Plant Physiol. Rep.
https://doi.org/10.1007/s40502-021-00637-6

SHORT COMMUNICATION

The antioxidative potential of sea grapes (*Caulerpa lentillifera*, Chlorophyta) can be triggered by light to reach comparable values of pomegranate and other highly nutritious fruits

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Received: 30 March 2021 / Accepted: 6 November 2021

Abstract The interest in edible sea grapes (*Caulerpa lentillifera*) is increasing due to their potentially beneficial effect on human health. This macroalga, already used for direct and indirect human consumption, is grown in aquacultures in Vietnam and The Philippines. Here, the edible fronds of sea grapes were examined for their antioxidant activity (AOA) at light intensities from 140 to 300 µmol photons m^{-2} s^{-1} and compared to commercially dehydrated *C. lentillifera* and the renowned highly antioxidative fruits Pomegranates (*Punica granatum*), Goji (*Lycium barbarum* and *L. chinense*) and Aronia (*Aronia melanocarpa)* berries, using an $ABTS^{+}$-assay for all samples. AOA of fronds exposed to 300 µmol photons m^{-2} s^{-1} for 14 days increased by about 320% from the initial value of 72.2 ± 5.6 to 232.2 ± 34.2 Trolox Equivalents (TE) mmol 100 g^{-1} dry weight (DW) onto the level of Pomegranates (272.8 ± 23.0 TE mmol 100 g^{-1} DW). This application could be used as a post-cultivation treatment in sea grape cultures to increase the quality and nutritional value of the product.

Keywords $ABTS^{+}$ assay · Antioxidant activity · Aronia · Goji · High light intensities · Post-harvest

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Introduction

While algae are traditionally consumed as sea vegetables in Asian countries (Fleurence, 2016), the rising global interest in a healthy diet has increased the popularity of seaweeds. Especially the edible green macroalga *Caulerpa lentillifera*, also known as 'sea grapes' or 'green caviar', is commercially cultured in several South East Asian countries (especially Vietnam and The Philippines), due to the ease of propagation, its high growth rate and potential health benefits (Paul et al., 2014). The thallus consists of a stolon with rhizoids and the edible fronds with vesiculate ramuli, evoking the association with caviar (Fig. 1) (Zubia et al., 2020).

The aquaculture of the benthic seaweed takes place in tidal ponds and after harvest, the fronds are cleaned and packed for transport or direct retail while still alive and photosynthetically active. During culture and storage of *C. lentillifera*, suitable light conditions have been shown to be a crucial factor for the shade-adapted light-sensitive seaweed, with irradiances higher than 100 µmol photons m^{-2} s^{-1} causing physiological stress reactions, reflected in e.g. lower maximum quantum yields of photosystem II (F_v/F_m) (Guo et al., 2015; Stuthmann et al., 2020). A smaller portion of the harvest is dehydrated with brine cure or salt for preservation before retail (Zubia et al., 2020). The special texture of sea grapes in combination with low levels of lipids (Niwano et al., 2009), multiple essential amino acids, polyunsaturated fatty acids (Saito et al., 2010) and diverse minerals have increased their popularity, even though nutritional studies on this organism are still rare. Furthermore, different preliminary studies have attributed a naturally high non-enzymatic antioxidant activity (AOA) to this species (Matanjun et al., 2009; Nguyen et al., 2011; Paul et al., 2014; Yap et al., 2019). *C. lentillifera* is rich in

