ORIGINAL ARTICLE

Does *Chlorella vulgaris* Modulate the Expression of *COL* and *MMP* Genes in Skin Ageing?

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ABSTRAK

Chlorella vulgaris, sejenis mikroalga unisel, menghasilkan banyak fitokimia intrasel seperti karotenoid, tokoferol, ubikuinon dan protein. Penuaan kulit diaruh oleh tekanan oksidatif melibatkan penurunan sintesis matriks ekstrasel dan peningkatan ekspresi enzim yang mendegradasikan matriks kolagen. Objektif kajian yang dijalankan ini adalah untuk menentukan kesan *C. vulgaris* terhadap ekspresi gen-gen yang mengkodkan kolagen (*COL*) dan matriks metaloproteinase (*MMP*) yang terlibat di dalam penuaan kulit. Sel fibroblas diploid manusia (HDF) diperolehi daripada kulit khatan kanak-kanak lelaki yang berusia 8-12 tahun. HDF telah dikulturkan kepada 3 kumpulan: sel kawalan tanpa rawatan, sel dengan penuaan pramatang aruhan tekanan oksidatif (SIPS; sel diaruh dengan H_2O_2 pada pasaj 6 selama 2 minggu) dan SIPS yang dirawat dengan *C. vulgaris* (rawatan *C. vulgaris* berpanjangan bermula pada pasaj 4 dan gabungan rawatan dengan H_2O_2 pada pasaj 6 selama 2 minggu). ß-Galaktosidase berkait-penuaan ditentukan menggunakan kit pewarnaan histokimia sel senesen (Sigma, USA). Ekspresi gen *COLI, COLIII, COLIV, MMPI, MMPII* dan *MMPIII* dianalisa secara kuantitatif menggunakan kaedah *real-time* RT-PCR (*iScript™ One Step real-time PCR with SYBR® Green;* Biorad). HDF yang dirawat dengan H_2O_2 (SIPS) menunjukkan morfologi sel senesen seperti sel menjadi rata dan membesar dengan peningkatan ekspresi ß-galaktosidase berkait-penuaan ($p<0.05$). Analisa ekspresi gen menunjukkan *COLI* menurun di dalam SIPS dan SIPS yang dirawat dengan *C. vulgaris* ($p<0.05$) manakala *COLIII* menurun di dalam SIPS dan meningkat di dalam SIPS yang dirawat dengan *C. vulgaris* ($p<0.05$). Ekspresi *MMPI* meningkat ($p<0.05$) di dalam SIPS dan SIPS yang dirawat dengan *C. vulgaris* menunjukkan kesan sinergistiknya bersama dengan rawatan H_2O_2. Sebagai kesimpulannya, pada penuaan kulit, ekspresi gen *COLI* dan *COLIII* menurun manakala *MMPI* meningkat. *C. vulgaris* telah memodulasikan ekspresi gen *COL* dan *MMP* dengan menurunkan ekspresi *COLI* dan meningkatkan ekspresi *COLIII* dan *MMPI* tetapi ia tidak menunjukkan kesan anti penuaan.

Kata kunci: *Chlorella vulgaris,* penuaan kulit, kolagen, MMP, ekspresi gen

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ABSTRACT

Chlorella vulgaris, a unicellular microalgae, produces many intracellular phytochemicals namely carotenoids, tocopherols, ubiquinone and protein. Skin ageing which is induced by oxidative stress involves decreased extracellular matrix synthesis and increased expression of enzymes that degrade the collagenous matrix. The objective of this study was to determine the effect of *C. vulgaris* on the expression of genes encoded for collagen (*COL*) and matrix metalloproteinases (*MMPs*) which are involved in skin ageing. Human diploid fibroblasts (HDFs) were obtained from circumcision foreskin of 8-12 year-old boys. HDFs were cultured into 3 groups: untreated control cells, cells with stress-induced premature senescence (SIPS; cells were induced with H_2O_2 at passage 6 for 2 weeks) and SIPS treated with *C. vulgaris* (prolonged *C. vulgaris* treatment started at passage 4 and combined treatment with H_2O_2 at passage 6 for 2 weeks). Senescence-associated ß-galactosidase (SA ß-gal) was determined using senescent cells histochemical staining kit (Sigma, USA). Expression of *COLI, COLIII, COLIV, MMPI, MMPII* and *MMPIII* genes was quantitatively analysed with real-time RT-PCR method (iScript™ One Step real-time PCR with SYBR® Green; Biorad). HDFs treated with H_2O_2 (SIPS) exhibited senescent morphological features of flattening and enlarged with increased expression of SA ß-gal ($p<0.05$). Gene expression analysis showed *COLI* was downregulated in SIPS and SIPS treated with *C. vulgaris* ($p<0.05$) while *COLIII* decreased in SIPS and increased in SIPS treated with *C. vulgaris* ($p<0.05$). Expression of *MMPI* was increased in SIPS and SIPS treated with *C. vulgaris* ($p<0.05$) indicating its synergistic effect with H_2O_2 treatment. In conclusion, in skin ageing, *COLI* and *COLIII* genes were downregulated while *MMPI* was upregulated. *C. vulgaris* modulated the expression of *COL* and *MMP* genes by downregulating *COLI* and upregulating *COLIII* and *MMPI* but it did not exert anti-ageing effect.

