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# Inhibitory Effect of *Sargassum patens* on Inflammation and Melanogenesis

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**Abstract:** Many seaweed species are used as traditional medicine in different parts of the world. However, data on the anti-inflammatory and anti-melanogenic effects of seaweeds are scarce. This study investigated the inhibitory activity of ethyl acetate extracts of *Sargassum patens* (ESP) on the production of inflammatory cytokines and melanin synthesis in cultured cell lines *in vitro*. ESP significantly inhibited the production of Nitric Oxide (NO), prostaglandin $E_2$ ($PGE_2$), interleukin (IL)-6 and tumor necrosis factor (TNF-$\alpha$) on the lipopolysaccharide (LPS)-stimulated RAW264.7 cells. In addition, ESP suppressed the protein expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in LPS-stimulated RAW264.7 cells. ESP markedly inhibited $\alpha$-melanocyte stimulating hormone ($\alpha$-MSH)-induced melanin synthesis and tyrosinase activity and also decreased $\alpha$-MSH-induced expression of microphthalmia-associated transcription factor (MITF), tyrosinase, tyrosinase-related protein (TRP)-1 and TRP-2 in B16 F10 cells. Furthermore, High Performance Liquid Chromatography (HPLC) fingerprinting of ESP revealed that fucoxanthin was the major component of these extracts.

**Key words:** Seaweed, *Sargassum patens*, anti-inflammatory, anti-melanogenic, fucoxanthin

## INTRODUCTION

Seaweeds or their extracts have long been used as novel sources of various biologically acitive compounds having potential medicinal value (Balboa *et al.*, 2013). Recently, considerable attention has been paid to the anti-tumor, anticholesterolemic and antioxidant activities of seaweed constituents. Seaweeds are commonly classified into 3 groups on the basis of their pigmentation: Brown (Phaeophyceae), red (Rhodophyceae) and green (Chlorophyceae) seaweeds.

Brown algae are under-exploited plant resources that are known to produce various secondary metabolites with different carbon skeletons (Blunt *et al.*, 2006). In particular, *Sargassum*, a genus of brown seaweed in the Sargassaceae family, contains approximately 400 species (Mattio and Payri, 2011). *Sargassum* is found across all oceans and consumed as food and medicine in many cultures. Currently, about 200 bioactive compounds such as meroterpenoids, carotenoids, phlorotannins, fucoidans, sterols and glycolipids have been identified from this genus (Heo *et al.*, 2011; Liu *et al.*, 2012). These compounds have been reported to possess biological activities, including anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, anticoagulant, antioxidant, hypoglycemic, hypolipidemic, antimelanogenic, anti-bone loss, hepatoprotective and neuroprotective activities (Balboa *et al.*, 2013; Heo *et al.*, 2011; Kim *et al.*, 2010a; Liu *et al.*, 2012). However, the anti-inflammatory and anti-melanogenesis activities of one of the species belonging to this genus, *Sargassum patens* have not yet been studied. Hence, in the present study, ethyl acetate extracts were obtained from *S. patens* (ESP) and their anti-inflammatory and anti-melanogenesis properties were assessed.

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## MATERIALS AND METHODS

**Reagents:** Dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT), L-DOPA, alpha-melanocyte stimulating hormone ($\alpha$-MSH) and Phosphate Buffered Saline (PBS) and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ELISA kits for $PGE_2$, TNF-$\alpha$, IL-1$\beta$ and IL-6 were purchased from R and D Systems, Inc. (St. Louis, MO, USA) and BD Biosciences (San Diego, CA, USA). Dulbecco's modified Eagle's medium (DMEM) and Fetal Bovine Serum (FBS) were obtained from Invitrogen-Gibco (Grand Island, NY, USA). Anti-inducible nitric oxide synthase (iNOS) and anti-cyclooxygenase-2 (COX-2) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies against microphthalmia-associated transcription factor (MITF), tyrosinase, tyrosinase-related protein (TRP)-1 and TRP-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

**Preparation of ESP:** *S. patens* was collected in Jeju Island on March 2010 and was then identified by Dong-Sam Kim. The samples were washed three times in tap water to remove the salt, epiphytes and sand attached to its surface, then rinsed carefully in fresh water and then dried at room temperature for 2 weeks. The dried samples were lyophilized and homogenized with a grinder prior to extraction. The powdered *S. patens* (100 g) was extracted with 80% ethanol (EtOH; 2L) at room temperature for 24 h and then evaporated in a vacuum. The evaporated EtOH extract was then suspended in water (4 L) and partitioned with ethyl acetate (EtOAc; 4L); this portioning was repeated 3 times.

