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The Protective Effects of Marine Microalgae Extract on Skin Cells-inducted Sun Ray

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ABSTRACT

Introduction: In the present study, two kinds of marine microalgae, *Tisochrysis lutea* (Coccolithophyceae) is a single microalga widely used as live food in aquaculture. *Tetraspora gelatinosa* (Tetrasporaceae) is spherical to elliptical cell, arranged two-by-two or four-by-four, with 2 pseudoflagella extending beyond the mucilage. They are able to produce high value chemicals which can be applied in feed and food, pharmaceutical, nutraceutical and cosmeceutical industries.

Objective: To determine effects of *T.lutea* and *T. gelatinosa* extracts on skin cells functions.

Methods: Dried microalgal biomass of microalgae were extract with ethanol. The biological activity of microalgae extracts was investigated on skin cells such as keratinocyte cells (HaCaT) and melanoma cells (B16F10). The protective effect against photo-inducted inflammation, oxidative stress and melanogenesis were investigated. Cytotoxicity of microalgae extracts were determined using WST-assay. Sun ray was treatment to HaCaT cells for inflammation and oxidative stress effect. and B16F10 cells was treated sun ray to inducing and melanin production.

Results: The content of the biochemical components in two marine microalgae showed total carbohydrate and protein content were the main component of *T. gelatinosa*. The effect of *T. lutea* and *T. gelatinosa* were exhibited to suppressing cytokine secretion, melanin content, and ROS production on sun ray-induced skin cells. Moreover, *T. gelatinosa* was showed significant protective effect on ROS scavenging.

Conclusion: *T. lutea* and *T. gelatinosa* are marine microalgae that have benefit on skin cells to protect sun ray induce cell damage and skin pigmentation. The protection activity on keratinocyte cells including anti-inflammation via cytokine PGE_2 suppressing and anti-oxidative by ROS scavenging from sun-ray stimulation. Therefore, both of marine microalgae may have the potential to use as an active ingredient in cosmetic or cosmeceutical products.

Keywords: *marine microalgae; sun-ray; anti-inflammation; ROS scavenging; melanin content*

Introduction

Currently, marine microalgae have been attracting attention as resources for new metabolites and biotechnologies. They are predominately aimed at applications with high added value given that algal biomass is composed of proteins, polysaccharides, essential fatty acids, pigments, vitamins, and minerals, all of which are of considerable interest in the preparation of natural products or bioactive compounds [1]. They can defend its cellular components by the counter production of primary metabolites such as chlorophyll, phycocyanin, palmitic acids, oleic acids, vitamin and etc [2]. Secondary metabolites are also generated under stress environment condition and accordingly produce various high-value metabolites such as antibiotic and antimicrobial agents, for example, working against inflammation and tumor, or with anti-viral and immunomodulating actions [3,4]. There is a

growing worldwide interest toward finding new, safe and powerful bioactive compounds from microalgae. Those metabolite productions can be applied in food, pharmaceutical, nutraceutical, cosmeceutical which can be applied to repair damaged skin, prevent blemishes, and inhibit the inflammatory process. Additionally, the various bioactive agents of microalgal extract could help to accelerate the healing process and maintain skin moisture [5,6].

The diverse pharmacological activities can be found in different species of microalgae, a specific marine microalgae species can be used as cosmetic applications from many different bioactive compounds. For instance, *Chlorella* is a good candidate for polysaccharide to help moisturize and thickener agent [7] and for chlorophyll to mask odors in dentifrices and deodorants [8]. *Dunaliella salina* can be used to produce carotenoids to support antiglycation and anti-inflammatory [9]. *Spirulina* can accumulate a large amount of phycocyanobilin and phycoerythrobilin to use as antioxidant for sunscreen formulation on health of the dermis and the skin elasticity, reduction of skin hyperpigmentation, protection against photoaging and inhibition of reactive oxygen species: ROS-induced damage to the dermis [10,11]. *Tisochrysis lutea* (also named as *Isochrysis galbana* T-Iso) [12] is widely used in aquaculture due to its high content in polyunsaturated fatty acids, particularly in docosahexaenoic acid (DHA). Additionally, this microalga is applied in cosmetics, particularly in skin photoaging protection and antiaging because of its high fucoxanthin content [13,14].

