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Original Article

Photoprotection of *Chlorella* sp. and *Tetradesmus dimorphus* Extracts on Ultraviolet-Irradiated Human Retinal Pigment Epithelial Cells

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Abstract

Introduction: Ultraviolet radiation induces retinal cell injury associated with age-related macular degeneration (AMD). Bioactive compounds from microalgae have demonstrated photoprotective properties against ultraviolet B (UVB)-induced injury, however their protective activity on the retinal pigment epithelium (RPE) remains unexplored. In this study, the photoprotective effects of *Chlorella* sp. and *Tetradesmus dimorphus* extracts on UVB-irradiated ARPE-19 cells were investigated.

Materials and Methods: ARPE-19 cells were irradiated with UVB, followed by treatment with *Chlorella* sp. and *T. dimorphus* extracts from distilled water, absolute methanol, 20% aqueous methanol (v/v), or 95% ethanol. Viability of ARPE-19 cells was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), while oxidative stress was evaluated with diacetyldichlorofluorescein (DCFH-DA). The presence of acidic vesicular organelles (AVOs) and apoptotic cells was detected through acridine orange and Hoechst 33258 staining.

Results: Aqueous-methanol (20%) extracts of *Chlorella* sp. (1 µg/ml) improved ARPE-19 cell viability by 23.26%, while other solvent extracts of *T. dimorphus* did not show significant improvement compared to the control group. Besides, aqueous extracts of *Chlorella* sp. (1 µg/ml) showed the greatest reduction in reactive oxygen species (ROS) level by 0.46-fold, while aqueous-methanol (20%) extracts of *T. dimorphus* (10 µg/ml) decreased the ROS level by 0.33-fold. Moreover, treatment with aqueous-methanol (20%) extracts of *Chlorella* sp. and *T. dimorphus* reduced autophagy by 38.52% and 36.26%, respectively. Apoptotic activity was attenuated by aqueous extracts of *Chlorella* sp. (37.33%) and *T. dimorphus* (46%).

Conclusions: *Chlorella* sp. and *T. dimorphus* extracts exert photoprotective effects on UVB-irradiated ARPE-19 cells, highlighting their potential as a novel approach for AMD treatment.

Keywords: Ultraviolet B, *Chlorella* sp., *Tetradesmus dimorphus*, Microalgae, Human Retinal Pigment Epithelial Cells, Photoprotection

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Introduction

Retinal pigment epithelial (RPE) cells are a group of specialised hexagonal cells located between the photoreceptors and Bruch's membrane, forming the outermost lining of the retina. RPE cells play a vital role in protecting the retina against photooxidative damage and performing phagocytosis to degrade damaged photoreceptor cells.[1,2] Ultraviolet (UV) radiation is a major risk factor for eye diseases such as cataracts and macular degeneration in the older age group. The primary source of UV radiation is natural sunlight, which can be divided into UVA, UVB and UVC based on their wavelength. Most of the UV rays emitted from sunlight are absorbed by the corneal epithelium to reduce harm to the lens and retina.[3,4] Although only low levels of UV rays reach the retina, excessive UV radiation due to ozone layer depletion and cumulative exposure can cause detrimental effects on RPE cells through various mechanisms.[5] Previous studies have proven that UVB irradiation induces the production of reactive oxygen species (ROS) and inflammation in RPE cells, as well as triggers cell apoptosis and autophagy, which can lead to macular degeneration.[5,6]

Age-related macular degeneration (AMD) is a neuro degenerative condition that affects the macula region of

the retina, resulting in permanent vision loss.[5] AMD is characterised by the degeneration of RPE cells and the loss of photoreceptors due to oxidative stress and impaired lysosomal function in RPE cells, leading to the accumulation of drusen and lipofuscin deposits.[7] Exposure to UV light has been proven to trigger oxidative stress in RPE cells, and prolonged UV exposure is highly associated with an increased risk of developing AMD.[8] The prevalence of AMD has significantly increased in recent years, making it the leading cause of irreversible blindness, particularly among individuals aged 60 and older.[9] However, current treatments such as anti-VEGF therapy, photodynamic therapy and dietary supplements have limitations, highlighting the need for an alternative treatment to better manage and slow the progression of AMD.[10]

With the increased interest in discovering natural products with photoprotective properties, microalgae have emerged as a potential source of compounds exhibiting protective effects against UV-induced cellular damage. Microalgae are a group of photosynthetic eukaryotic microorganisms that grow in freshwater and marine systems and produce various bioactive compounds.[11] Studies have revealed that bioactive compounds, carotenoids and phenolic compounds, extracted from *Chlorella* sp. demonstrate antioxidant and anti-inflammatory properties that protect human skin cells from UV-induced damage.[12,13] Moreover, *Scenedesmus quadricauda* also contains mycosporine-like amino acids (MAAs) and astaxanthin, which can reduce the generation of ROS and suppress DNA damage in cells induced by UVB irradiation.[14,15] These bioactive compounds have been widely used in cosmetic products, especially in sunscreen formulations, due to their promising UV protection abilities that help prevent skin aging and cancer.[16]

Despite the reported potential benefits of bioactive compounds derived from microalgae, limited studies have focused on the photoprotective effect of tropical microalgae extracts on UV-irradiated human RPE cells. Therefore, research into the potential uses of microalgae-derived extracts to protect human RPE cells against UV irradiation is crucial for advancing treatments and preventive measures for retinal degeneration. This study aims to investigate the photoprotective effects of *Chlorella* sp. and *Tetradesmus dimorphus* extracts on UV-irradiated ARPE-19 cell lines by assessing cell viability, apoptosis, autophagy, and oxidative stress levels.