**Cell culture:** The RAW 264.7 murine macrophage cells and B16F10 mouse melanoma cells were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). RAW 264.7 and B16F10 cells were cultured in DMEM supplemented with 100 U mL$^{-1}$ of penicillin, 100 µg mL$^{-1}$ of streptomycin and 10% FBS. The cells were then incubated in an atmosphere of 5% $CO_2$ at 37°C and were sub-cultured every 3 days.

**Lactate dehydrogenase (LDH) cytotoxicity assay:** RAW 264.7 cells ($1.5 \times 10^5$ cells mL$^{-1}$) plated on 96-well plates were pre-incubated and subsequently treated with LPS (1 µg mL$^{-1}$) coupled with aliquots of ESP at 37°C for 24 h. The medium was carefully removed from each well and the LDH activity in the medium was determined using an LDH cytotoxicity detection kit (Promega, Madison, WI, USA). In brief, 100 µL of reaction mixture were added to each well and the reaction was incubated for 30 min at room temperature in darkness. The absorbance of each well was measured at 490 nm with a UV spectrophotometer.

**Determination of NO production:** After a 24 h pre-incubation of RAW 264.7 cells ($1.5 \times 10^5$ cells mL$^{-1}$) with LPS (1 µg mL$^{-1}$), the quantity of nitrite accumulated in the culture medium was measured as an indicator of NO production. In brief, 100 µL of cell culture medium was mixed with 100 µL of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid), the mixture was incubated at room temperature for 10 min and the absorbance at 540 nm was measured in a microplate reader. Fresh culture medium employed as a blank in every experiment. The quantity of nitrite determined from a sodium nitrite standard curve.

**Measurement of $PGE_2$ and pro-inflammatory cytokines (IL-6 and TNF-$\alpha$) production:** The inhibitory effect ESP on prostaglandin $E_2$ ($PGE_2$), interleukin-6 (IL-6) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) production in LPS-treated RAW264.7 cells was determined as previously described (Kim *et al.*, 2013b). Supernatants were then harvested and assayed for $PGE_2$ and cytokines by ELISA.

**Measurement of cellular melanin contents:** Cellular melanin contents were measured using a previously described method (Yoon *et al.*, 2010). The cells ($2 \times 10^4$ cells mL$^{-1}$) were stimulated with $\alpha$-MSH (50 nM) and then incubated with ESP (0, 12.5, 25 and 50 µg mL$^{-1}$) for 72 h; the cells were then washed in ice-cold PBS. Briefly, the cells were incubated at 80°C for 1 h in 1 mL of 1N NaOH/10% DMSO and then vortexed to solubilize the melanin: the absorbance at 450 nm was then measured.

**Measurement of cellular tyrosinase activity:** Cellular tyrosinase activity was measured according to the previously reported method with slight modifications (Tomita *et al.*, 1992). Briefly, the cells were cultured at $2 \times 10^4$ cells mL$^{-1}$ in 24 well plates. The 16 h after cells seeding, the cells ($2 \times 10^4$ cells mL$^{-1}$) were stimulated with $\alpha$-MSH (50 nM) and then incubated with ESP (0, 12.5, 25 and 50 µg mL$^{-1}$) for 72 h. The cells were washed with PBS and lysed in PBS containing 1% Triton X-100 by freezing and thawing. The lysates were clarified by centrifugation at 13000 g for 10 min. After protein quantification and normalization, 90 µL of cell lyate (each sample contained the same amount of protein) was incubated in duplicate with 10 µL of 10 mM L-DOPA at 37°C for 1 h. After incubation, dopachrom was monitored by measuring the absorbance at 405 nm using and ELISA reader. The value of each measurement is expressed as percentage change from the control.