The sun ray comprises a various range of electromagnetic radiation [15], including ultraviolet (UV, approximate range of wavelength from 180 to 380 nm), visible (Vis, approximately from 380 to 800 nm), and infrared light (range 1–3 μm approximately). The sun's ultraviolet light can cause major damage to the skin cells. Too much exposure to UVB rays can lead to sunburn. UVA rays can travel more deeply into the skin than UVB rays, but both can affect your skin's health. UV light can lead to pathological UV-induced ROS production with affecting the enzyme catalase and up-regulating nitric oxide synthase (NOS) synthesis. It may also cause a decrease in protein kinase C (PKC) expression leading to increased ROS production [16]. Exposure the UV radiation triggers the release of prostaglandins (PGs), which is produced abundantly by keratinocytes in UV-exposed skin. This is the major and most effective metabolite generated by COX-2 activity and is considered to be a potent mediator of inflammatory responses. These data suggest that PGE2 plays a key role in UV radiation–induced immunosuppression [17]. UV radiation exposure is the stimulant for melanin synthesis. Enzyme tyrosinase is the key factor in melanogenesis, which catalyses tyrosine to L-DOPA and oxidation of this o-diphenol to dopaquinone. The oxidation of DOPA-quinone by cyclisation produces cyclo-DOPA and DOPA-chrome. Dopachrome continues the route for the formation of dark/brown eumelanin [18]. In this study, we investigated the effect of marine microalgae extracts from two marine microalgae on valuable application of cosmetics. The effects of *T.lutea* and *T. gelatinosa* extracts were determined on skin cell-induced sun ray. The anti-inflammatory and anti-oxidative effects were observed on keratinocyte (HaCaT) cells. While the anti-melanin production was determined on melanoma (B16F10) cells

Methodology

Microorganism

Two marine microalgae were obtained from the algal excellent center of Thailand institute of scientific and technological research (TISTR). *Tisochrysis lutea* TISTR 11470 (Haptophyta, Coccolithophyceae) and *Tetraspora gelatinosa* TISTR 11440 was collected from Mu Ko Chumphon National Park, Chumphon province and *Tetraspora gelatinosa* TISTR 11440 (Chlorophyta, Tetrasporaceae) was obtained from Cha-Am beach, Phetchaburi province. Those were located in the Gulf of Thailand.

Algal extraction

At the 10th day of cultivation, algal extraction was performed according to Maadane et al. [6] with some modifications. Dried microalgal biomass was ground well using a sterile mortar and pestle. 50 g of the dry cell was mixed with 100 ml of ethanol and placed at room temperature for 2 hours under dark. Cells were disrupted by a mechanical homogenizer (Daihan HG-15A, Korea) at a speed of 8,000 rpm for 10 minutes in an ice-bath at the controlled temperature of -5°C to avoid overheating. Samples were centrifuged at 6,000 rpm for 5 minutes and then the pellet was repeated extract. The pooled supernatant was evaporated to dryness at 40°C and the residue was kept at -20°C before analysis. The extracts were analysis chemical component: protein [19], carbohydrate [20] flavonoid [21], and phenolic content [6]. The bioactivity assay was analysis on skin cells including keratinocyte and melanocyte cell line.

Cell culture

Human keratinocyte cell line (HaCaT; CLS cell lines, Germany) and mouse skin melanoma cell (B16F10; ATCC® CRL-6475) were grown and maintained in Dulbecco's modified eagle medium (DMEM) (GIBCO, USA) containing 10% (v/v) fetal bovine serum (GIBCO, USA) and 1% of Antibiotic/ Antimycotic Solution (GIBCO, USA) in humidified atmosphere incubator with 5% CO_2 at 37°C. Cells were seed in 96 well plate at density of $5x10^4$ cells/well for 24 hours and incubated with various concentration of algae extract. Cells were treated with sun ray (UVA+B+IR) for 22 second before bioactivity analysis.