Published online: 07 January 2022

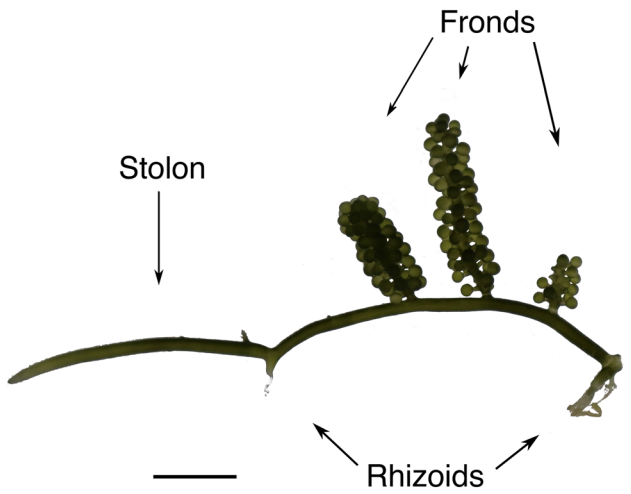

Fig. 1 *Caulerpa lentillifera* consists of edible fronds, which are connected by stolons with rhizoids. Scale bar, 1 cm

ascorbic acid (vitamin C), α-tocopherol (vitamin E) (Matanjun et al., 2009) and also its polyphenolic content is decisively correlating with their antioxidant activity (Nguyen et al., 2011). The essential importance of antioxidants is based on their ability to defuse reactive oxygen species (ROS), which are related to the pathogenesis of several human diseases such as diabetes mellitus, neurodegenerative disorders, cardiovascular diseases and cancer (Halliwell, 2000; Metodiewa & Kośka, 1999; Zampelas & Micha, 2015). In photosynthetically active organisms, the probability of ROS production in chloroplasts is increased during high light stress in the saturation region of photosynthesis. As a physiological response, the production of antioxidative compounds is expected to increase under stress conditions (Ito & Hori, 1989). The variety of assays and extraction methods to measure activity of all antioxidants present within cells of an organism is high and therefore direct comparisons between studies are difficult. One method commonly used in scientific studies to investigate and quantify the total antioxidant capacity of food sources is the 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay (Gülçin, 2012). Humans can obtain antioxidants through their diet, and some food products are especially known for their rich proportion. Pomegranate (*Punica granatum*), Goji or Wolfberry (*Lycium barbarum* and *L. chinense*) and Aronia (*Aronia melanocarpa)* berries are among those repeatedly reported as 'superfruits´ as a tool to highlight and promote their bioactive compounds and nutritional qualities, including their antioxidative activities (Sidhu & Zafar, 2012). For fruits the term 'fruit-quality´ is used mainly for the appearance and the taste, however, there has been an association with health benefits when consumed, mainly linked to antioxidative compounds like ascorbic acid (Atkinson et al., 2005). The opportunity to use physiological stress treatments as an opportunity for manipulation of the antioxidant potential of fruits and an inherent increase of the 'fruit-quality´ has been proposed and discussed (Atkinson et al., 2005). However, a successful manipulation requires a fundamental understanding of the organisms´ physiology. The present study was designed to apply this concept to the sea grape *C. lentillifera*, making a first attempt to increase the alga's quality as food product by triggering AOA, using irradiances of up to 300 µmol photons m^{-2} s^{-1} over a period of 14 days. Furthermore, the AOA of experimentally treated sea grapes was compared with the AOA of commercially cultured, purchasable preserved dehydrated fronds of *C. lentillifera*, as well as renowned highly antioxidative fruits like *P. granatum*, *L. barbarum/L. chinense*, and *A. melanocarpa*.

Material and methods

Sample material

C. lentillifera organisms used in this experiment were harvested in the Vietnamese sea grape-farm VIJA (Van Phong Bay, Vietnam) in June 2019 and were transported to the Marine Experimental Ecology (MAREE) aquaculture facility of the Leibniz Centre for Tropical Marine Research (ZMT) in Bremen, Germany. The algae were cultured in aquaria (130 cm × 36 cm × 80 cm) filled with artificial sea water at constant temperature (25.6 ± 1.3 °C), absolute salinity (S_A 34.5 ± 0.4), pH (8.1 ± 0.1) and irradiance (50 µmol photons m^{-2} s^{-1}). For comparison of the AOA, different commercial products were acquired in German supermarkets, namely *P. granatum*, dried *L. barbarum /L. chinense* and dried *A. melanocarpa* berries. Also, three commercially cultured and dehydrated types of sea grapes were tested for their AOA (SeA-VIET, Vietnam; VIJA, Vietnam; UMI, Korea). The dehydrated sea grape samples were re-hydrated in freshwater prior to the biochemical analysis, following the recommendations of the retailers.