Key words: *Chlorella vulgaris,* skin ageing, collagen, MMP, gene expression

INTRODUCTION

The ageing process is the accumulation of oxidative damage to cells and tissues, which is associated with a progressive increase in the chance of morbidity and mortality (Beckman & Ames 1998). Human ageing can be studied *in vitro*, specifically with normal human diploid fibroblasts (HDFs) which undergo a limited number of cell divisions in culture and progressively reach a state of irreversible growth arrest, a process termed as replicative senescence (Trougakos et al. 2006*)*. Senescent cells exhibit a gradual loss of replicative potential that results in reduced harvest cell densities and cell saturation densities (Cristofalo et al. 1998) and expressed senescence associated ß-galactosidase enzyme (SA β-gal) which can be detected by SA ß-galactosidase staining (Dimri et al. 1995). Since senescent cells have been shown to accumulate with age in human tissues, it has been proposed that they contribute to organismal ageing (Campisi 2000).

HDFs offer a typical model for stress induced premature senescence (SIPS) as it exhibits a finite potential of differentiation (Campisi et al. 2007). SIPS which shares the common features of replicative senescence can be defined as sustained effects of subcytotoxic stress on proliferative cell types, including irreversible growth arrest of the cell population. Various oxidative agents such as

hydrogen peroxide (H_2O_2) and ultraviolet light have been used to induce SIPS and H_2O_2 appeared to be the most commonly used agent.

Skin is the largest and outermost organ of the human body. Exposure to the environment resulted in the cellular components such as lipid, protein and DNA being targets to oxidative agents and susceptible to oxidative damage. Collagens are the main protein found in the skin of which collagen type I is the most abundant. It is incorporated into collagen type III, which is an important substance in reticular tissues in the dermis while collagen type IV is a crucial structure most commonly found in the basement membranes and is responsible for the intrinsic cohesiveness of functional basement membranes (Varani 2006). Three distinct collagenases are responsible for degrading connective tissues namely MMPI, MMPII and MMPIII (Shingleton et al. 1996). MMPI is also called collagenase 1 or interstitial collagenase which degrades type I collagen while MMPII is known as gelatinase A and readily digests the denatured collagens type I, II, III and gelatins. MMPIII which is also known as stromelysin I, digests extracellular matrix and activates proMMPI (Nagase & Woessner 1999).

Skin tissue is frequently and directly exposed to a pro-oxidative environment, including ultraviolet radiation (UV), drugs and air pollutants. Besides external inducers of oxidative attack, the skin has to cope with endogenous generation of reactive oxygen species (ROS) and other free radicals, which are continuously being produced and caused skin ageing (Suzanne et al. 2003).

Skin ageing is defined as structural changes of the skin as the age increases. It is indicated as the inability to balance the important functions of the skin leading to cell death (Chandrasoma & Taylor 2001). During the ageing process, there is an accumulation of oxidation products such as oxidized proteins, DNA adducts and lipid metabolites. In addition, there is a significant decrease in the defense system of the living cell, including a significant decrease in the antioxidant defense system (Beckman & Ames 1998). Balance between oxidants and antioxidants are needed to minimize molecular, cellular and tissue damage. However, if the balance is upset in favour of the oxidants, oxidative stress could occur and results in oxidative damage. Reactive oxygen species (ROS) are known to cause oxidative modification of DNA, proteins, lipids and small cellular molecules (Kang et al. 2005). To counteract the harmful effects of ROS, the various compartments of the skin are equipped with layer specific antioxidant systems to maintain equilibrium between ROS and antioxidant systems and thus prevent oxidative stress (Thiele & Ekanayake-Mudiyanselage 2007).

The unicellular algae *C. vulgaris* contains many bioactive substances such as carotenoids, chlorophyll, tocopherols, ubiquinone, and protein. C. *vulgaris* is capable of inhibiting lipid peroxidation and interacts with enzymatic and non-enzymatic antioxidants system attenuating and resisting the oxidative stress and further lipid peroxidation. A recent study has shown that *C. vulgaris* has great potential as an antioxidant with the ability to exert anti tumor effects against liver cancer (Mukti et al. 2009).

The ethanolic extract of *C. vulgaris* expressed significant antioxidant activity against naphthalene-induced oxidative stress in rat model (Vijayavel et al. 2007) while *Chlorella dichloromethane* extract ameliorates NO production and iNOS expression through the down-regulation of NFκB activity mediated by suppressed oxidative stress in RAW 264-7 macrophage (Park et al. 2005). Another study reported that administration of *C. vulgaris* inhibited MMPI activity (Cherng & Shih 2006).