Cytotoxicity assay

Cytotoxicity was determined using WST-1 assay (Bio Vision, Milpitas, CA, USA). Cells were treated with various concentrations of extracts for 24 hours. Then, 100 µl of WST-1 solution was added and incubated for 30 minutes before measuring the absorbance at 450 nm.

Evaluation of photo-induced cytokine production

After sun ray-inducted and 48 hours post-incubation, the supernatant of HaCaT cells were collected for cytokine assay. Prostaglandin E2 (PGE_2) were quantified via the enzyme-linked immunosorbent assay (ELISA) kit (Cayman, USA) according to the manufacturer's instruction and modified from Prasad et al [22].

Evaluation of photo-inducted ROS production

The ROS scavenging activity was performed according to Masaki et al. [23] with modifications. HaCaT cells were induced oxidative stress with sun ray and post-incubate for 5 minutes and added the ROS fluorescent dye solution (ROS detection kit, Abcam, USA). Cells were incubated in a 5% CO_2 incubator at 37°C for 45 minutes. Cellular ROS production were quantified the intensity of fluorescent using In Cell Analyzer (InCell Pro2000, GE Healthcare, UK).

Evaluation of photo-inducted melanin production

B16F10 cells were stimulated by sun ray and post-incubation for 48 hours. Cells were washed by PBS before added 100 µl of 2 M NaOH solution. Cell was incubated in a 5% CO_2 incubator at 60°C for 2 hours. The absorbance was measured at 405 nm following to Zhou et al. [24] with modification.

Results

Chemical compounds

The biochemical contents in two marine microalgae (figure 1) are shown in table 1. Total carbohydrate and protein contents were the main component of *T. gelatinosa* approximately 217.45±3.34 and 145.90±1.75 mg/g respectively. On the contrary, phenolic and flavonoid levels were higher in *T. lutea* compared with *T. gelatinosa*.

Table 1 Components of marine microalgae.

Components	*T. lutea*	*T. gelatinosa*
Total protein (mg/g)	8.99±0.63	145.90±1.75
Total carbohydrate (mg/g)	12.84±0.14	217.45±3.34
Phenolic (mg GAE/g)	5.36±0.39	0.09±0.02
Flavonoid (mg CE/g)	13.73±0.30	4.10±0.64

Results are expressed as mean ± SD (n = 3). Gallic acid equivalent (GAE) and catechin equivalent (CE).

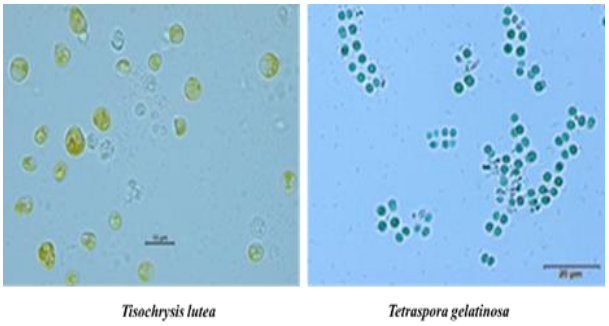

Figure 1 Morphology of marine microalgae.

Cytotoxicity on skin cells

Cytotoxicity of *T. lutea* and *T. gelatinosa* was measured cell viability using WST-1 assay. *T. lutea* and *T. gelatinosa* extracts did not show cytotoxicity to skin cells up to concentration of 0.50 mg/mL (data not shown).

Inhibitory effect on photo-induced cytokine production

The inhibitory effect of algae extract on cytokine (PGE_2) production was measured using ELIZA assay in sun ray-stimulated HaCaT cells. Sun ray-induced cells was increasing PGE_2 level more over than control cell. The protective effect of *T. lutea* and *T. gelatinosa* extracts at concentration of 0.125-0.5 mg/mL were shown to decreasing PGE_2 level from sun ray induction more than 30%. The inhibition ratio of *T. lutea* and *T. gelatinosa* on cell-induced inflammation was not significant difference (figure 2).