Experimental set-up

For testing the antioxidant potential in respect to different light intensities, five levels of irradiances of photosynthetically active radiation (PAR) were chosen: 140, 180, 220, 260 and 300 µmol photons m^{-2} s^{-1}. The sea grapes were held in 1 L glass beakers at constant temperature (24.0 ± 0.5 °C). For each treatment, five beakers containing four *C. lentillifera* organisms with approximately 2–4 fronds attached to the stolon were placed underneath a light-emitting diode (LED) bar (SolarStinger Sunstrip

800 mm, ECONLUX GmbH, Cologne, Germany) radiating white light in a 12:12 light:dark cycle using the standard settings. To adjust the different light intensities the irradiances were measured directly at the water surface using a LI-COR data logger (LI-189, Lincoln, USA). The seawater was stirred twice a day to evade gradient formation regarding nutrients and also to maintain a balanced exposure to the light. The seawater was exchanged after 7 days.

Sample preparations and extraction

Fresh and rehydrated *C. lentillifera* biomass was frozen (− 80 °C) directly after sampling and freeze-dried for 24 h at 1 mbar (ALPHA 1–4 LD plus; Christ GmbH, Osterode am Harz, Germany). The freeze-dried samples were ground to a powder for 20 s using a benchtop homogenizer (FastPrep-24, MP Biomedicals, Germany). The fruits were crushed in liquid nitrogen. Sea grapes *C. lentillifera* and *P. granatum* (0.05 g dry weight (DW)), as well as *L. barbarum*/*L. chinense* and *A. melanocarpa* (0.035 g DW) were dissolved in 1 mL ethanol (70%) and extracted in a water bath (47 °C) for 4 h, being vortexed hourly. Prior to analysis, samples were centrifuged (2500 g, 20 °C) for 5 min.

Analysis of AOA

The AOA was determined after a modified $ABTS^+$ assay (Re et al., 1999), also known as Trolox Equivalent Antioxidant Capacity (TEAC) assay. A stock solution of 2.45 mM $ABTS^+$ was obtained by oxidising 7.0 mM ABTS (Sherman Chemicals, Dorset, UK) with potassium disulfate for 16 h. By dilution with ethanol (absolute) a working solution with a consistent photometrically measured (Thermo Scientific Genesys 140/150, Fisher Scientific GmbH, Schwerte, Germany) absorption of 0.7 ± 0.02 at a wavelength of 734 nm was obtained. For analysis, 1 mL $ABTS^+$ working solution was added to 10 µL sample extract and the deradicalization was measured after 6 min. AOA of the samples was expressed as Trolox Equivalents (TE mmol 100 g^{-1} DW).

Statistical analysis

All statistical analyses and the creation of graphics were conducted using the statistic-software R (Version i386 4.0.2) combined with RStudio (Version 8.3) (R Core Team, 2019). For determination of significant differences, one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) tests were conducted. Quantitative data are presented as mean values with the respective standard deviation.