Since skin tissue is frequently and directly exposed to a pro-oxidative environment such as ultra violet radiation which results in oxidative damage and further causes cellular ageing, therefore in this study *C. vulgaris* was evaluated for its antioxidant properties in preventing skin ageing. Primary culture of HDFs which was exposed to H_2O_2 was treated with *C. vulgaris* and the expression of *COL* and *MMP* genes which are involved in skin ageing was determined.

MATERIALS AND METHODS

Cell culture, induction of senescence and treatment with C. vulgaris

Primary culture of HDFs was derived from circumcised foreskin of 9-12 year-old boys, from a local clinic. Written informed consents were obtained from all subjects. The samples were aseptically collected and washed several times with 75% alcohol and phosphate buffered saline (PBS) containing 1% antibiotic–antimycotic (PAA, Austria). After removing the epidermis, the pure dermis was cut into small pieces and transferred into a falcon tube containing 0.03 % collagenase type I solution (Worthington Biochemical Corporation, USA). Pure dermis was digested in an incubator shaker at 37ºC for 6-12 hours. Then, cells were rinsed with PBS before being cultured in Dulbecco Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) (PAA, Austria) and 1% antibiotic–antimycotic at 37ºC in a 5% CO_2 humidified incubator. After 5–6 days, the cultured HDFs were harvested by trypsinization and culture-expand into new T25 culture flasks (Nunc, Denmark) with expansion degree of 1:4. When the subcultures reached 80–90% confluence, serial passaging was done by trypsinization and the number of population doublings (PDs) was monitored.

HDFs were divided into three treatment groups viz; i) untreated control ii) H_2O_2-induced oxidative stress, SIPS (10 µM of H_2O_2 exposure for two weeks) iii) cells pretreated with *C. vulgaris* extract before oxidative stress induction. Treatment with *C. vulgaris* extract was started at passage 4 at dose 400 mg/ml based on the optimal cell viability that has been established by the Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia. It was a prolonged treatment whereby at passage 6, HDFs were induced with H_2O_2 and treated with *C. vulgaris* extract.

Preparation of C. vulgaris hot water extract

In this study, a green unicellular microalgae *C. vulgaris* Beijerinck grown in Bold's Basal Media was used. Dried *C. vulgaris* cells were suspended in distilled water at a concentration of 10% w/v, boiled at 100°C for 20 minutes and then centrifuged at 10000 rpm for 20 minutes. The supernatant was subsequently lyophilized to obtain *C. vulgaris* extract which was then added to cell culture growth medium as supplementation.

Morphology analysis and senescence-associated β-galactosidase (SA-β-gal) staining.

HDFs positive for SA-β-gal activity was determined as described by Dimri et al. (1995). SA-β-gal staining was performed with a senescent cell staining kit (Sigma, USA) according to the manufacturer's instructions. A total of $1X10^5$ cells were seeded in six-well plates and incubated with fixation buffer (2% formaldehyde/0.2% glutaraldehyde) for 6-7 mins at room temperature. Cells were then rinsed three times with PBS and incubated with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside at 1 mg/ml in a

buffer containing 40 mM citric acid/phosphate (pH 6.0), 5 mM K_3FeCN_6, 5 mM K_4FeCN_6, 150 mM NaCl, and 2 mM $MgCl_2$ for 4 h at 37°C in the absence of CO_2. Blue staining was visible after incubation and the percentage of blue cells observed in 100 cells under a light microscope was calculated.

Primer design

The expression level of each targeted gene was normalized to the housekeeping gene which is GAPDH. Primer 3 software was used to design the primers for each targeted gene and GADPH which were further blasted with GenBank database sequences from National Center of Biotechnology Information in order to obtain primers with high specificity. The sequence of the primers is shown in Table 1.