**Western blot analysis:** The effect of ESP on the expression of pro-inflammatory mediators and

melanogenesis-related protein such as iNOS, COX-2, tyrosinase, TRP-1, TRP-2 and MITF were assessed by Western blot analysis. Western blot was performed as described previously (Kim *et al.*, 2012). Briefly, nuclear proteins (40 µg) loaded into SDS-PAGE were electro-transferred onto nitrocellulose transfer membranes. After blocking with 5% nonfat milk for 2 h, blot were incubated with iNOS (1:1000 dilution), COX-2 (1:1000 dilution), tyrosinase (1:300 dilution), TRP1 (1:1000 dilution), TRP2 (1:1000 dilution), MITF (1:1000 dilution), or β-actin (1:3000 dilution) antibodies. β-actin was used as an internal control. Visualization was achieved by using ECL reagents.

**HPLC-fingerprint analysis:** HPLC analysis were carried out using an ACCELA system (ThermoFisher Scientific, USA), which included pump, auto sampler, column oven and PDA detector, connected to Xcalibur software. A Hypersil-Gold C18 column ($100 \times 2.1$ mm i.d., 1.9 um) from Thermo was used. The column temperature was maintained at $25°C$. The standards and samples were separated using an isocratic mobile phase consisting of 80% methanol. The flow rate was set at 0.2 mL $min^{-1}$ and the injection volume was 5 µL. The detection wavelength was set at 450 nm. Identification and quantification of fucoxanthin was based on retention time when co-injected with standards.

**Statistical analysis:** All data are expressed as Means±S.D. Significant differences between the groups were determined using the unpaired Student's t-test. A value of *p<0.05 was considered to be statistically significant.

## RESULTS

**Effect of ESP on NO and $PGE_2$ production in LPS-stimulated RAW264.7 cells:** The potential anti-inflammatory properties of ESP were investigated using RAW264.7 murine macrophage cells, which can produce NO and $PGE_2$ upon stimulation with LPS. Cells were pre-incubated with ESP for 1 h, following which they were stimulated with 1 µg $mL^{-1}$ LPS for 24 h. Both LPS and sample were not treated in control cells. LPS alone was able to markedly induce higher NO and $PGE_2$ production in the cells than that noted in control cells. However, ESP inhibited NO and $PGE_2$ production in LPS-stimulated RAW264.7 cells in a concentration-dependent manner (Fig. 1a, b). In addition, ESP did not show cytotoxic effects on RAW 264.7 cells

in the concentration range of 12.5-50 µg $mL^{-1}$ (Fig. 1a). Thus, doses of 12.5-50 µg $mL^{-1}$ were selected for use in further experiments.

**Effect of ESP on expression of iNOS and COX-2 proteins in LPS-stimulated RAW 264.7 cells:** Further determine whether inhibition of NO and $PGE_2$ production were associated with decreased levels of iNOS and COX-2 was determined by performing western blot analysis for the detection of protein levels. Untreated cells did not induce iNOS and COX-2 protein expressions, while LPS induced their expressions. However, ESP attenuated LPS-induced expressions of iNOS and COX-2 proteins in the cells (Fig. 2). Thus, the effects of EPS on the expression of the iNOS and COX-2 enzymes, which generate NO and $PGE_2$ as key mediators of inflammation, respectively, were evaluated in greater detail.

**Effect of ESP on IL-6 and TNF-α production in LPS-stimulated RAW264.7 cells:** Next, whether ESP suppresses the production of pro-inflammatory cytokines, IL-6 and TNF-α in LPS-stimulated RAW264.7 cells was investigated. The cells were incubated with ESP in the presence or absence of LPS for 24 h and cytokine levels in the culture media were measured. IL-6 was produced by treatment of LPS, which was inhibited to around 90% by the addition of ESP at 50 µg $mL^{-1}$ and LPS-induced IL-6 production decreased in a dose-dependent manner after treatment with ESP (Fig. 3a). Moreover, the results of the TNF-α assay suggested that 50 µg $mL^{-1}$ ESP significantly suppressed (25%) LPS-induced TNF-α production (Fig. 3b). Although ESP had an inhibitory effect on IL-6 production, the effect was not as strong as that exhibited during the inhibition of TNF-α production.