Materials and Methods

Cell Lines, Culture Media, and Reagents

The human retinal pigment epithelial cell line, ARPE-19, was purchased from the American Type Culture Collection (ATCC, USA). Dulbecco's Modified Eagle's medium/F-12 nutrient medium (DMEM/F-12) was obtained from Corning Inc., USA. Foetal bovine serum was purchased from Eurobio Scientific, France. Phosphate buffered saline (PBS) was obtained from MP Biomedicals, USA. Penicillin, streptomycin, acridine orange and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Medchem Express (MCE), USA. Hoechst 33258 was obtained from Sigma Aldrich, USA and 2′, 7′- dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from TCI, China. Dimethyl sulfoxide (DMSO) was purchased from Friedemann Schmidt, Germany.

Microalgae Culture

The tropical microalgae used in this study, *Chlorella* sp. (CHSS26-2), was isolated from a soil sample located in Cameron Highlands, Malaysia, while *Tetradesmus dimorphus* (N93) was purchased from the National Institute of Environmental Studies (NIES), Japan.[17] The microalgae were cultured in Bold's basal medium (BBM) and maintained in a controlled-environment incubator at 28 °C, supplied with cool white fluorescent lights (Philips, TLD 18W/54-765 providing 42 $\mu molm^{-2}s^{-2}$ PAR) on a 12h:12h light:dark cycle.

Microalgae Crude Extraction and Preparation

The solvent extraction protocol was adapted from Chaves-Peña et al. with modifications.[18] The freeze-dried microalgae were pulverised using mortar and pestle in the presence of liquid nitrogen. The pulverised samples were extracted in 50 ml screw-capped centrifuge tubes filled with different solvents (distilled water, absolute methanol, 20% aqueous methanol (v/v), 95% ethanol) in a water bath (Memmert, Germany) at 45 °C for 2 hours. Then, the microalgae crude extracts were centrifuged at 4000 rpm for 10 minutes (Eppendorf, USA). After centrifugation, the supernatant was collected, and the residue redissolved in specific solvents. Water bath extraction was repeated until the supernatant turned colourless.

The supernatants collected from absolute methanol and 20% methanol were dried using a rotary evaporator (Buchi, Switzerland), while the 95% ethanol extract was concentrated using a water bath at 60 °C. All the crude extracts were then subjected to freeze drying (Labconco Freezone, USA) overnight. The crude extracts containing organic solvents were dissolved in DMEM-F12 medium with 0.1% DMSO, while the aqueous extract was dissolved in DMEM-F12 medium. The microalgae extracts were then sterilised using a 0.22-micron filter to remove any impurities before cell treatment.

Retinal Cell Culture (ARPE-19 Cell Line)

The immortalised human retinal pigment epithelium (RPE) cell line, ARPE-19, was cultured in DMEM-F12 medium, supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were seeded at 2×10^4 cells/ml in a 96-well plate (NEST, USA) and 6×10^4 cells/ml in a 24-well plate (NEST, USA).

Cell Irradiation and Treatment

UVB irradiation was performed using a UVB lamp (Unitec, Elite Scientific Instrument Pte. Ltd.) with a wavelength of 312 nm and an intensity of 0.74 mW/cm^2. The cells were exposed to UVB irradiation for 19 minutes and 54 seconds which corresponded to their respective UVB dosage at 300 mJ/cm^2, as adapted from Oh et al. with modifications.[19] The section not exposed to UVB irradiation was covered with aluminium foil and served as a non-UV control. After UVB exposure, the cell culture medium was discarded, and the cells were treated with the extracts of *T. dimorphus* or *Chlorella* sp. The cells were divided into three groups: negative control, non-UV control, and treatment groups. The negative control group was exposed to UVB irradiation without microalgae extract treatment. Cells in the organic solvent control group were treated with DMEM-F12 medium containing 0.1% DMSO, while the aqueous control group was cultured with DMEM-F12 medium alone. The non-UV control group was not exposed to UVB irradiation and did not receive any microalgae extract treatment but was subjected to the same solvent conditions as the negative control. Treatment groups were exposed to UVB irradiation, followed by treatment with either organic solvent extracts dissolved in DMEM-F12 medium with 0.1% DMSO, or aqueous extracts in DMEM-F12 medium. Subsequently, all groups were incubated for 48 hours at 37 °C under a humidified 5% CO_2 atmosphere.

Cell Viability Assay

The cells were seeded in a 96-well plate, irradiated and treated with or without microalgae extracts (1, 10, 100 µg/ml) for 48 hours. Twenty microliters of MTT solution (5 mg/ml) were added into each well and incubated for 4 hours at 37 °C. After incubation, the culture medium was removed and replaced with DMSO to dissolve the purple formazan crystals formed. Finally, the absorbance of the solution was measured spectrophotometrically at 570/630 nm using a microplate reader (Tecan, Switzerland).

Measurement of ROS Level

The cells were seeded in a 96-well black plate (Sigma Aldrich, USA), irradiated and treated with or without microalgae extracts (1, 10, 100 µg/ml) for 48 hours. Subsequently, the microalgae extracts were removed, and 100 µl of 10 µM DCFH-DA reagent was added into each well and incubated for 30 minutes at 37 °C in the dark. ROS levels were detected using a microplate reader (Tecan, Switzerland) with excitation/emission wavelengths at 485/ 530 nm.