Total RNA extraction

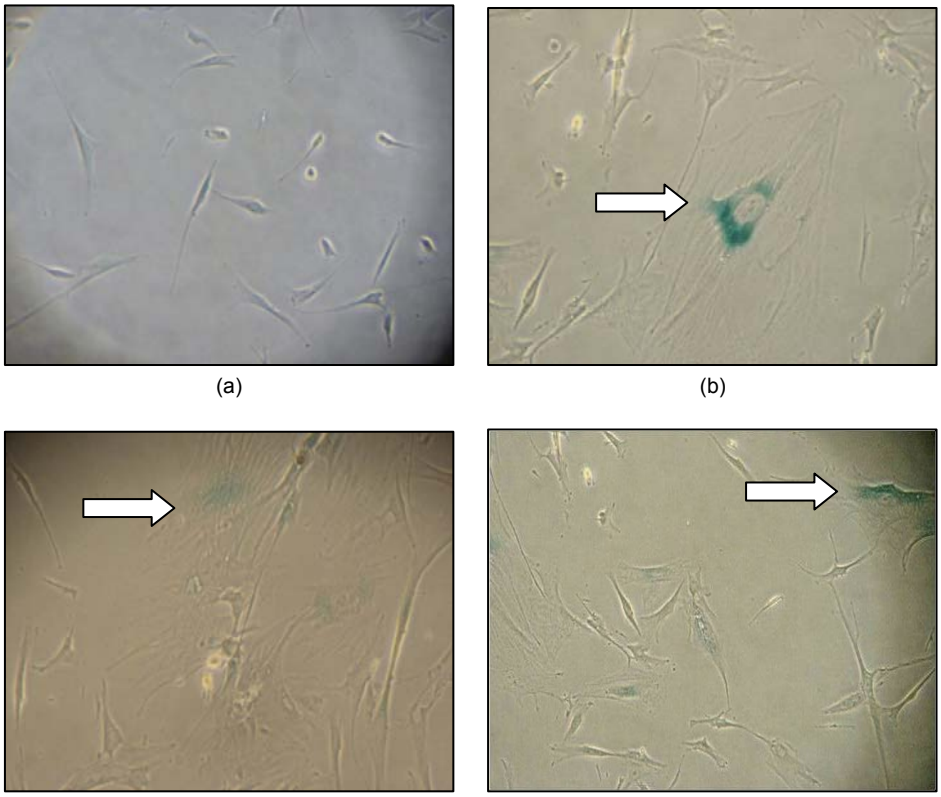
Total RNA from HDFs of each group was isolated using TRI Reagent (Molecular Research Centre, Inc.) according to the manufacturer's instruction. Polyacryl carrier was added to each extraction to precipitate the RNA and centrifuged for 8 mins at 12000g at 4°C. The pellet was washed with 75% ethanol. After another centrifugation, the extracted RNA pellet was left to dry at room temperature for 15 mins and then dissolved in RNase and DNase free distilled water. Total RNA was stored at -80°C immediately after extraction.

Quantitative real-time RT-PCR

Gene expression level of *COLI, COLIII, COLIV, MMPI, MMPII,* and *MMPIII* was quantitatively analysed with one-step real time RT-PCR technique. The expression level of each targeted gene was then normalized to GAPDH. Real-time PCR reaction was performed with 100 ng of total RNA, 400 nM of each primer and iScript One-Step RT-PCR kit with SYBR Green (Biorad, Canada) according to the manufacturer's instruction. Reactions were run in Bio-Rad iQ5 with reaction profile as follows; cDNA synthesis for 20 mins at 50°C; pre-denaturation for 4 mins at 95°C; PCR amplification for 38 cycles with 10 secs at 95°C, 30 secs at 61°C. This was followed by a melt curve analysis to determine the reaction specificity. Agarose gel electrophoresis was performed for confirmation of the PCR products.

Table 1: Primer sequences for quantitative gene expression analysis

Genes	Label	Primer sequence	PCR product size (bp)	Accession number
COLI	Forward	5'-agggctccaacgagatcgagatccg-3'	216	NM000088
	Reverse	5'-tacaggaagcagacagggccaacg-3'		
COLIII	Forward	5'-ccaggagctaacggtctcag-3'	103	NM_000090
	Reverse	5'-cagggtttccatctcttcca-3'		
COLIV	Forward	5'-ctggtccaagaggattcca-3'	193	NM_033641
	Reverse	5'-tcattgccttgacgtagag-3'		
MMPI	Forward	5'-acagcttcccagcgactcta-3'	200	NG_011740
	Reverse	5'-cagggtttcagcatctggtt-3'		
MMPII	Forward	5'-aacccagatgtggccaacta-3'	199	NM_001127891
	Reverse	5'-tgatgtctgcctctccatca-3'		
MMPIII	Forward	5'-ggccagggattaatggagat-3'	191	NG_011468
	Reverse	5'-ggaaccgagtcaggtctgtg-3'		
GAPDH	Forward	5'-tccctgagctgaacgggaag-3'	217	BC02030
	Reverse	5'-ggaggagtgggtgtcgctgt-3'		



(a) (b)

(c) (d)

Figure 1: Cell morphology and senescence-associated β-galactosidase expression. (a) Young untreated HDFs, (b) *Chlorella vulgaris* and H_2O_2-treated HDFs, (c) senescent HDFs at passage 30 and (d) H_2O_2-treated HDFs. Young HDFs was spindle shaped while senescent and H_2O_2-treated HDFs lost their original fibroblastic shape by acquiring a flattened and elongated morphology with increased in size of nucleus and cells. Positive blue stain of SA-β-galactosidase appeared in senescent and H_2O_2-treated HDFs as indicated by arrow. Micrographs are shown at 200X magnification.