**Effect of ESP on tyrosinase activity and melanin synthesis in α-MSH-stimulated B16F10 cells:** The hypopigmentary effect of ESP was investigated by performing melanin content and tyrosinase activity assays in α-MSH-stimulated B16F10 cells. ESP significantly inhibited both melanin production and tyrosinase activity in a concentration-dependent manner (Fig. 4). In addition, MTT assay results suggested that there was no cytotoxicity effect of ESP on B16F10 cells at the tested concentration (data not shown). These results suggested that ESP inhibits α-MSH-induced melanin synthesis with down-regulation of tyrosinase activity in B16F10 cells.
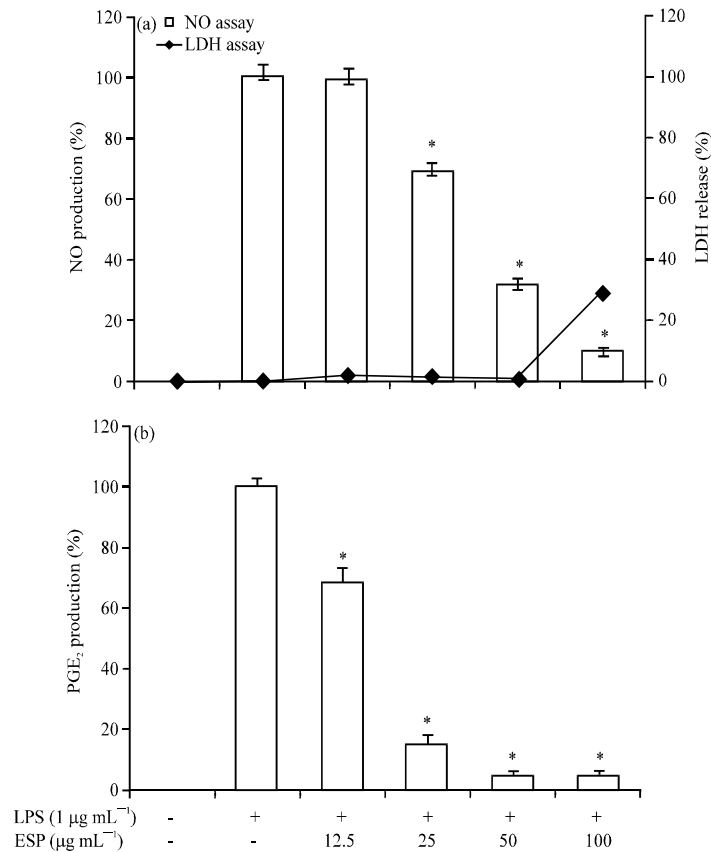
Fig. 1(a-b): Effects of ethyl acetate extracts of *S. patens* (ESP) on (a) Nitric oxide (NO) and (b) prostaglandin $E_2$ (PGE$_2$) production in lipopolysaccharide (LPS)-induced RAW264.7 cells. Cells were pretreated for 1h with different concentrations (12.5, 25, 50 and 100 $\mu$g mL$^{-1}$) of ESP and then LPS (1 $\mu$g mL$^{-1}$) was added and incubated for 24 h, (a) Cytotoxicity was determined using the Lactate dehydrogenase (LDH) method, values are expressed as Means$\pm$S.D of triplicate experiments, *p<0.05 indicate significant differences from the LPS-stimulated group. Parameters on x-axis denote as follows, LPS (-): Without LPS-treatment; LPS (+): LPS-treated cells; ESP (-): Without EPS treatment; ESP 12.5, 25, 50 and 100: 12.5, 25, 50 and 100 $\mu$g mL$^{-1}$ ESP-treated cells, respectively

**Effect of ESP on the expression of tyrosinase, TRP-1, TRP-2 and MITF proteins in α-MSH-stimulated B16F10 cells:** Next whether ESP could modulate melanogenesis-related protein expression under α-MSH stimulation was investigated. B16F10 cells were exposed to ESP in the presence of α-MSH and the changes in the levels of tyrosinase, TRP-1, TRP-2 and MITF expression were analyzed by western blot analysis. B16F10 cells treated with ESP showed significantly decreased tyrosinase, TRP-1, TRP-2 and MITF protein expression levels compared with those of the α-MSH group (Fig. 5). These results indicated that ESP decreases melanin synthesis via the down-regulation of TRP and MITF expression.