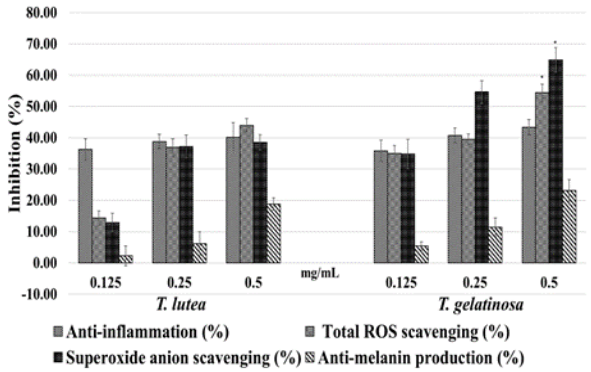

Figure 2 Effects of algae extracts on photo-induced skin cells. Values are expressed as mean±SD. Statistical significance was evaluated using the turkey test. $p < 0.05$ compared between group.

Inhibitory effect on photo-induced cellular ROS production

To determine the effect of algae extracts on anti-oxidative in HaCat cells was performed with cellular ROS scavenging activity. Total ROS (green) and superoxide anion (red) was detected with fluorescence intensity. HaCat cells induced with sun ray showed increasing of total ROS and superoxide anion

production (Figure 3). The result showed that *T. lutea* and *T. gelatinosa* were suppressing the intensity of ROS production after sun ray induction. In additional, radical scavenging effect of *T. gelatinosa* extracts at 0.5 mg/mL was significantly greater than *T. lutea* (figure 2).

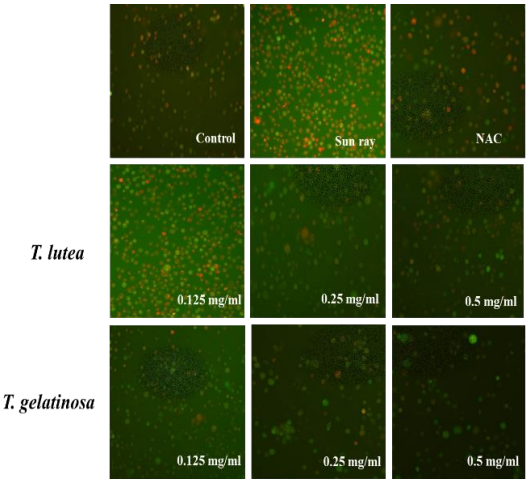

Figure 3 Effects of microalgae on sun ray induced oxidative stress in keratinocyte (HaCaT) cells, stained with immunofluorescence. Fluorescence images were merged, in the presence of total ROS (green) and superoxide anion (red).

Inhibitory effect on photo-inducted melanin production

To investigate the anti-melanogenic effect of algae extract, cell was treated with sun ray and analyzed for melanin content. The result examined *T. lutea* and *T. gelatinosa* were decreasing melanin content after stimulated with sun ray in dose dependent manner as show in figure 4. The result showed that the inhibition effect on B16F10 cells melanin secretion of *T. lutea* slightly higher than *T. gelatinosa* extracts but not significant difference (figure 2).

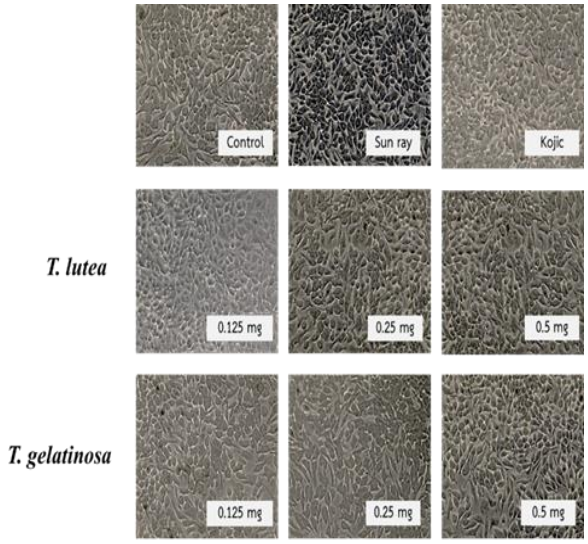

Figure 4 Effects of microalgae on sun ray induced melanin production in melanoma (B16F10) cells.