Intracellular Detection of Acidic Vesicles

ARPE-19 cells were seeded in a 24-well plate and treated with 1 µg/ml of microalgae extracts after UVB irradiation. After 48 hours of incubation, the extracts were discarded, and the cells were washed twice with PBS. The cells were stained with 250 µl of 5 µg/ml acridine orange for 10 minutes in the dark at room temperature, then washed with PBS twice. The presence of acidic vesicles was examined using an inverted fluorescence microscope (Olympus Optical, Japan) and images were captured at 200× magnification. The quantification of autophagic cells was performed using the Image J software.

Apoptosis Assay

To determine apoptotic nuclear morphology, Hoechst 33258 staining was used. Cells were seeded in a 24-well plate and treated with 1 µg/ml of microalgae extracts for 48 hours after UVB irradiation. Then, the extracts were removed, and the cells were washed with PBS and fixed with 4% formaldehyde for 20 minutes. The cells were stained with 5 µg/ml of Hoechst 33258 for 10 minutes in the dark, followed by PBS wash. The cells were observed under a fluorescence microscope (Olympus Optical, Japan) and fluorescence images were captured at 200× magnification. The number of apoptotic cells was counted using Image J software.

Statistical Analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). The results were analysed using paired *t*-test for comparison between the non-UV and UV control groups, and two-way ANOVA followed by Tukey's multiple comparison test for comparison among the treatment and control groups. The analysis was carried out using GraphPad Prism 10 software and a *p*-value less than 0.05 was considered statistically significant.

Results

Effect of Microalgae Extracts on UVB-Irradiated ARPE-19 Cell Viability

ARPE-19 cells were exposed to UVB (300 mJ/cm^2) and treated with *Chlorella* sp. and *T. dimorphus* extracts at concentrations ranging from 1 µg/ml to 100 µg/ml. As shown in Figure 1a, UVB irradiation caused a significant decrease in cell viability by 19.87% in the UV-irradiated organic solvent control group (medium with DMSO) and 21% in the aqueous control (medium only) compared to the non-UV control groups. Treatment with 1 µg/ml of 20% methanolic extracts of *Chlorella* sp. increased cell viability by 23.26% relative to the UV negative control, with a significance level of $p < 0.01$; however, no significant improvement was observed with extracts from distilled water, absolute methanol and 95% ethanol (Figure 1b). Similarly, treatment with *T. dimorphus* extracts from all four solvents did not result in a significant increase in cell viability

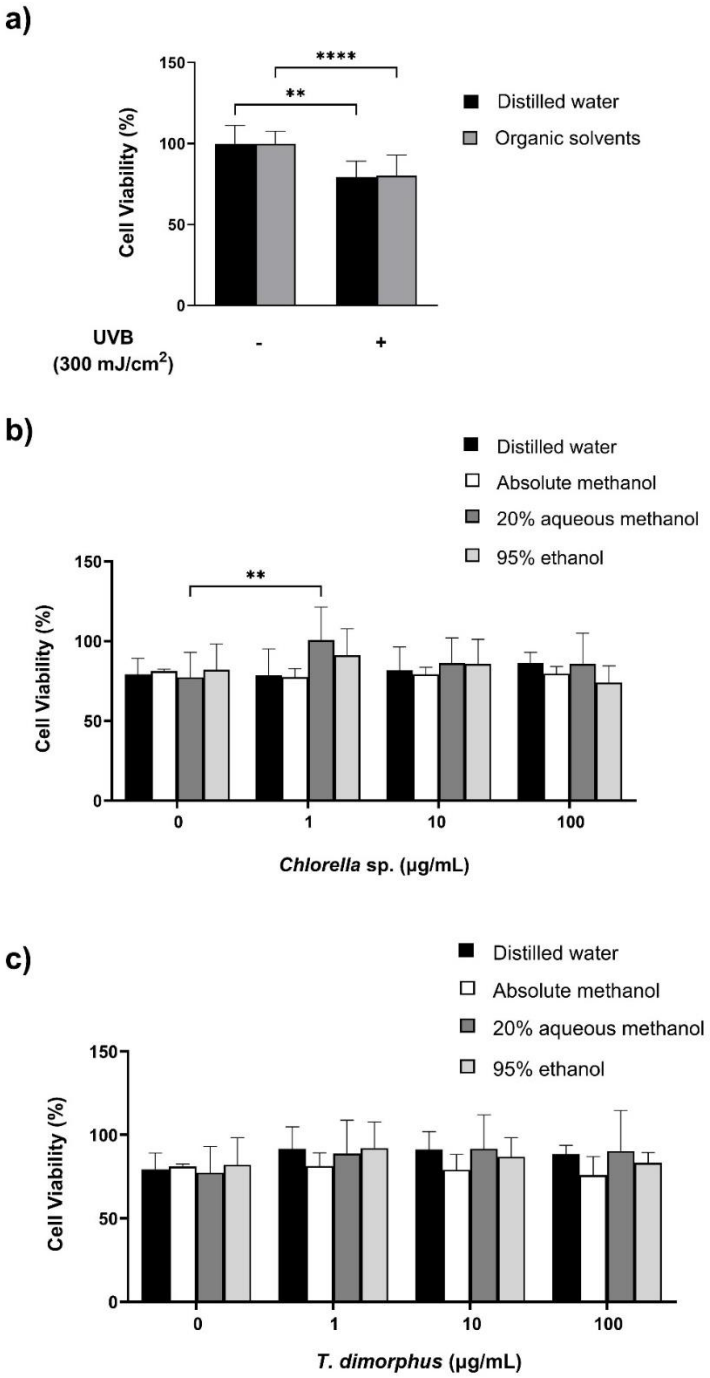

Figure 1. Effect of *Chlorella* sp. and *Tetradesmus dimorphus* Extracts on the Viability of ARPE-19 Cells after UVB Irradiation. **(a)** ARPE-19 cell viability after UVB irradiation; **(b)** ARPE-19 cell viability after treatment with *Chlorella* sp. extracts; **(c)** Viability of ARPE-19 cells treated with *T. dimorphus* extracts. Data are shown as the mean percentage of cell viability compared to the non-UV control group. The *p*-values were determined using paired t-test (a) and two-way ANOVA followed by Tukey's multiple comparison test (b,c) with significance levels of $p < 0.01$ (**) and $p < 0.0001$ (****).

compared to the UV-irradiated control group (Figure 1c). These data suggest that UVB irradiation markedly reduced ARPE-19 cell viability, and the 20% methanolic extract of *Chlorella* sp. shows a protective effect on cell viability against UV irradiation.