Statistical analysis

Each experiment was carried out in duplicates. Data were reported as mean ± SD. Comparison between groups was made by Student t-test (two-tailed). $p<0.05$ was considered statistically significant.

RESULTS

Cell morphology and senescence-associated β-galactosidase expression.

Morphological changes were observed with ageing of the HDFs. Young HDFs displayed the normal spindle shape characteristic of fibroblast cells. However, in SIPS, the original fibroblastic shape was lost and HDFs became larger and flattened with accumulation of cytoplasmic granular inclusions. Similar cell morphology was observed for SIPS cells which were treated with *C. vulgaris* (Figure 1).

A positive blue stain of SA-β-gal appeared mainly in H_2O_2-treated HDFs (SIPS cells) suggesting the presence of senescent cells. Quantitative analysis showed the percentage of cells positive for SA-β-gal staining was increased ($p<0.05$) in SIPS compared to young untreated HDFs (Figure 2). Similar results

were observed for senescent cells at passage 30 and SIPS treated with *C. vulgaris* ($p<0.05$).

Specificity of primers and real-time RT-PCR

Six senescence-associated gene primers were designed. Agarose gel electrophoresis showed that each PCR product appeared as single bands (Figure 3). The melting curve analysis showed a single and narrow peak of each PCR product (Figure 4) indicating that the primers designed and the real-time RT-PCR protocols were specific.

Expression of COL and MMP genes

Treatment with H_2O_2 caused a downregulation of *COLI* and *COLIII* genes (Figure 5) and upregulation of *MMPI* gene (Figure 6) compared to untreated control HDFs ($p<0.05$). H_2O_2-treated HDFs (SIPS cells) which were incubated with *C. vulgaris* extract showed downregulation of *COLI* gene and upregulation of *MMPI* gene compared to untreated control HDFs ($p<0.05$). *COLIII* gene was upregulated with *C. vulgaris* treatment compared to H_2O_2-treated HDFs ($p<0.05$).

DISCUSSION

The present study evaluated the effects of *C. vulgaris* extract in possibly modulating H_2O_2-induced cellular senescence (SIPS) in skin HDFs. Our results showed that when HDFs reached senescence, there were clear changes in cell morphology, decreased cell proliferation and increased senescence associated β-galactosidase activity. Morphological changes are typical features of senescent phenotype that can occur at both cellular and organism level (Cho et al. 2004). Senescent HDFs showed morphological changes of flattened and enlarged cell shapes. This shift is accompanied by changes in nuclear structure, gene expression, protein processing and metabolism (Campisi 2000).

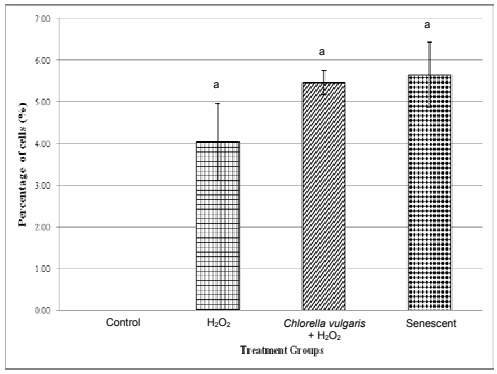


Figure 2: Quantitative analysis of positive β-galactosidase stained HDFs. The percentage of cells positive for SA-β-gal staining was significantly increased in H_2O_2-treated HDFs. Incubation of H_2O_2-treated HDFs with *Chlorella vulgaris* failed to cause a reduction in the percentage of cells positive for SA-β-gal staining. [a] Denotes $p<0.05$ compared to control. Data is presented as mean ± SD.

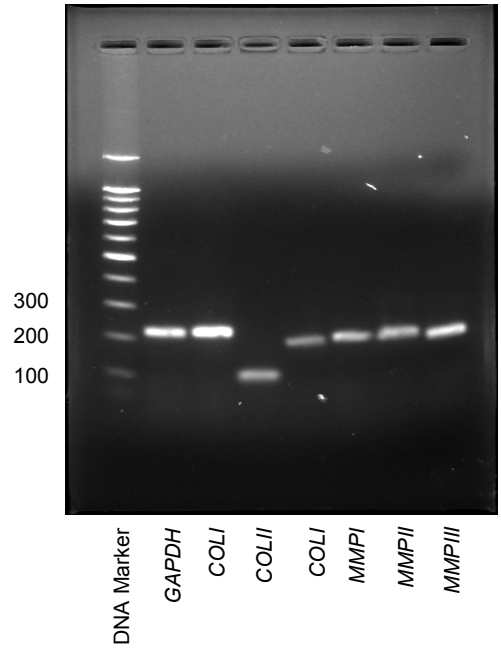


Figure 3: Agarose gel electrophoresis for PCR products of *COLI, COLIII, COLIV, MMPI, MMPII* and *MMPIII* genes in HDFs demonstrated the specificity of primers used.