**HPLC fingerprint of ESP:** According to traditional oriental medicine, the therapeutic actions of herbal medicines are based on integral interaction of many ingredients combined. With the development of analytical technology, chromatographic methods are highly recommended for developing fingerprints of traditional oriental medicine and their raw materials. Thus, interest in HPLC fingerprint analysis has increased in not only Asia but also other countries worldwide (Kim *et al.*, 2009; Yoon *et al.*, 2009; Zhang *et al.*, 2007). Therefore, simple HPLC fingerprints of the algae were explored in this study. Since fucoxanthin has been reported to be an ingredient in the *Sargassum* species that has effective anti-inflammatory and anti-melanogenesis effects, this
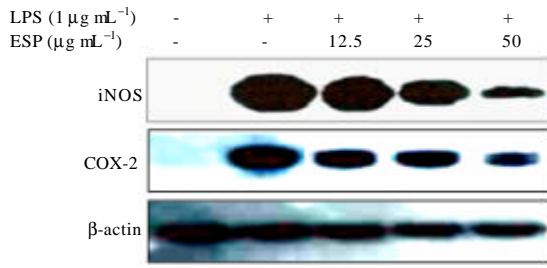
Fig. 2: Effects of ethyl acetate extracts of *S. patens* (ESP) on lipopolysaccharide (LPS)-induced inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expressions in RAW 264.7 cells. Cells were treated for 1h with different concentrations (12.5, 25 and 50 μg mL$^{-1}$) of ESP, LPS (1 μg mL$^{-1}$) was then added and cells were incubated for 24 h. iNOS and COX-2 protein level were determined via Western blotting and β-actin was used as an internal control

compound was used as a standard substance. The conditions described in the methods section led to better resolution of fucoxanthin from ESP, with excellent peak shapes. The contents in the ESP were 11.2 mg g$^{-1}$ (Fig. 6).

## DISCCUSION

*Sargassum* species extracted using various solvents showed anti-inflammatory, anti-allergic, antimicrobial, antiviral, antiplasmodial, anticancer, liver protective, gastric protective, bone protective, skin-whitening, anti-alzheimer and antioxidant activities (Liu *et al.*, 2012). This is the first report of evaluation of the anti-inflammatory and anti-melanogenesis activities of *S. patens* in RAW264.7 cells and B16F10 cells, respectively.

Inflammatory action is an essential aspect of host response to bacterial and viral infections and injury and is required for the maintenance of good health. However, chronic and uncontrolled inflammation has been implicated as the pathophysiological mechanism underlying many chronic diseases, including diabetes, cardiovascular disease, cancer, arthritis and neurodegenerative diseases (Lawrence *et al.*, 2002; Libby, 2007).

Macrophages actively participate in inflammatory responses by releasing pro-inflammatory cytokines (TNF-α and IL-6) and inflammatory factors (NO and PGE$_2$) that recruit additional immune cells to sites of infection or tissue injury (Bosca *et al.*, 2005; Kim *et al.*, 2010a). The
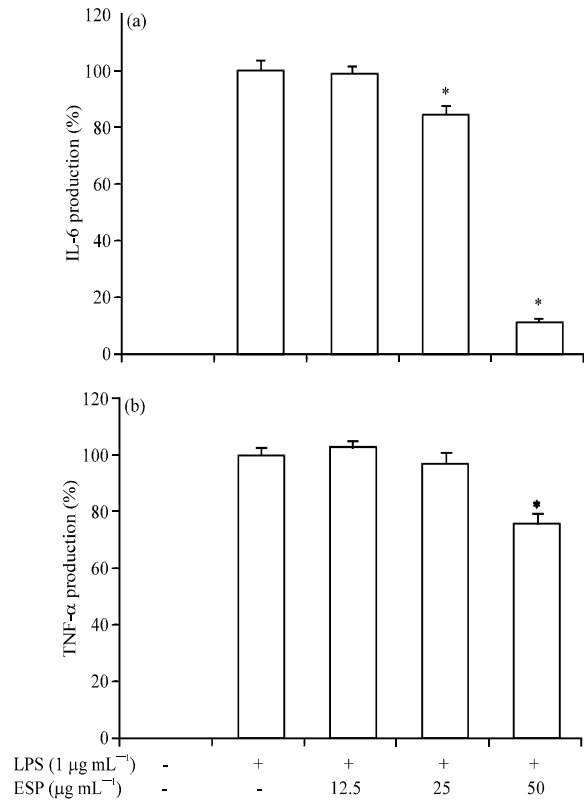


