Research Article

Algae 2013, 28(1): 111-119
http://dx.doi.org/10.4490/algae.2013.28.1.111
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In vitro studies of anti-inflammatory and anticancer activities of organic solvent extracts from cultured marine microalgae

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Marine microalgae are a promising source of organisms that can be cultured and targeted to isolate the broad spectrum of functional metabolites. In this study, two species of cyanobacteria, *Chlorella ovalis* Butcher and *Nannchloropsis oculata* Droop, one species of bacillariophyta, *Phaeoductylum tricornutum* Bohlin, and one species of Dinophyceae, *Amphidinium carterae* (Hulburt) were cultured and biomasses used to evaluate the proximate comical compositions. Among the determined proximate chemical compositions of the cultured marine microalgae, the highest content of crude proteins and lipids were exhibited in *P. tricornutum* and *A. carterae*, respectively. Solvent-solvent partition chromatography was subjected to fractionate each of the cultured species and separated *n*-hexane, chloroform, ethyl acetate, and aqueous fractions. Nitric oxide production inhibitory level (%) and cytotoxicity effect on lipo-polysaccharide–induced RAW 264.7 macrophages were performed to determine the anti-inflammatory activity. *N. oculata* hexane and chloroform fractions showed significantly the strongest anti-inflammatory activity at 6.25 μg mL^{-1} concentration. The cancer cell growth inhibition (%) was determined on three different cell lines including HL-60 (a human promyelocytic leukemia cell line), A549 (a human lung carcinoma cell line), and B16F10 (a mouse melanoma cell line), respectively. Among the extracts, *C. ovalis* ethyl acetate and *A. carterae* chloroform fractions suppressed the growth of HL-60 cells significantly at 25 and 50 μg mL^{-1} concentrations. Thus, the cultured marine microalgae solvent extracts may have potentiality to isolate pharmacologically active metabolites further using advance chromatographic steps. Hence, the cultured marine microalgae can be described as a good candidate for the future therapeutic uses.

Key Words: *Amphidinium carterae*; anticancer; anti-inflammatory effect; *Chlorella ovalis*; cultured marine microalgae; *Nannchloropsis oculata*; *Phaeoductylum tricornutum*

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; DPBS, Dulbecco's phosphate-buffered saline; DMSO, dimethyl sulfoxide; DMRT, Duncan's multiple range test; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; LPS, lipo-polysaccharide; NO, nitric oxide; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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INTRODUCTION

Microalgae, as an evolutionary form of organism, are showing an extraordinary adaptation in the ocean. The oldest fossil evidence has shown that rise of environment oxygen that is contributed by eukaryotes and cyanobacteria appeared over 2.45-2.32 billion years ago (Rasmussen et al. 2008). In fact, blue-green algae (cyanobateria), diatoms (Bacillariophyta), and dinoflagellates (Dinophyceae) have evolved from their origins in a primitive age to contemporary times without much change in structure. It is this fascinating group of single-celled, microscopic organisms, which have been at the bottom of the food chain and have long served as a primary producer in aquatic sources either fresh or marine, contributing to almost 70% of global oxygen demand, even though abiotic factors such as, light intensity, temperature, nutrients, and salinity levels influence their biological functions. Furthermore, extreme fluctuations in climate changes according to the seasons have given a major outbreak through the variations and required for survival in the competitive environmental conditions (Plaza et al. 2008).

As a consequence, biochemically and ecologically significant differences have been gained a vast microalgae diversity and associated a broad spectrum of secondary metabolites. Thus, researchers are continuously mining the bioactive components from marine microalgae to determine pharmacological and medicinal values in many parts of the world (Borowitzca 1995). Hence, marine microalgae have emphasized that research on their natural products are useful for the cure and for the alleviation of human diseases (Imhoff et al. 2011). Screening of marine microalgae has received much attention as a rich source for the exploration of pharmaceutical and other biological active components in last decades (Borowitzca 1995). In this regard, microalgae bear an unusual quality of nutrients that include a rich source of protein, poly unsaturated fatty acids, carbohydrates, minerals, vitamins, pigments, and secondary metabolites (Becker 2007). Thus, many of the prominent microalgae natural sources have targeted for the research and revealed the health effects, for human well-being (Liang et al. 2004) as well as for domestic animal applications (Becker 2007). However, there are some potential benefits in addition to the nutritional value of microalgae, including antioxidant (Sheih et al. 2009), antitumor (Sheih et al. 2010), immunostimulant (Morris et al. 2007), antibacterial (Desbois et al. 2009), hypocholesterolemic effect (Dvir et al. 2009), and angiotensin-converting enzyme inhibitor-I inhibition activity (Samarakoon et al. 2013).