Results and discussion

AOA of sea grapes

Following exposure of *C. lentillifera* fronds to treatment irradiances (140–300 µmol photons m^{-2} s^{-1}), an enrichment of antioxidants was observed. The rise of the AOA was significantly dependent on the applied light intensity ($F = 19.93$; $p < 0.001$) and also on time ($F = 24.08$; $p < 0.001$; Fig. 2). *C. lentillifera* showed an initial value of 72.2 ± 5.6 TE mmol 100 g^{-1} DW after a cultivation at a light intensity of 50 µmol photons m^{-2} s^{-1}. The maximum AOA´s were detected in sea grapes exposed to 300 µmol photons m^{-2} s^{-1} with 169.9 ± 24.5 TE mmol 100 g^{-1} DW after 7 and 232.2 ± 34.2 TE mmol 100 g^{-1} DW after 14 days of exposure, translating to an increase of about 320% compared to initial AOA values. The ABTS assay does not indicate specifically which compounds are responsible for the antioxidant activity, but sea grapes cultured under 50 µmol photons m^{-2} s^{-1} showed values of total phenolic content (TPC) of 124.5 ± 25.5 Gallic acid equivalents (GAE) mg 100 g^{-1} DW. The TPC values increased under 14 days exposure to irradiances of 100, 200 and 400 µmol photons m^{-2} s^{-1} to 152.6 ± 23.2, 221.9 ± 25.7 and 241.7 ± 85.2 GAE mg 100 g^{-1} DW, respectively (Stuthmann et al. in prep., Ascending irradiances increase Antioxidant Activity and Total phenolic Content and affect photosynthesis of sea grapes (*Caulerpa lentillifera*, Ulvophyceae, Caulerpacae)). The simultaneous increase of AOA and TPC indicates that polyphenolics are one major group of antioxidative compounds of sea grapes quantified in this study. The measured AOA was expected to increase as a reaction of the exposure to irradiances above the photon saturation limit of photosynthesis and therefore as a protection against produced ROS (Hajiboland, 2014). The saturation irradiance of shade-adapted seaweeds, like *Caulerpa* is in general lower than for other seaweeds (García-Sánchez et al., 2012). The results suggest that the increase in AOA can be triggered by irradiances of 140 µmol photons m^{-2} s^{-1} and higher. However, Kang et al. (2020) cultured *C. lentillifera* under ascending irradiances (50, 100, 150 µmol photons m^{-2} s^{-1}, 16.7% blue + 83.3% red spectral distribution) over 12 days and found similar AOAs for all treatments, with significantly higher reducing power for *C. lentillifera* at 150 µmol photons m^{-2} s^{-1}. This indicates that an irradiance of approximately 150 µmol photons m^{-2} s^{-1} might be a tipping point. However, despite the steady increase of AOA with light treatment and exposure time, no saturation region of the AOA was detectable in this study and therefore it is assumed that the antioxidant potential of *C. lentillifera* fronds could be triggered even higher (Fig. 2).

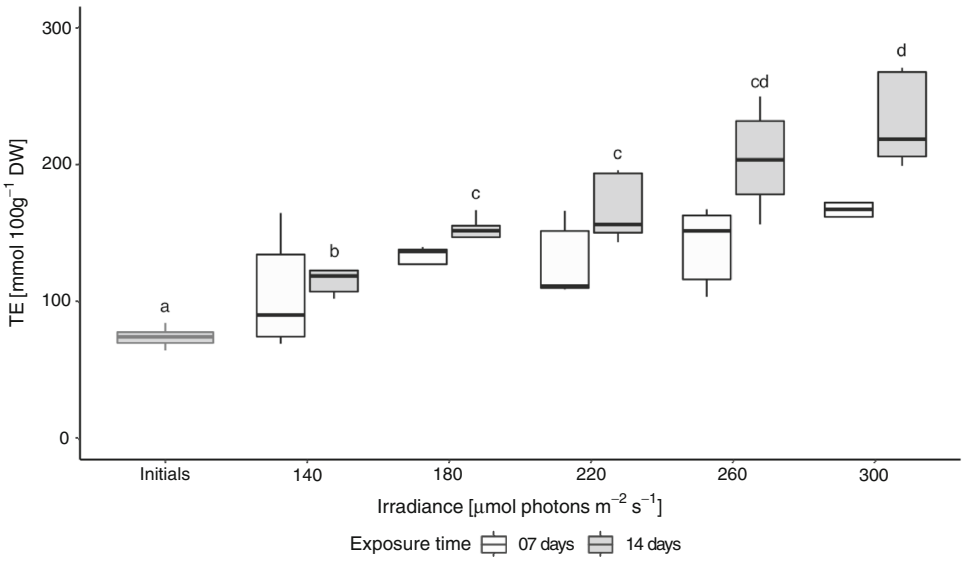

Fig. 2 Effects of different light intensities on the antioxidant activity of *Caulerpa lentillifera* fronds expressed as Trolox Equivalents (TE mmol 100 g^{-1} dry weight (DW)) applied for 7 and 14 days, respectively (n = 5–6). Letters indicate significant differences between initials and associated 14 days treatments (one-way ANOVA followed by Tukey's HSD, $p < 0.05$)