Antioxidant Effect of Microalgae Extracts in UVB-Irradiated ARPE-19 Cells

To evaluate the antioxidative effect of *Chlorella* sp. and *T. dimorphus* extracts on ARPE-19 cells after UVB exposure, the intracellular ROS level was measured using DCFH-DA assay. After UVB exposure, the ROS production significantly increased to 1.09 and 1.89-fold in the UV-irradiated organic solvent control group and aqueous control group, respectively, compared to the non-UV control group (Figure 2a). Based on the results (Figure 2b), aqueous extracts

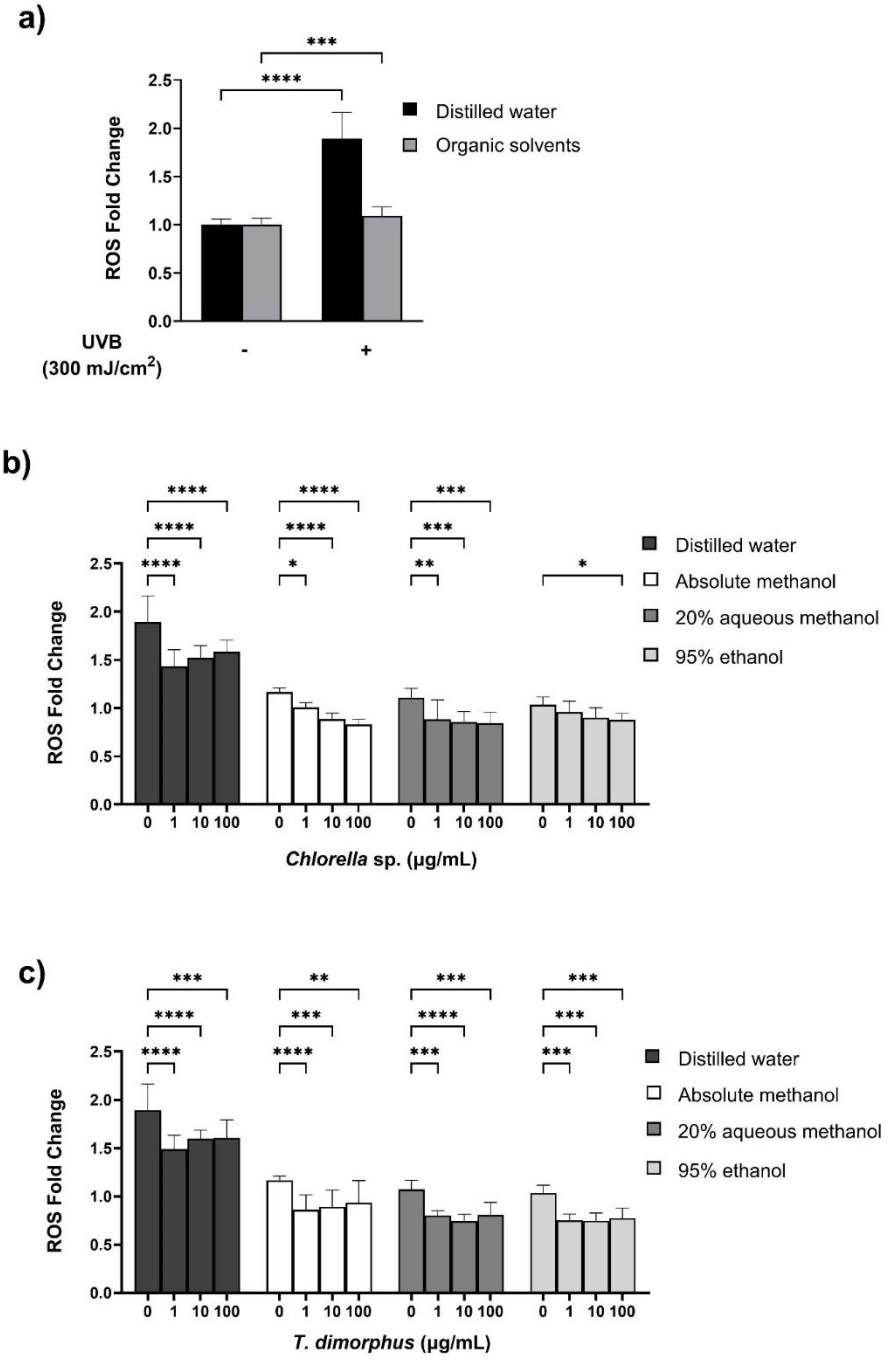

Figure 2. Intracellular ROS Levels in UVB-Irradiated ARPE-19 Cells Treated with Microalgae Extracts. **(a)** Generation of ROS levels in ARPE-19 cells after UVB exposure; **(b)** Generation of ROS levels in ARPE-19 cells after treatment with *Chlorella* sp. extracts; **(c)** Generation of ROS levels in ARPE-19 cells after treatment with *T. dimorphus* extracts. Data are expressed as the average fold changes in ROS levels ± SD of 3 independent experiments. The *p*-values were determined using paired t-test (a) and two-way ANOVA followed by Tukey's multiple comparison test (b,c) with significance levels of $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

of *Chlorella* sp. showed the greatest reduction in ROS level, with decreases of 0.46, 0.37, and 0.31-fold at concentrations of 1, 10, and 100 µg/ml, respectively ($p < 0.0001$), compared to the other solvents. Additionally, treatment with absolute methanol and 20% aqueous methanol extracts of *Chlorella* sp. similarly demonstrated a significant decrease in the ROS levels ($p < 0.001$), while the 95% ethanol extract only reduced the ROS level at a concentration of 100 µg/ml with a significance level of $p < 0.05$.