The cultivation of marine microalgae has been practiced for some time. In particular, there can be described a couple of algae systems that culture as open, closed (photobioreactors) or artificial culture. Each one of the culturing systems has either a desirable or undesirable effect, since the photosynthetic organisms and their mass production may directly correlate with the source of energy. Nevertheless, only a select few microalgae strains are used in controlled conditions for cultivation in particular environments (Hong et al. 2012). The controlled optimum condition with the operational inputs such as salt, dissolved CO_2, water, nutrients, pH, and O_2 providing a great opportunity for the steady environment without being contaminated in photobioreactors (Plaza et al. 2009). Hence, researchers have been interested in cultured marine microalgae in order to reveal the biochemical constituents of their crude extracts and to determine which components have pharmacological effects (Kim and Wijesekara 2010).

In this study, four different microalgae strains belonging to the three main classes of marine microalgae including cyanobateria (*Chlorella ovalis*, *Nannchloropsis oculata*), Bacillariophyta (*Phaeoductylum tricornutum*), and Dinophyceae (*Amphidinium carterae*) were cultured, appropriately. Then, we attempted to screen the anti-inflammatory and anticancer activities of the crude organic solvent extracts from the cultured marine microalgae using *in vitro* assays.

MATERIALS AND METHODS

Chemicals and reagents

The murine macrophage cell line (RAW 264.7), a human promyelocytic leukemia cell line (HL-60), a mouse melanoma cell line (B16F10), and a human lung carcinoma cell line (A549) were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). Roswell Park Memorial Institute (RPMI-1640) medium, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco/BRL (Burlington, ON, Canada). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in these investigations were of analytical grade.

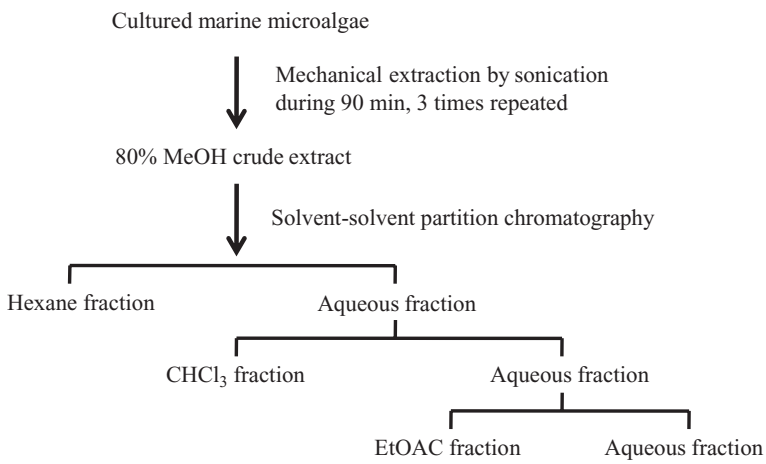

Fig. 1. Extraction approaches of cultured marine microalgae samples using solvent-solvent partition chromatography.

Culture of marine microalgae

Marine microalgae *Phaeodactylim tricornutum* Bohlin, *Chlorella ovalis* Butcher, and *Nannchloropsis oculata* Droop were kindly provided by the Korea Marine Microalgae Culture Center (KMMCC). The algae were inoculated in 30-L plastic cylinders at 20°C after pre-cultivated in 5 L glass vessels (medium 4 L), and air was continuously supplied at 5 L min^{-1} by air-lift. Light was provided by 60-W fluorescent lamps at an intensity of 34 µmol photons m^{-2} s^{-1} (light : darkness = 24 : 0). Microalgae species were cultured in Conway medium (Walne 1966) prepared from filter-sterilized seawater and the culture were continuously active during the 8-10 days after inoculation. The cells were flocculated with 200 ppm $Al_2(SO_4)_3$ (v/v) (Magicpool-99; Ilshin Biochemical, Hanam, Korea) and then recovered with centrifugation at 2,000 rpm using a basket centrifuge (Hanseong Co., Ansan, Korea). The harvested diatom biomass was frozen at -80°C and preserved until freeze dry.