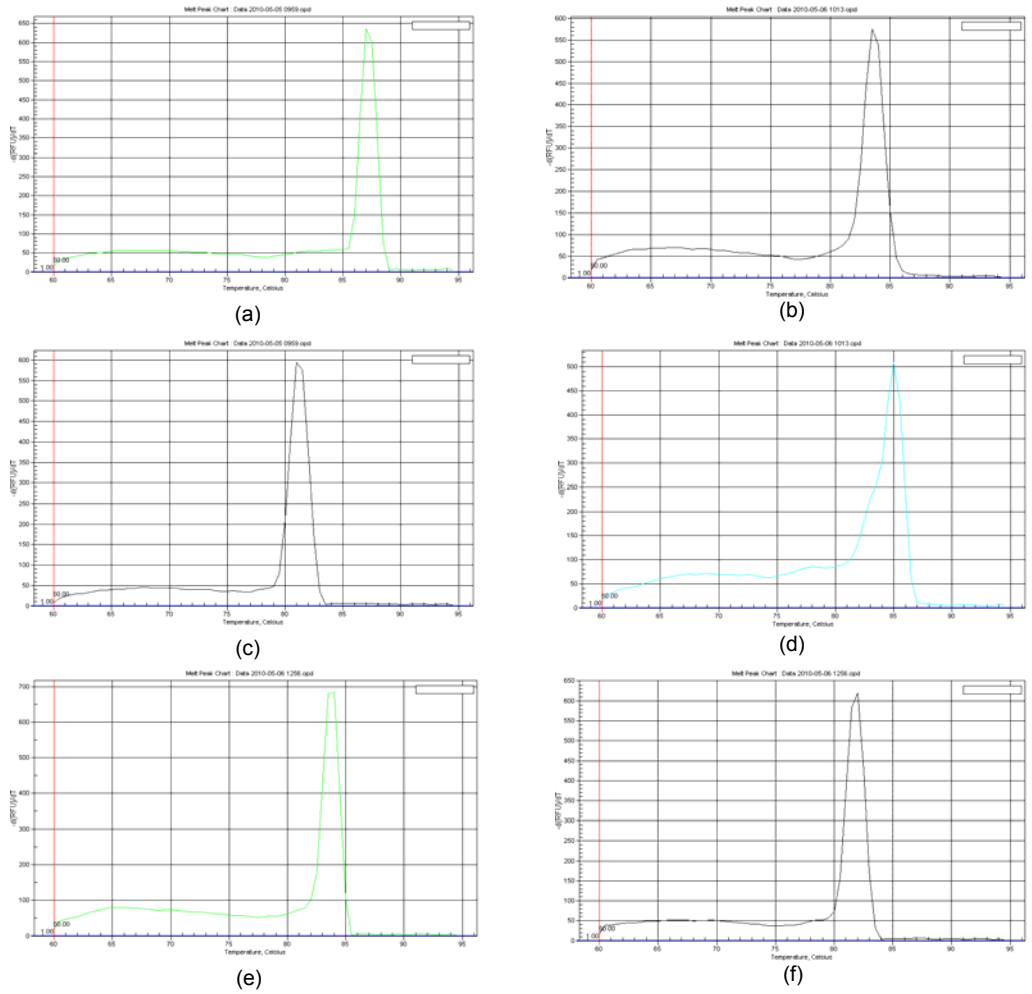
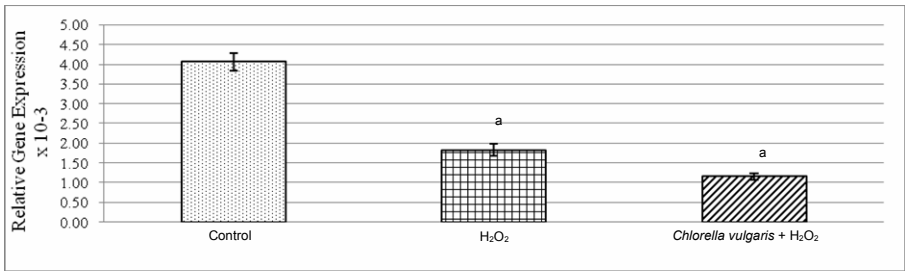


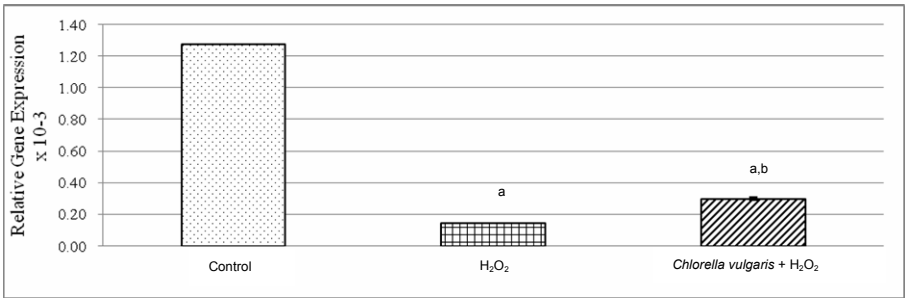
Figure 4: Melting curve analysis showed single and narrow peak indicating the primers and reactions were specific for (a) *COLI* gene, (b) *COLIII* gene, (c) *COLIV* gene, (d) *MMPI* gene, (e) *MMPII* gene and (f) *MMPIII* gene.