Fig. 3(a-b): Inhibitory effect of ethyl acetate extracts of *S. patens* (ESP) on the pro-inflammatory cytokine production in RAW264.7 cells. The production of (a) IL-6 and (b) TNF-α were assayed in the culture medium of cells stimulated with lipopolysaccharide (LPS) (1 μg mL$^{-1}$) for 24 h in the presence of ESP (12.5, 25 and 50 μg mL$^{-1}$). Supernatants were collected and the tumor necrosis factor (TNF-α) and interleukin (IL)-6 concentration in the supernatants were determined by ELISA, values are expressed as Means±S.D of triplicate experiments. *p<0.05 indicate significant differences from the LPS: Stimulated group, LPS (-): Without LPS-treatment; LPS (+): LPS-treated cells, ESP (-): Without EPS treatment; ESP 12.5, 25 and 50: 12.5, 25 and 50 μg mL$^{-1}$ ESP-treated cells, respectively

production of these inflammatory molecules by macrophages can be induced in response to LPS stimulation.

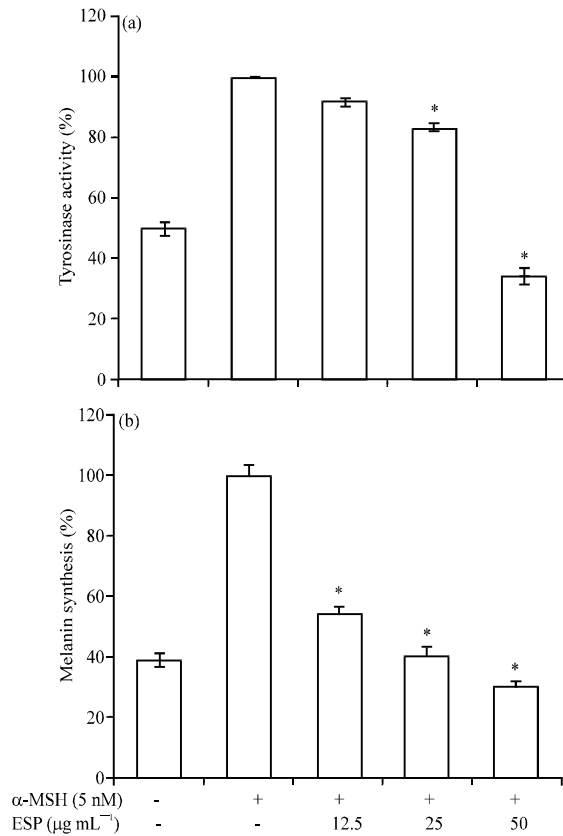NO is an important regulatory molecule in a range of physiological and pathological processes

Fig. 4(a-b): Effect of ethyl acetate extracts of *S. patens* (ESP) on cellular (a) Tyrosinase activity and (b) Melanin synthesis in B16F10 cells, cells were exposed to 50 nM α-melanocyte stimulating hormone (α-MSH) in the presence of 12.5-50 μg mL$^{-1}$ ESP. The percentage values of the treated cells are expressed relatively compared to that in the control cells. Data are reported as the Mean±S.D of three independent experiments carried out in triplicate. *p<0.05 indicate significant differences from the the α-MSH treated one. α-MSH (-): Without α-MSH-treatment, α-MSH (+): α-MSH-treated cells; ESP (-): Without EPS treatment; ESP 12.5, 25 and 50: 12.5, 25 and 50 μg mL$^{-1}$ ESP-treated cells, respectively
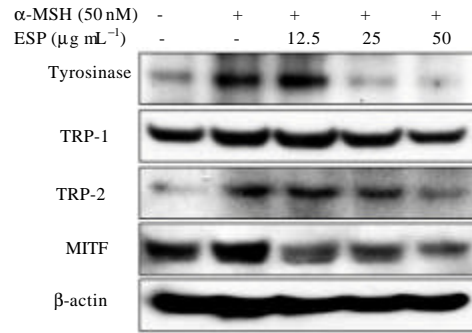