Collected benthic dinoflagellate strains from different coastal sites of Jeju Island and established as single cell cultures. The culture of *Amphidinium carterae* Hulburt were scaled up from 15 to 30 mL test tube, and 0.3, 1, 3, and 20 L under laboratory condition using 1/4 IMK medium (Nihon Pharmaceutical Co., Ltd., Osaka, Japan) at 22°C, 30 ppt salinity, 12 : 12 h light : dark cycle using fluorescent lamp at an intensity of 40 µmol photons m^{-2} s^{-1} with a medium level of aeration. After 30-40 days of culture, the cells were harvested by centrifuging (5,000 rpm for 10 min using 250 mL centrifuge bottle) the cultures and kept at -80°C until freeze dried.

Determination of proximate chemical compositions of the cultured marine microalgae

Proximate chemical composition of cultured marine microalgae was determined according to the Association of Official Analytical Chemists (AOAC) method (AOAC 1990). Total nitrogen content was analyzed by the Kjeldahl method (Kejltec 8400; FOSS, Eden Prarie, MN, USA). Crude protein was determined by calculating a conversion factor of 6.25. Crude lipid was performed by soxhelt method extraction with diethyl ether solvent (Soxtec 2050; FOSS) and crude ash was determined by incineration of samples at 550°C in the muffle furnace (B180; Nabertherm GmbH, Lilienthal, Germany). Moisture was determined by oven-drying method at 105°C in the moisture analyzer (mb45; OHAUS, Nanikon, Switzerland). Crude fiber content was measured by fibertec system (Fibertec 2010 Analyzer; FOSS).

Solvent extractions and preparation of samples from the cultured marine microalgae

After freeze-drying, the cultured marine microalgae were grounded into fine powder and each of the materials was homogenized separately. Then, the homogenized marine microalgae samples were sonicated at 25°C for 90 min, 3 times each using (80%) methanol. Crude methanol extracts were concentrated by evaporating the solvent under reduced pressure using rotary evaporator and further subjected to solvent-solvent partition chromatography. Four different fractions such as *n*-hexane, chloroform, ethyl acetate, and aqueous extracts were fractionated with the varying of polarity as presented in Fig. 1. Each

of the separated fractions from different cultured marine microalgae evaporated the solvents further using rotary evaporator. Prior to the in vitro assays, the solvent (*n*-hexane, chloroform, ethyl acetate, and aqueous) extractions from the four different samples, such as *P. tricornutum*, *C. ovalis*, *N. oculata*, and *A. carterae* were prepared 100 mg mL^{-1} concentration, each using DMSO. Further dilution was carried out using Dulbecco's phosphate-buffered saline solvent to gain the desired concentration for the *in vitro* assay as appropriately.

Cell culture

HL-60 (a human promyelocytic leukemia cell line) and A549 (a human lung carcinoma cell line) cells were grown in RPMI-1640 medium, B16F10 (a mouse melanoma cell line), and RAW 264.7 (a murine macrophage cell line) were cultured in DMEM. Both culture media were supplemented with 100 U mL^{-1} of penicillin, 100 μg mL^{-1} of streptomycin and 10% FBS. The cells were incubated and maintained in an atmosphere of 5% CO_2 at 37°C. The cells were subcultured every 2 days and exponential phase cells were used throughout the experiments.

Determination of nitric oxide (NO) production

RAW 264.7 cells (1×10^5 cells mL^{-1}) were placed in a 24-well plate and after 24 h the cells were pre-incubated with various concentrations of the sample at 37°C for 1 h. Then further incubation was done for another 24 h with lipo-polysaccharide (LPS; 1 μg mL^{-1}) at the same temperature. After the incubation, a quantity of nitrite that had accumulated in the culture medium was measured as an indicator of NO production (Lee et al. 2007). Briefly, 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 optical density at 540 nm was measured using an enzyme-linked immunosorbent assay (ELISA) microplate reader (Sunrise; Tecan Co. Ltd., Salzburg, Australia). The fresh culture medium was used as a blank in every experiment.

Cytotoxicity assessment using MTT assay

The cytotoxicity of the crude solvent extracts against the RAW 264.7 cells was determined using the colorimetric MTT assay. Cells were seeded in a 24-well plate at a concentration of 1×10^5 cells mL^{-1}. After 24 h, the seeded cells were treated with extracts. Then, all of the cells were incubated for an additional 24 h at 37°C. MTT stock solution (50 μL; 2 mg mL^{-1} in phosphate-buffered saline [PBS]) and was added to each well to a total reaction volume of 250 μL. After incubating for 3 h, the supernatants were aspirated. The formazan crystals in each well were dissolved in 200 μL of DMSO. The resulting absorbance was measured with an ELISA plate reader set at 540 nm.