As shown in Figure 2c, aqueous extracts of *T. dimorphus* at concentrations of 1, 10, and 100 µg/ml exhibited significant decreases in ROS levels by 0.4, 0.3 and 0.29-fold ($p < 0.001$), respectively. In contrast, absolute methanol extracts reduced the ROS level by 0.31, 0.28 and 0.23-fold, while 20% aqueous methanol extracts showed a reduction in ROS level by 0.27, 0.33 and 0.26-fold ($p < 0.001$), respectively.

Treatment with ethanol extracts also lowered the ROS level by 0.28, 0.29, and 0.26-fold ($p < 0.001$), respectively compared to the UV-irradiated control. These results highlight that extracts from *Chlorella* sp. and *T. dimorphus* may alleviate oxidative stress in UVB-irradiated ARPE-19 cells.

Effect of Microalgae Extracts on Autophagy in UVB-Irradiated ARPE-19 Cells

After UVB irradiation, the cells were treated with 1 µg/ml of microalgae extracts and incubated for 48 hours. The presence of acidic vesicular organelles (AVOs) in the cells was detected using acridine orange (AO) dye. The nuclei and cytoplasm are stained green, while the AVOs appear bright red or orange under AO staining. As shown in Figure 3, the UV-irradiated control groups displayed a larger proportion of cells emitting bright orange fluorescence, indicating increased autophagy compared to the non-UV control groups. The four extracts of *Chlorella* sp. (distilled water, absolute methanol, 20% aqueous methanol and 95% ethanol) noticeably reduced the number of autophagic cells by 29.33%, 21.38%, 38.52%, and 23.67% respectively, after UV exposure. Whereas, the same solvent extracts of *T. dimorphus* reduced

