs.jafc.8b01849
8. Wang, Z. J., Si, Y. X., Oh, S., Yang, J. M., Yin, S. J., Park, Y. D., & Qian, G. Y. (2012). The effect of fucoidan on tyrosinase: Computational molecular dynamics integrating inhibition kinetics. *Journal of Biomolecular Structure and Dynamics, 30*(4), 460–473. https://doi.org/10.1080/07391102.2012.682211
9. Ephrem, E., Elaissari, H., & Greige-Gerges, H. (2017). Improvement of skin whitening agents efficiency through encapsulation: Current state of knowledge. *International Journal of Pharmaceutics, 526*(1–2), 50–68. https://doi.org/10.1016/j.ijpharm.2017.04.020
10. Kose, A., & Oncel, S. S. (2015). Properties of microalgal enzymatic protein hydrolysates: Biochemical composition, protein distribution and FTIR characteristics. *Biotechnology Reports, 6*, 137–143. https://doi.org/10.1016/J.BTRE.2015.02.005
11. Garc ıa, J. L., de Vicente, M., & Gal an, B. (n.d.). Microalgae, old sustainable food and fashion nutraceuticals. https://doi.org/10.1111/1751-7915.12800
12. Nishigaki, I., Peramaiyan, R., Ramachandran, V., Gnapathy, E., Dhanapal, S., & Yutaka, N. (2010). Cytoprotective role of astaxanthin against glycated protein/iron chelate-induced toxicity in human umbilical vein endothelial cells. *Phytotherapy Research, 24*(June), 54–59. https://doi.org/10.1002/ptr
13. Jain, S., Prajapat, G., Abrar, M., Ledwani, L., Singh, A., & Agrawal, A. (2017). Cyanobacteria as efficient producers of mycosporine-like amino acids. *Journal of Basic Microbiology*. https://doi.org/10.1002/jobm.201700044
14. Pratoomthai, B., Songtavisin, T., Gangnonngiw, W., & Wongprasert, K. (n.d.). In vitro inhibitory effect of sulfated galactans isolated from red alga Gracilaria fisheri on melanogenesis in B16F10 melanoma cells. https://doi.org/10.1007/s10811-018-1469-3
15. Rao, A. R., Sindhuja, H. N., Dharmesh, S. M., Sankar, K. U., Sarada, R., & Ravishankar, G. A. (2013). Effective inhibition of skin cancer, tyrosinase, and antioxidative properties by astaxanthin and astaxanthin esters from the green alga haematococcus pluvialis. *Journal of Agricultural and Food Chemistry, 61*(16), 3842–3851. https://doi.org/10.1021/jf304609j
16. Custódio, L., Soares, F., Pereira, H., Rodrigues, M. J., Barreira, L., Rauter, A. P., & Varela, J. (2015). Botryococcus braunii and Nannochloropsis oculata extracts inhibit cholinesterases and protect human dopaminergic SH-SY5Y cells from H2O2-induced cytotoxicity. *Journal of Applied Phycology, 27*(2), 839–848. https://doi.org/10.1007/s10811-014-0369-4
17. Levy-Ontman, O., Huleihel, M., Hamias, R., Wolak, T., & Paran, E. (2017). An anti-inflammatory effect of red microalga polysaccharides in coronary artery endothelial cells. *Atherosclerosis, 264*, 11–18. https://doi.org/10.1016/j.atherosclerosis.2017.07.017
18. Isleten-Hosoglu, M., & Elibol, M. (2017). Improvement of medium composition and cultivation conditions for growth and lipid production by Crypthecodinium cohnii. *Romanian Biotechnological Letters, 22*(6), 13086–13095.
19. Sahin, D., Tas, E., & Altindag, U. H. (2018). Enhancement of docosahexaenoic acid (DHA) production from Schizochytrium sp. S31 using different growth medium conditions. *AMB Express*, *8*(1). https://doi.org/10.1186/s13568-018-0540-4
20. Ando, H., Watabe, H., Valencia, J. C., Yasumoto, K., Furumura, M., Funasaka, Y., & Hearing, V. J. (2004). Fatty acids regulate pigmentation via proteasomal degradation of tyrosinase. *Journal of Biological Chemistry, 279*(15), 15427–15433. https://doi.org/10.1074/jbc.m313701200
21. Balcos, M. C., Kim, S. Y., Jeong, H., Yun, H., Baek, K. J., Kwon, N. S., & Kim, D. (2014). Docosahexaenoic acid inhibits melanin synthesis in murine melanoma cells in vitro through increasing tyrosinase degradation. *Nature Publishing Group, 1*, 489–495. https://doi.org/10.1038/aps.2013.174
22. Cui, H. X., Duan, F. F., Jia, S. S., Cheng, F. R., & Yuan, K. (2018). Antioxidant and tyrosinase inhibitory activities of seed oils from Torreya grandis Fort. ex Lindl. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/5314320
23. Yildiztekin, F., Nadeem, S., Erol, E., Yildiztekin, M., Tuna, A. L., & Ozturk, M. (2016). Antioxidant, anticholinesterase and tyrosinase inhibition activities, and fatty acids of Crocus mathewii – A forgotten endemic angiosperm of Turkey. *Pharmaceutical Biology, 54*(9), 1557–1563. https://doi.org/10.3109/13880209.2015.1107746