Cell growth inhibitory assay for anticancer activity

The cell growth inhibitory activity of cultured marine microalgae (*C. ovalis* and *A. carterae*) crude solvent extracts against the cancer cells (HL-60, B16F10, and A549) were determined by the colorimetric MTT assay. Suspension cells (HL-60 cells) were seeded (2×10^4 cells mL^{-1}) together with the samples and incubated for 48 h before MTT treatment. Attached cells (B16F10 and A549) were seeded in a 96-well plate at a concentration of 2×10^4 cells mL^{-1}. At 16 h after seeding, the cells were treated with the samples. MTT stock solution (50 μL; 2 mg mL^{-1} in PBS) was added to each well to achieve a total reaction volume of 250 μL. After 4 h of incubation, the plates were centrifuged for 10 min at 2,000 rpm and the supernatants were aspirated. The formazan crystals in each well were dissolved in DMSO. The amount of purple formazan was assessed by measuring the absorbance at 540 nm.

Table 1. Proximate chemical compositions of marine microalgae sample crude dry weight basis

Microalgae	Proximate chemical composition dry weight basis[a] (%)					
	Protein	Lipid	Carbohydrate	Fiber	Ash	Moisture
Chlorella ovalis	32.10 ± 0.13	0.91 ± 0.02	27.44 ± 0.06	2.94 ± 0.05	34.03 ± 0.15	2.58 ± 0.06
Nannchloropsis oculata	30.97 ± 0.14	1.30 ± 0.07	17.78 ± 0.01	4.38 ± 0.08	32.87 ± 0.15	12.70 ± 0.10
Amphidinium carterae	21.50 ± 0.05	6.31 ± 0.01	25.03 ± 0.04	2.45 ± 0.06	41.15 ± 0.2	3.57 ± 0.07
Phaeoductylum tricornutum	34.75 ± 0.25	2.47 ± 0.05	15.75 ± 0.03	3.65 ± 0.08	35.70 ± 0.03	7.68 ± 0.04

[a]Analysis was according to the Association of Official Analytical Chemists (AOAC) methods. Values are mean ± standard deviation of three determinations.

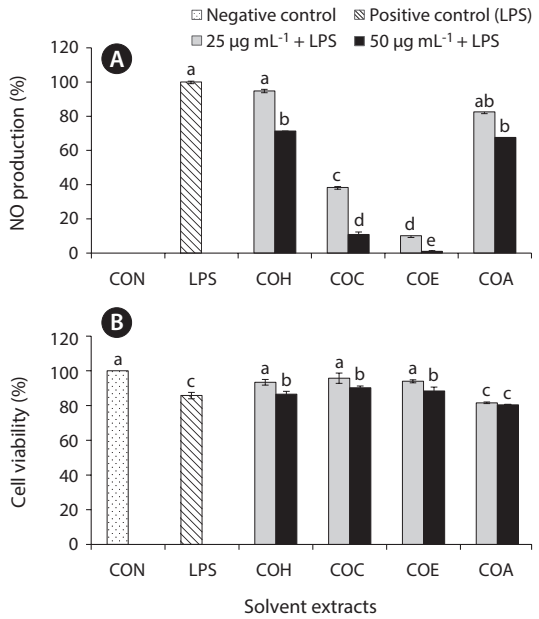

Fig. 2. Inhibitory effect of cultured marine microalga *Chlorella ovalis* solvent extracts by solvent-solvent partition chromatography on lipo-polysaccharide (LPS)–induced nitric oxide (NO) production (%) (A) and cell viability (%) (B) in RAW 264.7 macrophages. The incubation of extracts (COH, *C. ovalis* hexane fraction; COC, *C. ovalis* chloroform fraction; COE, *C. ovalis* ethyl acetate fraction; COA, *C. ovalis* aqueous fraction) with cells in response to LPS (1 μg mL^{-1}) for 24 h, the NO levels in the medium was measured. CON, negative control (no LPS treated); LPS, positive control (LPS 1 μg mL^{-1} treated). Concentration of sample treated 25 μg mL^{-1} + LPS and 50 μg mL^{-1} + LPS, respectively. Values are mean ± standard deviation of three determinations. Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