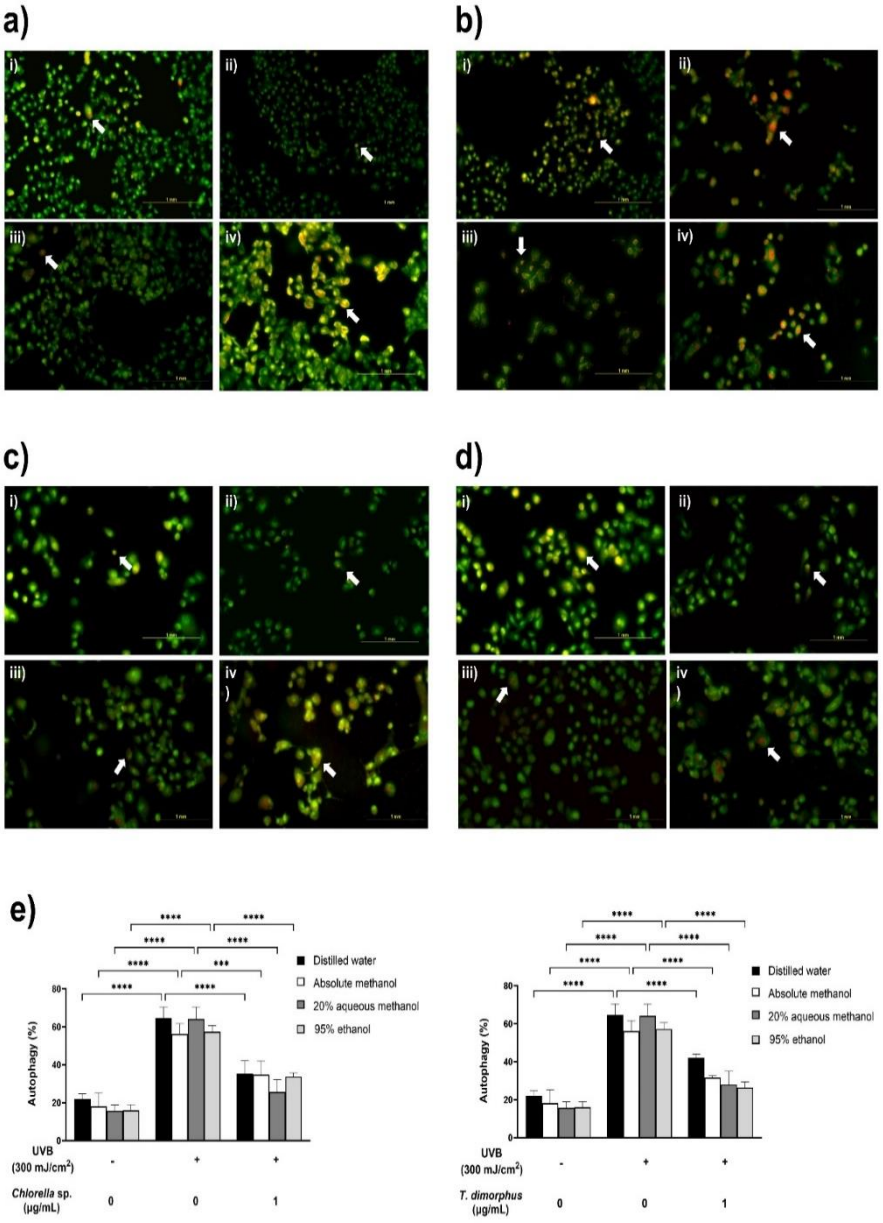

Figure 3. Detection of Acidic Vesicular Organelles (AVOs) in ARPE-19 Cells after UVB Exposure. **(a)** Non-UV irradiated cells; **(b)** UVB-irradiated cells without treatment; **(c)** UVB-irradiated cells treated with 1 µg/ml of *Chlorella* sp. extract; **(d)** UVB-irradiated cells treated with 1 µg/ml of *T. dimorphus* extract. Each group was treated with microalgae extracts obtained using the following solvents: (i) distilled water; (ii) absolute methanol; (iii) 20% aqueous methanol; (iv) 95% ethanol. The white arrows indicate red or bright orange-stained AVOs, while the nuclei and cytoplasm appear green. The images were captured at 200× magnification using an inverted fluorescence microscope. (Scale bar: 1 mm) **(e)** Quantification of autophagic cells was performed using Image J software. The data were expressed as an average ± SD from three randomly selected fields captured at 200× magnification. The *p*-values were determined using two-way ANOVA followed by Tukey's multiple comparison test. ***$p < 0.001$, ****$p < 0.0001$.

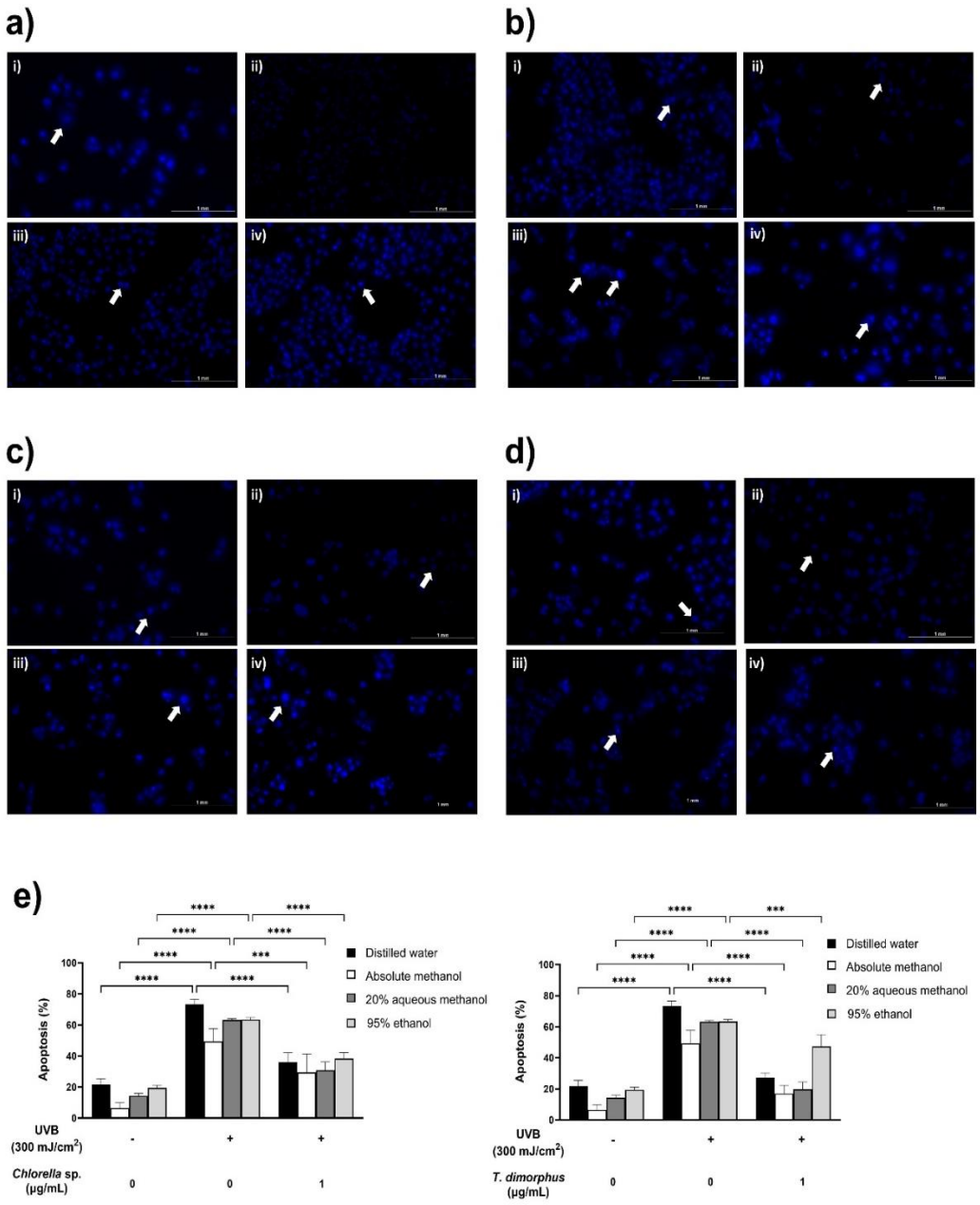

Figure 4. Effect of Microalgae Extracts on UVB-Induced Apoptosis in ARPE-19 Cells. (**a)** Non-UV irradiated cells; **(b)** UVB-irradiated cells without treatment; **(c)** UVB-irradiated cells treated with 1 µg/ml of *Chlorella* sp. extract; **(d)** UVB-irradiated cells treated with 1 µg/ml of *T. dimorphus* extract. Each group was treated with microalgae extracts obtained using the following solvents: (i) distilled water; (ii) absolute methanol; (iii) 20% aqueous methanol; (iv) 95% ethanol. The nuclear changes in ARPE-19 cells were captured using an inverted fluorescence microscope at 200× magnification (Scale bar: 1mm). The white arrows indicate cells that undergo apoptosis, characterised by nuclear condensation and fragmentation. (e) Quantification of apoptotic cells was performed using Image J software. The data were expressed as an average ± SD from three randomly selected fields captured at 200× magnification. The *p*-values were determined using two-way ANOVA followed by Tukey's multiple comparison test. ***$p < 0.001$. ****$p < 0.0001$.