Fig. 5: Effect of ethyl acetate extracts of *S. patens* (ESP) on the expression of melanogenesis-related proteins in B16F10 cells. Cells were exposed to 50 nM α-melanocyte stimulating hormone (α-MSH) in the presence of 12.5-50 μM ESP for 72 h. The expression levels of the microphthalmia-associated transcription factor (MITF), tyrosinase, tyrosinase-related protein (TRP)-1 and TRP-2 proteins were examined by Western blot and β-actin was used as an internal control

(Ajizian *et al.*, 1999; Kim *et al.*, 2013b). It is generated by 3 isoforms of NO Synthase (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS) and iNOS (Nathan and Xie, 1994). iNOS is expressed in response to various inflammatory stimuli and generates high levels of NO in macrophages during the inflammatory process (Laskin and Pendino, 1995). PGE$_2$ is also a well-known inflammatory mediator derived from arachidonic acid via the action of cyclooxygenases. Growth factors, cytokines and pro-inflammatory molecules-mediated overproduction of PGE$_2$ is known to be associated with the up-regulation of COX-2 (Minghetti, 2004; St-Onge *et al.*, 2007). The results obtained in this study suggested that ESP inhibits NO and PGE$_2$ production via the suppression of iNOS and COX-2 expression, respectively.

Furthermore, ESP also reduced the LPS-induced production of TNF-α and IL-6. Pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 are known to contribute to tissue damage and multiple organ failure. They are considered to be important initiators of inflammatory response and mediators of the development of various inflammatory diseases (Glauser, 1996; Mannel and Echtenacher, 2000). Moreover, the production of TNF-α is crucially required for the synergistic induction of NO synthesis in LPS-stimulated macrophages (Aggarwal and Natarajan, 1996). IL-6 is a cytokine released by LPS-activated monocytes and plays a crucial role in the immune response (Ghosh and Karin, 2002). Therefore, anti-inflammatory drugs are developed using inhibitors of these inflammatory molecules (Andreakos *et al.*, 2004) to alleviate various disorders caused by the activation of macrophages.

Melanogenesis is a physiological process resulting in the production of melanin pigment, which plays an
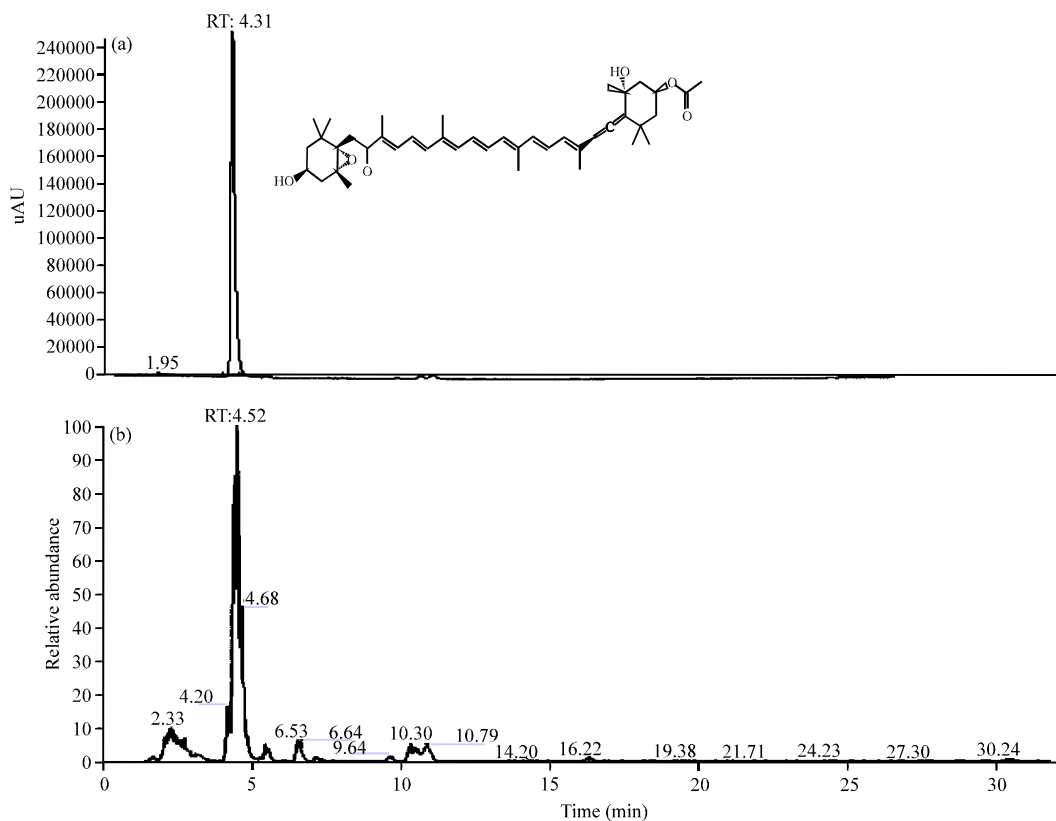
Fig. 6(a-b): High-performance liquid chromatography (HPLC) fingerprinting analysis of the ethyl acetate extracts of *S. patens* (ESP), HPLC profiles of (a) Authentic fucoxanthin and (b) ESP, respectively, the wavelength of fucoxanthin is 440 nm