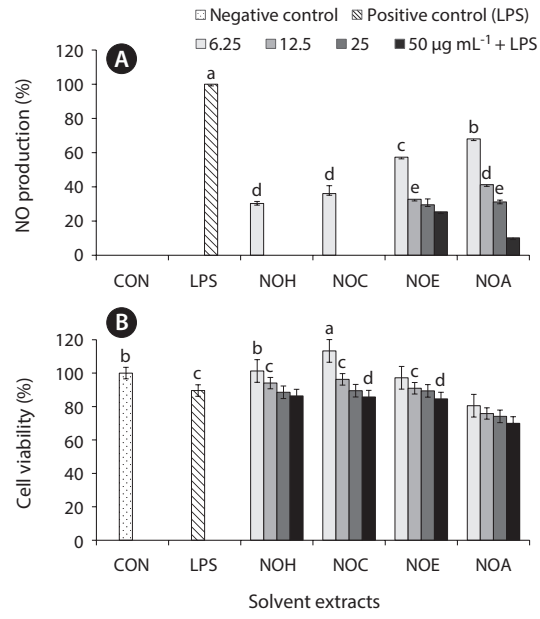

Fig. 3. Inhibitory effect of cultured marine microalga *Nannchloropsis oculata* solvent extracts by solvent-solvent partition chromatography on lipo-polysaccharide (LPS)–induced nitric oxide (NO) production (%) (A) and cell viability (%) (B) in RAW 264.7 macrophages. The incubation of extracts (NOH, *N. oculata* hexane fraction; NOC, *N. oculata* chloroform fraction; NOE, *N. oculata* ethyl acetate fraction; NOA, *N. oculata* aqueous fraction) with cells in response to LPS (1 μg mL^{-1}) for 24 h, the NO levels in the medium was measured. CON, negative control (no LPS treated); LPS, positive control (LPS 1 μg mL^{-1} treated). Concentration of sample treated 6.25, 12.5, 25, and 50 μg mL^{-1}, and add LPS, respectively. Values are mean ± standard deviation of three determinations. Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

Statistical analysis

All the data are expressed as mean ± standard deviation of three determinations. Statistical comparison was performed via a one-way analysis of variance followed by Duncan's multiple range test (DMRT). p-values of less than 0.05 ($p < 0.05$) were considered as significant.

RESULTS AND DISCUSSION

Proximate chemical composition and solvent extraction of the cultured marine microalgae

According to the AOAC (1990) method, the proximate chemical composition (%) of the cultured marine microalgae samples were determined by crude dry weight basis. In this study, two cyanobacteria species (*C. ovalis* and *N. oculata*), one diatom species (*P. tricornutum*), and one dinoflagellate species (*A. carterae*) were cultured and the lyophilized samples were used for chemical composition analysis. Thus, all samples demonstrated the high content of ash (%) compared to the other nutrients. Furthermore, *A. carterae* possessed the highest ash content, at 41.2%. However, the crude protein content of the cultured marine microalgae was as high as 1/3 of the total ash composition (Table 1). This is evidence that the microalgae can be cultivated in closed culturing systems and is able to gain a high content of proteins in biomass (Plaza et al. 2009). Although, the cultured marine dinoflagellate (*A. carterae*) from the Jeju Island was shown to have significantly lower content of crude proteins as 21.5% compared to the other cultured species. In fact, the *A. carterae* species has been reported to contain a higher amount of crude lipids at 6.3% than other cultured microalgae. Thus, this species rendered a positive indication