autophagy by 22.67%, 24.53%, 36.26%, and 31% compared to the UV-irradiated control group. These results imply that both *Chlorella* sp. and *T. dimorphus* extracts effectively reduce autophagy in UVB-irradiated ARPE-19 cells.

Effect of Microalgae Extracts on UVB-Irradiated ARPE-19 Cell Apoptosis

To evaluate the anti-apoptotic effect of microalgae extracts on UVB-irradiated cells, Hoechst 33258 staining was performed to visualise the morphological changes in the nuclei. As shown in Figure 4, the non-UV control group showed normal cell morphology, while UVB-irradiated cells without treatment exhibited apoptotic characteristics, such as nuclear condensation and fragmentation. Treatment with 1 µg/ml of *Chlorella* sp. and *T. dimorphus* extracts resulted in a significant reduction in apoptotic cells after UVB exposure. Specifically, aqueous extracts of *Chlorella* sp. reduced the number of apoptotic cells by 37.33%, whereas

absolute methanol extracts decreased the apoptosis rate by 19.92%. Moreover, treatment with 20% aqueous methanol extracts of *Chlorella* sp. demonstrated a 32.40% reduction in the number of apoptotic cells, while treatment with 95% ethanol extracts showed a 25.12% decrease in apoptosis. On the other hand, aqueous extracts and absolute methanol extracts of *T. dimorphus* significantly reduced the apoptosis activity by 46% and 32.31%, respectively. Besides, extracts obtained using 20% aqueous methanol and 95% ethanol showed a 43.41% and 16.12% decrease in apoptotic cells, respectively. These data indicate that the extracts from *Chlorella* sp. and *T. dimorphus* substantially reduced apoptosis activity in UVB-irradiated ARPE-19 cells.

Discussion

In the present study, the photoprotective effects of different solvent extracts of microalgae on UVB-induced damaged ARPE-19 cells were investigated. The cells were treated with microalgae extracts at concentrations of 1, 10, and 100 µg/ml, as previous studies reported that extracts from *Desmodesmus quadricauda*, *Microcystis aeruginosa*, *M. viridis*, *Planktothrix agardhii*, and *Limnothrix redekei* demonstrated high toxicity to cells at concentrations ranging from 0.04 mg/ml to 2 mg/ml.[20] Based on our results, UVB irradiation markedly reduced cell viability and increased ROS levels, autophagic activity, and apoptotic activity in ARPE-19 cells. However, treatment with *Chlorella* sp. aqueous methanol extract improved cell viability after UVB exposure. Furthermore, all solvent extracts from *Chlorella* sp. and *T. dimorphus* showed prominent effects in reducing ROS levels in ARPE-19 cells after UVB exposure. Autophagy and apoptosis in UVB-irradiated ARPE-19 cells are minimised by treatment with *Chlorella* sp. and *T. dimorphus* extracts.

The finding is supported by Chen et al., who reported that the presence of *Chlorella*-derived peptide increased the proliferation of skin fibroblasts after UVB irradiation.[21] Moreover, a study on UVB-irradiated human keratinocytes revealed that the extract of *Nannochloropsis oceanica* using a mixture of dichloromethane and methanol (2:1, v/v) significantly increased cell viability and promoted cell survival to approximately 100%.[22] Furthermore, another study on the protective effects of methanolic extracts of *Chlorella vulgaris* against sodium nitrite-induced cytotoxicity on the male rat reproductive system showed a 12.17% improvement in sperm viability, which could be attributed to the antioxidant properties of *C. vulgaris* extracts.[23] However, the lack of a significant effect of *T. dimorphus* extract on cell viability in this study contrasts with the previous study conducted by Campiche et al., which used UVA-irradiated human dermal fibroblasts pre-treated with *Scenedesmus rubescens* dry extract. The study revealed that *S. rubescens* improved the cell viability of UVA-irradiated human dermal fibroblasts by 56%.[24] This discrepancy might be due to differences in the microalgae species used and experimental design, as the other study pre-treated the cells with the extract one hour before UVA irradiation, whereas, in the current study, treatment was given after UVB exposure.

Furthermore, multiple studies have revealed that high levels of flavonoids and phenolic compounds can be extracted from *C. vulgaris* using solvents such as distilled water, methanol and 96% ethanol.[25–27] Phenolic compounds such as caffeic acid, ferulic acid and p-coumaric acid have been proven to exhibit antioxidant properties, by scavenging free radicals and reducing the production of ROS.[28] Another study carried out by Park et al., reported that the methanolic extract of *Chlorella ellipsoidea* possesses antioxidative activity that inhibits nitric oxide production and suppresses intracellular oxidative stress in lipopolysaccharides (LPS)-stimulated murine macrophage cells.[29] Moreover, the aqueous extract of *Chlorella pyrenoidosa* showed a higher yield of phenols and flavonoids compared to other organic solvents,[30] corresponding to our findings that the aqueous extract of *Chlorella* sp. exhibited the greatest inhibitory effect on ROS production. Alternatively, a previous study found that *S. quadricauda* contains an abundance of flavonoids, including hesperidin, quercetin, and naringin, which exhibit DPPH radical scavenging capabilities.[31] Hesperidin has been proven to effectively reduce the generation of ROS and promote antioxidative activity in UVA-irradiated HaCaT cells.[32] These findings align with our results, where both *Chlorella* sp. and *T. dimorphus* extracts reduced oxidative stress caused by UV irradiation in ARPE-19 cells.