Bayreuther et al. (1988) stated that progressive morphological changes occur while cells aged *in vitro*. Senescent cells are bigger and a senescent population has more diverse morphotypes than cells at earlier cumulative population doublings. In fact, a confluent senescent culture has a smaller cellular density than a confluent young culture, though this also occurs because senescent cells are more sensitive to cell-cell contact inhibition. These changes are concomitant with increased activity of β-galactosidase measured at pH 6 (Dimri et al. 1995; Krishna et al. 1999). In the present study, *C. vulgaris* extract however was not able to show any morphological effects on H_2O_2-induced cellular senescence.

Extracts of *C. vulgaris* has previously been reported to show antitumour effect in fibrobsarcomas (Konishi et al. 1985; Hasegawa et al. 2002) and liver cancer (Mukti et al. 2009). The algal extract has also been shown to exhibit possible anticancer properties in hepatocellular carcinoma cell lines, HepG2 (Llovet et al. 2003; Md. Saad et al. 2006). There was also a report of using *C. vulgaris* as a



(a)



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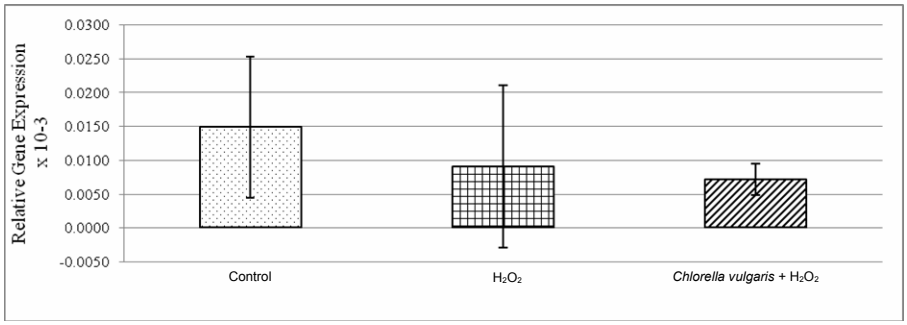
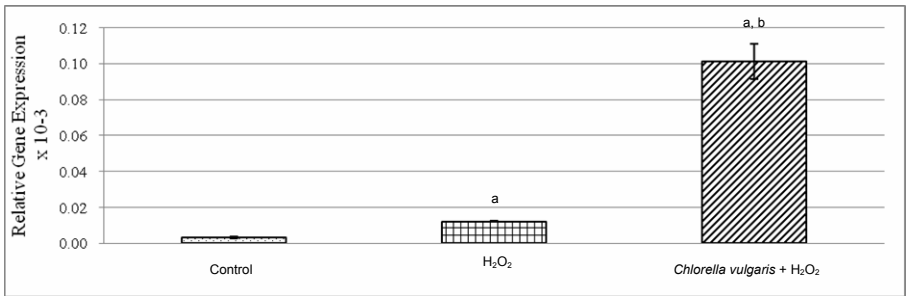


(c)

Figure 5: Relative mRNA expression of (a) *COLI* gene, (b) *COLIII* gene, (c) *COLIV* gene. [a]Denotes $p<0.05$ compared to control. [b] $p<0.05$ compared to H_2O_2-treated HDFs. Data is presented as mean ± SEM.

potential therapeutic agent against advanced glycation end product (AGE), in which AGE has long been recognized and implicated in the development of age related disorders such as atherosclerosis and diabetes (Yamagishi et al. 2005). *C. vulgaris* also had been shown to modulate H_2O_2-induced DNA damage and telomere shortening of human fibroblasts derived from different aged individuals whereby it exhibited bioprotective effects against free radical attacks (Makpol et al. 2009).