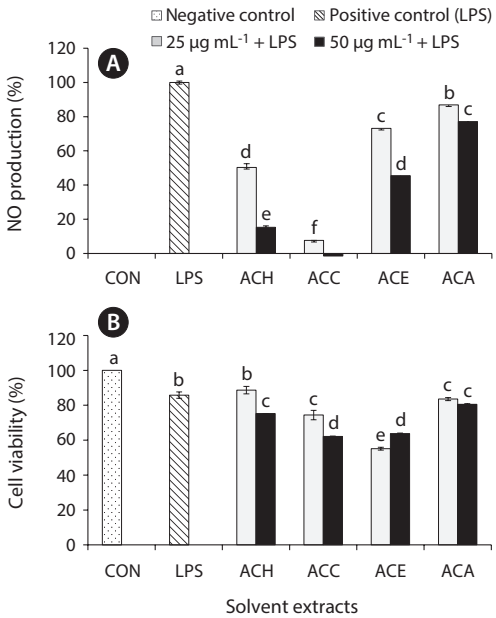

Fig. 4. Inhibitory effect of cultured marine microalga *Amphidinium carterae* solvent extracts by solvent-solvent partition chromatography on lipo-polysaccharide (LPS)–induced nitric oxide (NO) production (%) (A) and cell viability (%) (B) in RAW 264.7 macrophages. The incubation of extracts (ACH, *A. carterae* hexane fraction; ACC, *A. carterae* chloroform fraction; ACE, *A. carterae* ethyl acetate fraction; ACA, *A. carterae* aqueous fraction) with cells in response to LPS (1 μg mL^{-1}) for 24 h, the NO levels in the medium was measured. CON, negative control (no LPS treated); LPS, positive control (LPS 1 μg mL^{-1} treated). Concentration of sample treated 25 μg mL^{-1} + LPS and 50 μg mL^{-1}+ LPS, respectively. Values are mean ± standard deviation of three determinations. Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

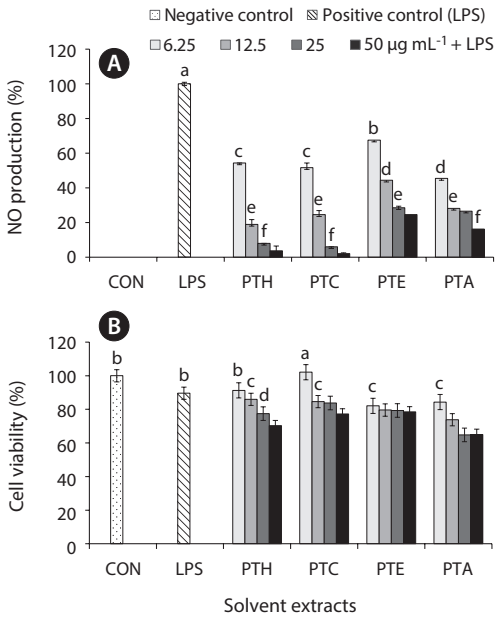

Fig. 5. Inhibitory effect of cultured marine microalga *Phaeoductylum tricornutum* solvent extracts by solvent-solvent partition chromatography on lipo-polysaccharide (LPS)-induced nitric oxide (NO) production (%) (A) and cell viability (%) (B) in RAW 264.7 macrophages. The incubation of extracts (PTH, *P. tricornutum* hexane fraction; PTC, *P. tricornutum* chloroform fraction; PTE, *P. tricornutum* ethyl acetate fraction; PTA, *P. tricornutum* aqueous fraction) with cells in response to LPS (1 μg mL^{-1}) for 24 h, the NO levels in the medium was measured. CON, negative control (no LPS treated); LPS, positive control (LPS 1 μg mL^{-1} treated). Concentration of sample treated 6.25, 12.5, 25, and 50 μg mL^{-1}, and add LPS, respectively. Values are mean ± standard deviation of three determinations. Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

to isolate the possible bioactive lipids than other cultured species. In addition, a recent publication has been reported that the high content of proteins as the range of 40-60% of their dry weight in marine microalgae, which included *Chlorella pyrenoidosa*, *Arthrospira maxima*, *Anabaena cylindrica*, and *Spirulina platensis* (Becker 2007).

Solvent-solvent partition chromatography was subjected to fractionation of the crude samples of (80%) methanol extracts further from the cultured marine microalgae as shown in Fig. 1. The chemistry of this approach was based on the variation in organic solvent polarity to separate different functional metabolites in the crude solvent extracts. Therefore, more significant exploration was achieved with the organic solvent fractionations in order to gain a different polarity of crude extracts from the cultured marine microalgae samples.

Anti-inflammatory effect of the extracts and fractions from cultured marine microalgae

Inflammation is a physiological process that is initiated due to the pathogenic invasion or injury to cells and tissues (Wadleigh et al. 2000). This can be influenced by the activation of various immune cells such as macrophages, neutrophils and lymphocytes. Inflammatory mediators, such as NO, play an important role as the signaling molecules that are induced in macrophages. LPS acts as endotoxins for mammals and stimulation of the RAW cells in terms of enhancing the NO concentration in the medium. Hence, the inhibition of NO production (%) level was indicated that the positive evidence of anti-inflammatory activity on LPS-induced RAW macrophages. Figs 2A, 3A, 4A, and 5A are showing the inhibitory effect of NO pro-