important role in the prevention of sun-induced skin injury and contributes to skin and hair color (Gilchrest and Eller, 1999; Sturm *et al.*, 1998). However, the accumulation of excess epidermal melanin can induce cosmetic problems, such as melasma, lentigo and age spots and cause various hyperpigmentation disorders such as malignant melanoma (Gupta *et al.*, 2006; Narayanan *et al.*, 2010). Therefore, many plant parts have been investigated to determine their potential for use as anti-melanogenesis agents.

Melanogenesis is directly regulated by melanogenic enzymes such as tyrosinase, TRP-1 and TRP-2. Tyrosinase is a rate-limiting enzyme of melanogenesis, which first catalyzes the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and then catalyzes the oxidation of DOPA to DOPA-quinone. TRP-1 oxidizes 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to a carboxylated indole-quinone (Hearing and Jimenez, 1987). Tyrosinase and TRP-1 complex is important for tyrosinase activation and stability (Kobayashi *et al.*, 1998). TRP-2, which functions as a DOPA-chrome tautomerase, catalyzes the rearrangement of DOPA-chrome to DHICA

(Park *et al.*, 2009). These 3 enzymes have been recognized as the most critical regulators of melanin biosynthesis. The tyrosinase family genes tyrosinase, TRP-1 and TRP-2 responsible for pigmentation are transcriptionally regulated by microphthalmia-associated transcription factor (MITF) (Levy *et al.*, 2006; Tachibana, 2001; Ye *et al.*, 2010). In the present study, ESP was found to markedly inhibit $\alpha$-MSH-induced melanin synthesis and tyrosinase activity in a dose-dependent manner and these doses were not cytotoxic to B16F10 cells. Further, ESP significantly suppressed the protein levels of tyrosinase, TRP-1, TRP-2 and MITF in $\alpha$-MSH-stimulated B16F10 cells.

*Sargassum* is widely distributed in tropical areas and is reported to possess diverse compounds, including triterpenoids, carotenoids, phlorotannins, fucoidans, sterols and glycolipids, with various biological activities (Heo *et al.*, 2011; Liu *et al.*, 2012). Among the carotenoids, fucoxanthin-one of the major xanthophylls in brown alga-has been identified in several *Sargassum* species. Recent pharmacological research indicates that fucoxanthin has anticancer, anti-pigmentary,

anti-inflammatory, anti-oxidant, anti-obesity and antidiabetic activities (Kim *et al.*, 2013a; Kim *et al.*, 2010a; Kim *et al.*, 2010b; Liu *et al.*, 2012; Shimoda *et al.*, 2010). *Sargassum* contains 1-8 mg of fucoxanthin per gram dry weight, with seasonal variation (Terasaki *et al.*, 2009). In the present study, ESP showed high fucoxanthin contents (11.2 mg $g^{-1}$). These facts suggest the anti-inflammatory and anti-melanogenesis effects of ESP might primarily be attributed to fucoxanthin.

## CONCLUSION

In conclusion, ESP was found to significantly attenuate the release of inflammatory mediators (NO, $PGE_2$, TNF-α and IL-6) in LPS-stimulated RAW264.7 cells. Additionally, ESP inhibited cellular melanin synthesis and tyrosinase activity by suppressing MITF, tyrosinase, TRP-1 and TRP-2 in α-MSH-stimulated B16F10 cells. Further studies are necessary to isolate the bioactive compounds from ESP in order to elucidate the mechanism of melanogenesis and inflammation inhibition.

## ACKNOWLEDGMENT

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