Our data showed that the mRNA expression of *COLI* and *COLIII* decreased in H_2O_2-induced cellular senescence of skin HDFs while the expression of matrix metalloproteinase 1 mRNA (*MMP1*) was increased when cells aged. Previous study reported among the changes in gene expression in senescent cells, the over expression of matrix metalloproteinases (MMPs) was striking resulting in lost of proteins maintaining ultra structural shape (Fisher et al. 2008). Relative overproduction of collagenase in ageing



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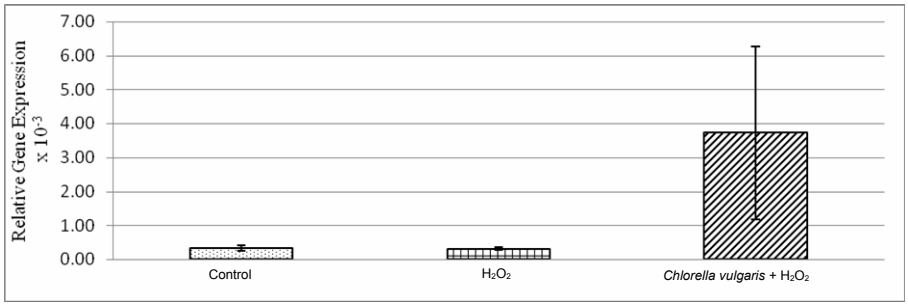

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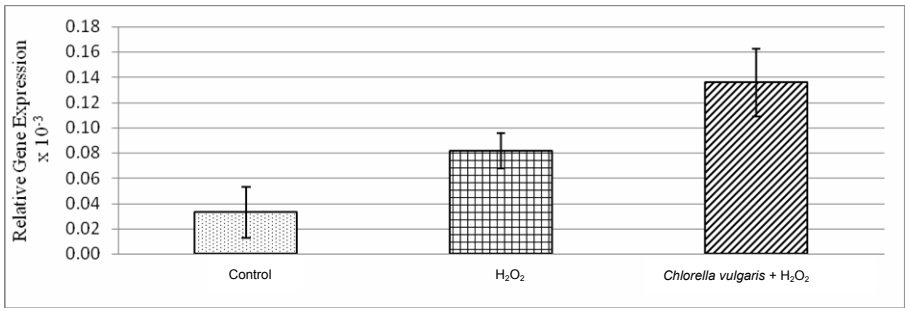

(c)

Figure 6: Relative mRNA expression of (a) *MMPI* gene, (b) *MMPII* gene and (c) *MMPIII* gene. [a] Denotes $p<0.05$ compared to control, [b] $p<0.05$ compared to H_2O_2-treated HDFs. Data is presented as mean ± SEM.

cells was proposed as the matrix-degrading phenotype of senescent cells (Mawal-Dewan et al. 2002). In addition of that, it has been reported that senescent HDFs had decreased expression of several extracellular matrix components such as collagen I-1α , collagen III-1α and elastin besides increased expression of collagenase and stromelysin; two enzymes that serve to breakdown the extracellular matrix (Fisher et al. 2008).

In this study, prolonged *C. vulgaris* treatment was not able to upregulate the mRNA expression of *COLI* or downregulate the mRNA expression of *MMP1* in H_2O_2-induced senescent HDFs eventhough the expression of *COLIII* was increased. These findings could be due to the irreversible effects of H_2O_2 in skin HDFs. According to studies by Chen et al. (2004) H_2O_2 induced senescent morphological changes and irreversible arrest in cell replicative capacity in HDFs F65 cells. With H_2O_2 treatment, the cells could not enter the cell cycle again even with mitogen stimulation. The effects of

H_2O_2 were cumulative and irreversible. This might explain the reason why *C. vulgaris* could not reverse the effects of H_2O_2 in our study. In addition to that, H_2O_2 was reported to increase the steady-state mRNA levels of collagenase or *MMPI* in HDFs. It was an important intermediate in the downstream signaling pathway which leads to the induction of increased steady state *MMPI* mRNA levels (Olsen et al. 1989).

Compared to other antioxidants, relatively few researches had been done to explore the components of *C. vulgaris.* Therefore the chemical composition of *C. vulgaris* and its beneficial potential especially in preventing skin ageing have not been fully recognized. Various bioactive compounds in *C. vulgaris* may react to inhibit growth in cells that had been damaged by the oxidative stress induced by H_2O_2. *C. vulgaris* was reported to induce apoptosis in order to maintain normal cell homeostasis (Md Saad et al. 2006).

In our study, the bioprotective effect of *C. vulgaris* extract in preventing skin ageing was determined. H_2O_2 was added at passage 6 to induce cellular ageing of the skin while at the same time HDFs were treated with *C. vulgaris* extract. Unfortunately there was no extensive study on the influence of H_2O_2 towards the effectiveness of prolonged *C. vulgaris* extract treatment. Therefore for future studies, we would like to suggest that normal replicative senescence model shall be used in order to elucidate the protective effects of *C. vulgaris* extract against skin ageing.

CONCLUSION

In skin ageing, *COLI* and *COLIII* genes were downregulated while *MMPI* was upregulated. *C. vulgaris* extract did modulate the expression of *COL* and *MMP* genes by downregulating *COLI* and upregulating *COLIII* and *MMPI* but it did not exert anti-ageing effect.

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