Autophagy is characterised by increased formation of AVOs, which can be visualised using acridine orange (AO) staining.[33] UVB radiation causes oxidative damage in DNA, forming photoproducts and triggering autophagy in retinal cells to enhance photoproduct repair.[34] Autophagy protects cells against oxidative stress and promotes cell survival, but excessive autophagic activity can result in autophagic cell death.[35] Our results showed that UVB irradiation elevated autophagic activity in the cells, as evidenced by the increased formation of AVOs. However, this condition is inhibited after treatment with microalgae extracts. Our findings correspond to a study that revealed that flavonoids, particularly quercetin, markedly reduce the expression of autophagy-related proteins, LC3B-II, Beclin-1, and p62 in ARPE-19 cells treated with $NaIO_3$.[36] In addition, another study conducted by Nakashima et al. also showed that *Chlorella* sp. extract significantly suppresses NLRP3-induced autophagy in mouse macrophage cells, due to its high concentration of carotenoids.[37] Therefore, it can be deduced that the bioactive compounds extracted from microalgae may protect ARPE-19 cells against UVB-induced damage by regulating the autophagy process.

Besides, research by He et al. showed that UVB irradiation triggered cell apoptosis by downregulating PTEN expression and upregulating p53 protein level.[38] This finding is consistent with the current study, where the number of apoptotic cells significantly increased upon UVB exposure. After treatment with microalgae extracts, the number of apoptotic cells decreased, indicating that extracts from *Chlorella* sp. and *T. dimorphus* exert an anti-apoptotic effect in UV-irradiated ARPE-19 cells. This hypothesis is supported by a previous study that reported that quercetin reduced the protein expression of Bax and increased the level of Bcl-2, thereby suppressing $NaIO_3$-induced apoptosis in ARPE-19 cells.[39] Similarly, treatment with dry extract of *S. rubescens* also exhibited protective effects on UV-induced keratinocytes, resulting in a notably reduced formation of sunburn cells, which are cells undergoing apoptosis.[24] Additionally, the extracts from brown seaweed, *Sargassum horneri* significantly decreased nuclear fragmentation and condensation in UVB-irradiated HaCaT cells.[40] Furthermore, ethanolic extract from freshwater algae, *Prasiola japonica* demonstrated anti-apoptotic effects on UVB-induced damaged skin keratinocytes by inhibiting the activation of proteolytic caspases, particularly caspase-3, -8, and -9.[41]

With the current findings, extracts from the tropical microalgae *Chlorella* sp. and *T. dimorphus* have shown significant photoprotective effects against UVB-induced damage on human retinal cells, highlighting their potential as therapeutic agents in treating AMD and associated retinopathies. Previous studies have proven that the extract of *C. vulgaris* showed protective effects against retinal degeneration in *N*-methyl-D-aspartate (NMDA)-induced retinal ganglion cells. This may be attributed to the anti-apoptotic properties of *C. vulgaris* extract, which down regulates the expression of apoptotic proteins such as PARP, caspase-9, and caspase-3, making it a promising approach for preventing glaucoma.[42] Besides that, research conducted by Okamoto et al. reported that dietary supplementation with *Spirulina*, a blue-green microalga, mitigated light-induced visual impairment in mice by suppressing the retinal ROS level, photoreceptor apoptosis and retinal degeneration.[43] Moreover, the green microalgae *Dunaliella salina* has been shown to improve the antioxidant defense system and suppress lipid peroxidation to protect mice against UVB-induced corneal injury.[44]

However, a limitation of this study is the lack of identification and quantification of bioactive compounds extracted from microalgae. Hence, it may be challenging to identify which bioactive compounds contributed to the significant photoprotective effect observed against UVB-induced cellular damage. Further research should involve the characterisation of compounds extracted from microalgae and their mechanism of action for potential therapeutic options in managing AMD.

Conclusion

In summary, this study evaluated the photoprotective effects of different solvent extracts of tropical microalgae in protecting retinal cells against UV radiation associated with AMD. The significant decrease in ROS levels, autophagy, and apoptosis indicates that both *Chlorella* sp. and *Tetradesmus dimorphus* extracts exhibit protective effects on UVB-induced damage in ARPE-19 cells, with aqueous and 20% aqueous methanol microalgae extracts demonstrating greater effectiveness compared to absolute methanol and 95% ethanol. Moreover, further investigation on the characterisation of bioactive compounds found in microalgae should be performed to gain a better understanding of the potential effects of microalgae in maintaining ocular health.

Authors' Contributions

CLL, RYK, CYW, and CWM designed the experiments, provided the resources and edited the manuscript, YTT, YZC, CYL, FAME, and KFC conducted the experiments, analysed and interpreted the data. YTT, YZC, CYL, and FAME wrote and edited the manuscript. All authors approved the final version of the manuscript.

Ethical Approval

Ethical approval was obtained from the IMU Joint-Committee on Research and Ethics (IMU-JC), IMU University on 9th May 2024 (Project Number: IM297).

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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