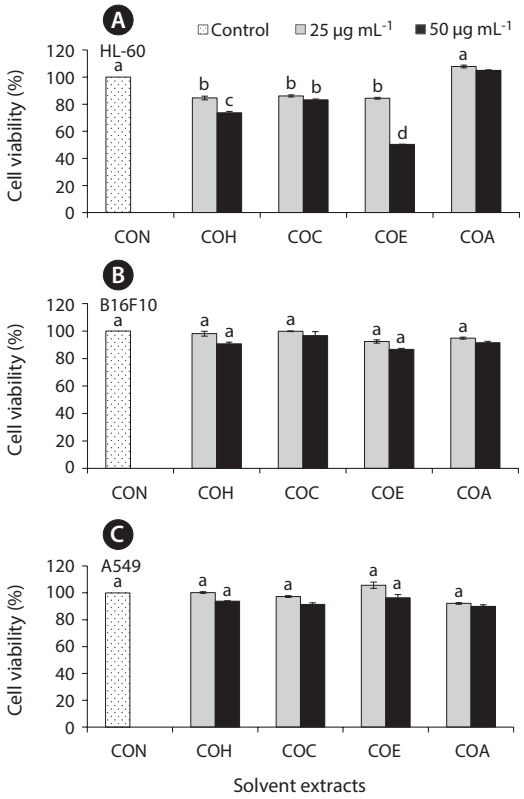

Fig. 6. Inhibitory effect of the growth of cancer cells against cultured marine microalga *Chlorella ovalis* solvent extracts by solvent-solvent partition chromatography on HL-60 (A), B16F10 (B), and A549 (C) cell lines. Cells were treated with the extracts (COH, *C. ovalis* hexane fraction; COC, *C. ovalis* chloroform fraction; COE, *C. ovalis* ethyl acetate fraction; COA, *C. ovalis* aqueous fraction) at the indicated concentrations denoted as 25 and 50 μg mL^{-1}, respectively. CON, control. After 24 h to treat the extracts cell viability was assessed by MTT assay. Values are expressed as means ± standard deviation in triplicate experiments. Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

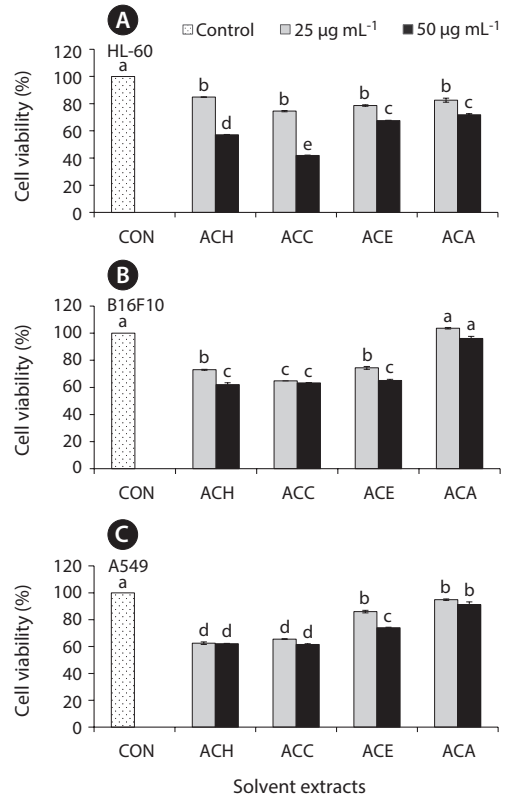

Fig. 7. Inhibitory effect of the growth of cancer cells against cultured marine microalga *Amphidinium carterae* solvent extracts by solvent-solvent partition chromatography on HL-60 (A), B16F10 (B), and A549 (C) cell lines. Cells were treated with the extracts (ACH, *A. carterae* hexane fraction; ACC, *A. carterae* chloroform fraction; ACE, *A. carterae* ethyl acetate fraction; ACA, *A. carterae* aqueous fraction) at the indicated concentrations denoted as 25 and 50 μg mL^{-1}, respectively. CON, control. After 24 h to treat the extracts cell viability was assessed by MTT assay. Values are expressed as means ± standard deviation in triplicate experiments. Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

duction level of marine microalgae including *C. ovalis*, *N. oculata*, *A. carterae*, and *P. tricornutum* against LPS-induced RAW macrophages in a dose-dependent manner. According to the results, the *n*-hexane and chloroform fractions from *N. oculata* extract significantly inhibited the NO productions at all the concentrations against LPS-induced RAW 264.7 cells in vitro (Fig. 3A) compared to the positive control. In particular, at the lowest concentration as 6.25 μg mL^{-1} of both extracts, *N. oculata* hexane (NOH) and *N. oculata* chloroform (NOC) exhibited less than 40% of NO production against LPS-induced RAW 264.7 cells. Moreover, *C. ovalis* ethyl acetate (COE), *A. carterae* chloroform (ACC), *P. tricornutum* hexane (PTH), and *P. tricornutum* chloroform (PTC) fractions showed a significant inhibition of NO production compared to the other fractions extracted from the cultured marine microalgae (Figs 2, 4 & 5), respectively.