24. Isleten-Hosoglu, M., Gultepe, I., & Elibol, M. (2012). Optimization of carbon and nitrogen sources for biomass and lipid production by Chlorella saccharophila under heterotrophic conditions and development of Nile red fluorescence based method for quantification of its neutral lipid content. *Biochemical Engineering Journal, 61*, 11–19. https://doi.org/10.1016/J.BEJ.2011.12.001

25. Chan, Y. Y., Kim, K. H., & Cheah, S. H. (2011). Inhibitory effects of Sargassum polycystum on tyrosinase activity and melanin formation in B16F10 murine melanoma cells. *Journal of Ethnopharmacology*. https://doi.org/10.1016/j.jep.2011.07.050
26. Sun, L., Guo, Y., Zhang, Y., & Zhuang, Y. (2017). Antioxidant and anti-tyrosinase activities of phenolic extracts from rape bee pollen and inhibitory melanogenesis by cAMP/MITF/TYR pathway in B16 mouse melanoma cells. *Frontiers in Pharmacology*, *8*(MAR), 1–9. https://doi.org/10.3389/fphar.2017.00104
27. Fistarol, S. K., & Itin, P. H. (2010). Disorders of pigmentation, *2010*(Band 8), 187–202. https://doi.org/10.1111/j.1610-0387.2009.07137.x
28. Chang, T. S. (2009). An updated review of tyrosinase inhibitors. *International Journal of Molecular Sciences, 10*(6), 2440–2475. https://doi.org/10.3390/ijms10062440
29. Wang, L. X., Qian, J., Zhao, L. N., & Zhao, S. H. (2018). Effects of volatile oil from ginger on the murine B16 melanoma cells and its mechanism. *Food and Function, 9*(2), 1058–1069. https://doi.org/10.1039/c7fo01127j
30. Tel, G., Öztürk, M., Duru, M. E., Doğan, B., & Harmandar, M. (2013). Fatty acid composition, antioxidant, anticholinesterase and tyrosinase inhibitory activities of four Serratula species from anatolia. *Records of Natural Products, 7*(2), 86–95.
31. Shabani, F., & Sariri, R. (2010). Increase of melanogenesis in the presence of fatty acids. *Pharmacologyonline, 1*, 314–323.
32. Jeon, S., Kim, N. H., Koo, B. S., Kim, J. Y., & Lee, A. Y. (2009). Lotus (Nelumbo nuficera) flower essential oil increased melanogenesis in normal human melanocytes. *Experimental and Molecular Medicine, 41*(7), 517–524. https://doi.org/10.3858/emm.2009.41.7.057
33. Chaikul, P., Sripisut, T., Chanpirom, S., Sathirachawan, K., & Ditthawuthikul, N. (2017). Melanogenesis inhibitory and antioxidant effects of Camellia oleifera seed oil. *Advanced Pharmaceutical Bulletin*, *7*(3), 473–477. https://doi.org/10.15171/apb.2017.057
34. Matsuura, R., Ukeda, H., & Sawamura, M. (2006). Tyrosinase inhibitory activity of citrus essential oils. *Journal of Agricultural and Food Chemistry, 54*(6), 2309–2313. https://doi.org/10.1021/jf051682i
35. Zajdel, A., Wilczok, A., Chodurek, E., Gruchlik, A., & Dzierzewicz, Z. (2013). Polyunsaturated fatty acids inhibit melanoma cell growth in vitro. *Acta Poloniae Pharmaceutica - Drug Research, 70*(2), 365–369.
36. Tan, R., Wang, F., Fan, C., & Zhang, X. (2018). Function suppresses B16F10 melanoma lung metastasis by autophagy induction. 6179–6186. https://doi.org/10.1039/c8fo01617h
37. Mericli, F., Becer, E., Kabadayi, H., Hanoglu, A., Hanoglu, D. Y., Yavuz, D. O., & Vatansever, S. (2017). Fatty acid composition and anticancer activity in colon carcinoma cell lines of Prunus dulcis seed oil. *Pharmaceutical Biology, 55*(1), 1239–1248. https://doi.org/10.1080/13880209.2017.1296003
38. Schauss, A. G. (2016). Advances in the study of the health benefits and mechanisms of action of the pulp and seed of the Amazonian palm fruit, Euterpe oleracea Mart., known as "Açai." *Fruits, Vegetables, and Herbs: Bioactive Foods in Health Promotion, 48*, 179–220. https://doi.org/10.1016/B978-0-12-802972-5.00010-X
39. Santos, A., Andres, E., Medina, S. P. H., Ferrari, C. R., & Lourenc, C. B. (2012). Schinus terebinthifolius Raddi extract and linoleic acid from Passiflora edulis synergistically decrease melanin synthesis in B16 cells and reconstituted epidermis. 435–440. https://doi.org/10.1111/j.1468-2494.2012.00736.x
40. Kose, A., & Oncel, S. S. (2017). Algae as a promising resource for biofuel industry: Facts and challenges. *International Journal of Energy Research, 41*(7), 924–951. https://doi.org/10.1002/er.3699
41. Borowitzka, M. A. (2013). High-value products from microalgae-their development and commercialisation. *Journal of Applied Phycology, 25*(3), 743–756. https://doi.org/10.1007/s10811-013-9983-9
42. Shigeta, Y., Imanaka, H., Ando, H., Ryu, A., Oku, N., Baba, N., & Makino, T. (2004). Skin whitening effect of linoleic acid is enhanced by liposomal formulations. *Biological and Pharmaceutical Bulletin, 27*(4), 591–594. https://doi.org/10.1248/bpb.27.591

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.