AOA of dehydrated sea grapes and fruits

The AOAs of the freshly harvested organisms and the purchased dehydrated sea grapes were similar, indicating that the post-harvest processing and dehydrating of the sea grapes did not negatively affect the antioxidative potential (Fig. 3). However, the light triggered fronds exposed to 300 µmol photons m^{-2} s^{-1} for 14 days were significantly enriched in antioxidants compared to the other sea grape categories ($p < 0.05$, Fig. 3). These results introduce the possibility for farmers to expose sea grapes before retail or dehydration to higher light irradiances if an enrichment in AOA is desired. Overall, the light triggered sea grapes showed similar antioxidative levels compared to *P. granatum* (272.8 ± 23.0 TE mmol 100 g^{-1} DW, $p > 0.05$). *L. barbarum/L. chinense* and *A. melanocarpa* exhibited a significantly higher AOA compared to all measured sea grape fronds ($p < 0.001$), with 408.5 ± 27.6 and 435.9 ± 46.1 TE mmol 100 g^{-1} DW, respectively. Intra-study comparisons like this are important, since methodological parameters, like assay and extraction method, are varying widely among studies investigating AOA of *C. lentillifera* (Matanjun et al., 2008; Nguyen et al., 2011; Yap et al., 2019) and other food products (Gülçin, 2012) and direct comparisons between results are therefore hardly possible. Therefore, this study provides a unique comparison of *C. lentillifera* with several 'superfruits´. The competitiveness regarding AOA of *C. lentillifera* with renowned 'superfruits´ in combination with all the other specific health beneficial compounds (such as

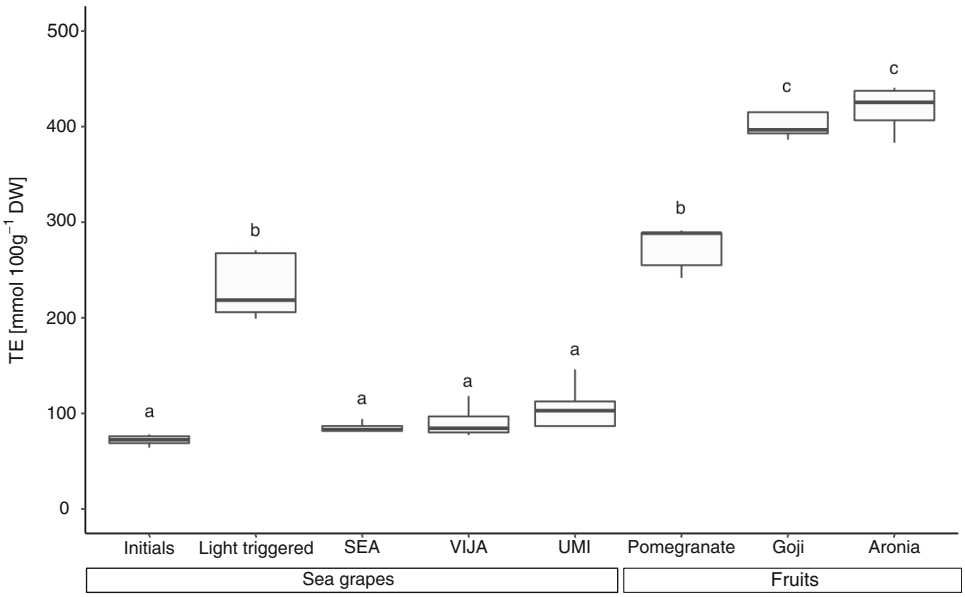

Fig. 3 Levels of antioxidant activities of *Caulerpa lentillifera* initials and after exposure to 300 µmol photons m^{-2} s^{-1} for 14 days in comparison to purchasable dried sea grapes from farms in Vietnam (SEA, VIJA) and Korea (UMI) and the highly nutritious fruits Pomegranate, dried Goji berries and Aronia berries (n = 4–6). Values are expressed as Trolox Equivalents (TE mmol 100 g^{-1} dry weight (DW)). Letters indicate significant differences between associated categories (one-way ANOVA followed by Tukey's HSD, $p < 0.05$)