In addition, the cytotoxic effects on RAW 264.7 cells with the treated samples were performed by MTT assay. The cell viability (%) of the extracted samples from the cultured marine microalgae was shown in the Figs 2B, 3B, 4B & 5B. According to the results, NOH and NOC fractions were reported to have the high cell viability against RAW 264.7 cells compared to the other fractions followed by LPS induced toxicity. More importantly, the concentration of NOC at 6.25 μg mL^{-1} was shown the highest cell viability as nearly 120% compared to the negative control. In addition, same the concentration of PTC and NOH are also reported more than 100 (%) of cell viability with comparatively significant differences. The fractions

of *C. ovalis* chloroform (COC) and COE were shown to be nearly 100% of cell viability at 25 μg mL^{-1} and 50 μg mL^{-1} concentrations, respectively. These results are evidenced that the increased cell viability of the samples against LPS treated toxicity followed by significant suppression of NO production as well. Recently reported many publications have indicated that containing of anti-inflammatory components in *P. tricornutum* and *Chlorella stigmatophora* organic solvent extracts (Guzmán et al. 2003). Therefore, these results were suggested that the strong anti-inflammatory activities were observed in the extracts and the fractions derived from the cultured marine microalgae *in vitro* assay.

Anticancer effect against HL-60, B16F10, and A549 cell lines

The cell growth inhibitory effect was examined against different cancer cell lines followed by pre-treated cultured marine microalgae solvent extracts and fractions for screening anticancer activity of the samples. Figs 6 and 7 represent the cell viability against treated samples from cultured marine microalgae including *C. ovalis* and *A. carterae* extracts on the different cell lines such as a human promyelocytic leukemia cell line (HL-60) (Figs 6A & 7A), a mouse melanoma cell line (B16F10) (Figs 6B & 7B) and a human lung carcinoma cell line (A549) (Figs 6C & 7C), respectively. In this study, significant growth inhibitory effects were observed from COE and ACC against HL-60 cell lines and the anticancer activities were dose dependent at 25 μg mL^{-1} and 50 μg mL^{-1} concentrations. The cultured dinoflagellate, *A. carterae* extracts such as *A. carterae* hexane fraction (ACH), ACC, and *A. carterae* ethyl acetate fraction (ACE) also effectively suppressed the growth of HL-60, B16F10, and A549 cell lines in vitro compared to the control. To our knowledge, few of reports have been published with the extracts of bioactive metabolites from the cultured marine microalgae. Recently, two monogalactosyl diacylglycerols were isolated from the cultured marine *P. tricornutum* and reported as inducing apoptosis in two genetically-matched immortal mouse epithelial cell lines (Andrianasolo et al. 2008). Moreover, these results indicate the potential of cultured marine microalgae species for anticancer activities and should lead to study further on secondary metabolites.

CONCLUSION

In the present study, four different marine microalgae species including *Chlorella ovalis*, *Nannchloropsis oculata*, *Amphidinium carterae*, and *Phaeoductylum tricornutum* were cultured under optimum growth conditions. Crude solvent extracts and fractions from the cultured marine microalgae were screened to determine the anti-inflammatory and anticancer activities *in vitro*. Among the solvent extracts of the cultured microalgae species, NOH and NOC fractions showed the strongest inhibitory activity of NO production level against LPS-induced RAW macrophages and did not show the significant cytotoxicity at all the concentrations. Significant suppression of HL-60 cancer cell growth was observed; COE and ACC extracts recorded the highest anticancer activity among the tested anticancer screenings. Proximate chemical composition appeared to show the nutrient profile of each of the cultured marine microalgae. Therefore, active fractions that have demonstrated anti-inflammatory and anticancer activity should be subjected to further isolation in order to determine the bioactive functional ingredients.

ACKNOWLEDGEMENTS

This research was sincerely supported by the Jeju Sea Grant Program and funded by the Ministry of Land, Transport and Maritime, Korea.

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