Discussion

The beneficial effects of marine algal extracts showed the various biological activities against skin disorders including wrinkles, skin inflammation, hyperpigmentation [25,26,27] The content of the main biochemical components in microalgal cells varies depending on the investigated microalga species [28,29]. This study showed that *T. gelatinosa* gave the highest total protein and carbohydrate. This reported also similar the total carbohydrates in the four marine microalgae varied from 54 to 235 mg g^{-1} DW [30] and total protein content of marine microalgae *Chlorella* was 45 percent of total organic matter [31]. The high total carbohydrate of *T. gelatinosa* may relate with polysaccharide or sulphate polysaccharide that was found in marine microalgae. Polysaccharide from most marine microalgae are heteropolymers, constituted mainly of xylose, galactose, and glucose in different proportions [32].

The significant anti-oxidant activity of *T. gelatinosa* which contain highest carbohydrate was supported in numerous reported. Dvir et al. [33] claimed that sulphate polysaccharides released by marine microalgae may not only function as dietary fibre, but have also illustrated the ability to prevent the accumulation and the activity of free radicals and reactive chemical species. According to Park et al. [34] demonstrated that sulphate polysaccharide from green microalgae *Haematococcus lacustris* had anti-inflammatory activity. Moreover, bioactive peptide and proteins from *Chlorella* exhibited anti-inflammatory activity [35,36] and the possibility of the glycoprotein from the red marine microalga *Porphyridium* sp. contributed the antioxidant properties [37]. The polysaccharides isolated from red green and brown

marine macroalgae/seaweed possess antioxidant potential [38]. Sun et al. [39] found that polysaccharides degradation from algae *Pavlova viridis* and *Sarcinochrysis marina* Geitler et al. showed antioxidant activity in DPPH radical and hydroxyl free radical scavenging. Luo et al. [40] found that *Spirulina platensis* polysaccharide has a strong antioxidant activity and could be used as an antioxidant in Sausage to extend its shelf life. However, although diverse biological activities of marine carbohydrates have been determined, their detailed molecular mechanisms and target proteins are not fully understood [41]. The results of *T. lutea* and *T. gelatinosa* were showed inhibition effect on melanin content in B16F10 cells similar with fresh water green algae (*Prasiola japonica*). *P. japonica* ethanol extract (Pj-EE) was suppressed the transcription of genes encoding matrix metalloproteinases (MMPS), which were induced in HaCaT cells by hydrogen peroxide (H_2O_2) treatment and reduced the melanin secretion and content in B16F10 cells [42]. Study from Wu and co-workers found that C-PC from *Spirulina* sp., was inhibits melanin biosynthesis in B16F10 murine melanoma cells [43]. A novel peptide isolated from *Pavlova lutheri* demonstrated inhibitory properties against a-Melanocyte Stimulating Hormone-induced melanogenesis via melanin content, tyrosinase inhibition in B16F10 melanoma cells, and also decreased melanogenesis-related proteins [44]. However, the color and the active ingredients of the algae should be related to the protective effect from photo induction such as the green color, phenolic and flavonoid. The sun protection factor (SPF) should be further determined to clarified the mechanism of action. Moreover, in view of the industrial scale culture of microalgae have the potential to become antioxidants in foods and cosmetics.

Conclusion

T. lutea and *T. gelatinosa* are marine microalgae that have benefit on skin cells to protected sun ray induce cell damage. Melanin content from sun ray induction on melanoma cells was decreasing from protective effect of both marine microalgae. The protection activity on keratinocyte cells including anti-inflammation via cytokine PGE_2 suppressing and anti-oxidative by ROS scavenging from sun-ray stimulation. While, *T. gelatinosa* showed stronger effect of ROS scavenging activity than *T. lutea*. However, both of marine microalgae may have the potential to use as an active ingredient in cosmetic or cosmeceutical products.

Competing Interests

The authors declare that they have no competing interests

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Author contributions

TM participated in research design, discussion of result and final revision. NC prepared the sample extract and also aided in the discussion of result and manuscript drafting. IP contributed to research design, performed the experiment, and analyzed the result.

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