polyunsaturated fatty acids, proteins etc.) makes this macroalga an exceptional food, also aiming at a commercial application in both human (as nutraceuticals for novel food) and animal health (feed additives for e.g. shrimps and fish in aquaculture approaches). The demonstrated potential of post-harvest manipulations needs closer investigations, but might be a useful and comparatively easy tool for farmers and retailers to further increase the value of this seaweed.

Conclusion

The successful increase of sea grapes AOA by exposure to increased irradiances on the level of 'superfruit´ Pomegranate (*P. granatum*), introduces the possibility to establish light treatments as post-harvest processing before sea grape dehydration or fresh retail, for example during cleaning process or transport. To determine the saturation region of AOA in *C. lentillifera,* even higher irradiances need to be applied in further studies.

Authors contributions JS, AK, LS and KS designed the study, JS carried out the experiments and wrote the initial draft of the manuscript, all authors contributed to improving the manuscript, AK and KS secured funding.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by Leibniz Centre for Tropical Marine Research inhouse funding.

Data availability Raw data were generated at University of Bremen. Derived data supporting the findings of this study are available from the corresponding author on request.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Atkinson, C. J., Nestby, R., Ford, Y. Y., & Dodds, P. A. A. (2005). Enhancing beneficial antioxidants in fruits: A plant physiological perspective. *BioFactors, 23*(4), 229–234. https://doi.org/10.1002/biof.5520230408

Fleurence, J. (2016). Seaweeds as food. In Joel Fleurence & I. A. Levine (Eds.), *Seaweed in health and disease prevention* (pp. 149–167). https://doi.org/10.1016/B978-0-12-802772-1.00005-1

García-Sánchez, M., Korbee, N., Pérez-Ruzafa, I. M., Marcos, C., Domínguez, B., Figueroa, F. L., & Pérez-Ruzafa, Á. (2012). Physiological response and photoacclimation capacity of *Caulerpa prolifera* (Forsskål) J.V. Lamouroux and *Cymodocea nodosa* (Ucria) Ascherson meadows in the Mar Menor lagoon (SE Spain). *Marine Environmental Research, 79*, 37–47. https://doi.org/10.1016/j.marenvres.2012.05.001

Gülçin, İ. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology, 86*(3), 345–391. https://doi.org/10.1007/s00204-011-0774-2

Guo, H., Yao, J., Sun, Z., & Duan, D. (2015). Effect of temperature, irradiance on the growth of the green alga *Caulerpa lentillifera* (Bryopsidophyceae, Chlorophyta). *Journal of Applied Phycology, 27*(2), 879–885. https://doi.org/10.1007/s10811-014-0358-7

Hajiboland, R. (2014). Reactive oxygen species and photosynthesis. In P. Ahmad (Ed.), *Oxidative damage to plants* (pp. 1–63). https://doi.org/10.1016/B978-0-12-799963-0.00001-0

Halliwell, B. (2000). The antioxidant paradox. *The Lancet, 355*(9210), 1179–1180. https://doi.org/10.1016/S0140-6736(00)02075-4

Ito, K., & Hori, K. (1989). Seaweed: Chemical composition and potential food uses. *Food Reviews International, 5*(1), 101–144. https://doi.org/10.1080/87559128909540845

Kang, L.-K., Huang, Y.-J., Lim, W.-T., Hsu, P.-H., & Hwang, P.-A. (2020). Growth, pigment content, antioxidant activity, and phytoene desaturase gene expression in *Caulerpa lentillifera* grown under different combinations of blue and red light-emitting diodes. *Journal of Applied Phycology, 32*(3), 1971–1982. https://doi.org/10.1007/s10811-020-02082-8

Matanjun, P., Mohamed, S., Mustapha, N. M., Muhammad, K., & Ming, C. H. (2008). Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *Journal of Applied Phycology, 20*(4), 367–373. https://doi.org/10.1007/s10811-007-9264-6

Matanjun, P., Mohamed, S., Mustapha, N. M., & Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystrum*. *Journal of Applied Phycology, 21*(1), 75–80. https://doi.org/10.1007/s10811-008-9326-4

Metodiewa, D., & Kośka, C. (1999). Reactive oxygen species and reactive nitrogen species: Relevance to cyto(neuro)toxic events and neurologic disorders. An overview. *Neurotoxicity Research, 1*(3), 197–233. https://doi.org/10.1007/BF03033290

Nguyen, V. T., Ueng, J.-P., & Tsai, G.-J. (2011). Proximate composition, total phenolic content, and antioxidant activity of seagrape (*Caulerpa lentillifera*). *Journal of Food Science, 76*(7), C950–C958. https://doi.org/10.1111/j.1750-3841.2011.02289.x

Niwano, Y., Beppu, F., Shimada, T., Kyan, R., Yasura, K., Tamaki, M., Nishino, M., Midorikawa, Y., & Hamada, H. (2009). Extensive screening for plant foodstuffs in Okinawa, Japan with anti-obese activity on adipocytes in vitro. *Plant Foods for Human Nutrition, 64*(1), 6–10. https://doi.org/10.1007/s11130-008-0102-z

Paul, N. A., Neveux, N., Magnusson, M., & de Nys, R. (2014). Comparative production and nutritional value of "sea grapes" - the tropical green seaweeds *Caulerpa lentillifera* and *C. racemosa*. *Journal of Applied Phycology, 26*(4), 1833–1844. https://doi.org/10.1007/s10811-013-0227-9

R Core Team. (2019). *A language and environment for statistical computing*. Austria.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Saito, H., Xue, C., Yamashiro, R., Moromizato, S., & Itabashi, Y. (2010). High polyunsatturated fatty acid levels in two subtropical macroalgae, *Cladosiphon okamuranus* and *Caulerpa lentillifera*. *Journal of Phycology, 46*(4), 665–673. https://doi.org/10.1111/j.1529-8817.2010.00848.x

Sidhu, J. S., & Zafar, T. A. (2012). Super fruits: pomegranate, Wolfberry, Aronia (Chokeberry), Acai, Noni, and Amla. In N. K. Sinha, J. S. Sidhu, J. Barta, J. S. B. Wu, & M. P. Cano (Eds.), *Handbook of fruits and fruit processing* (pp. 653–679). https://doi.org/10.1002/9781118352533.ch35

Stuthmann, L. E., Springer, K., & Kunzmann, A. (2020). Cultured and packed sea grapes (*Caulerpa lentillifera*): Effect of different irradiances on photosynthesis. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-020-02322-x

Yap, W. F., Tay, V., Tan, S. H., Yow, Y. Y., & Chew, J. (2019). Decoding antioxidant and antibacterial potentials of Malaysian green seaweeds: *Caulerpa racemosa* and *Caulerpa lentillifera*. *Antibiotics, 8*(3), 152. https://doi.org/10.3390/antibiotics8030152

Zampelas, A., & Micha, R. (Eds.). (2015). *Antioxidants in health and disease*. CRC Press. https://doi.org/10.1201/b18539

Zubia, M., Draisma, S. G. A., Morrissey, K. L., Varela-Álvarez, E., & De Clerck, O. (2020). Concise review of the genus *Caulerpa* J.V. Lamouroux. *Journal of Applied Phycology, 32*(1), 23–39. https://doi.org/10.1007/s10811-019-01868-9