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RESEARCH ARTICLE

Efficacy of Seaweed Liquid Extract from *Ulva* spp. in Improving Growth and Chlorophyll-*a* Content of *Eucheuma denticulatum* in Tissue Culture

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ABSTRACT

The addition of biostimulants in seaweed tissue culture helps produce a large number of good-quality, fast-growing, and uniform seaweed seedlings that could support the eucheumatoid farming industry. Various *Ulva* species already gained recognition for their potential use as phycobiostimulants in terrestrial crops; however, its growth-promoting potential on cultured seaweed species is not yet fully assessed and subject to further studies. This study aimed to examine the efficacy of a liquid extract (SLE) from the green seaweed *Ulva* spp. in improving the growth and chl-*a* content of *Eucheuma denticulatum* in tissue culture. Seaweed explants were cultured for 45 days in varying concentrations of *Ulva* SLE (0.01, 0.1, 1, 2, and 3 mL L^{-1}) and one control group (UV-filtered seawater). Among all the treatments, the 1 and 2 mL L^{-1} concentrations had the highest direct axes formation (99.8–100%) and longest shoot measurements (3.6–3.8 mm). The highest chl-*a* content was observed in the 2 and 3 mL L^{-1} *Ulva* SLE with values of 93.5 ± 10.1 and 90.1 ± 3.0 μg g_{fw}^{-1}, respectively. The present study also revealed the presence of macro- and micronutrients in *Ulva* SLE, thereby improving the success rates of tissue culture. Thus, it can be a potential alternative to existing nutrient or biostimulant enrichment techniques, which could help address phyconomic issues concerning the availability of good quality and high-yielding eucheumatoid cultivars for large-scale production and minimize losses in seaweed production.

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1. INTRODUCTION

Eucheumatoid (*Eucheuma* and *Kappaphycus*) farming is an essential livelihood for marginalized coastal communities in developing countries in Southeast Asia for human consumption and carrageenan production. Carrageenan is one of the most common and crucial seaweed-derived hydrocolloids because they are used as a thickening, gelling, and emulsifying agent in various industries (Qin 2018; Lomartire et al. 2021). However, conventional eucheumatoid farming utilizes seedlings from vegetative cuttings of the previous harvest, which results in low genetic variability and increased vulnerability of seaweed seedstocks to pests and diseases, such as the "ice-ice" disease (Hayashi et al. 2017; Hurtado et al. 2019; Jiksing et al. 2022). Because of this, there is a problem concerning the availability of good quality and high-yield seaweed seedlings for large-scale production, which could pose a threat to the seaweed farming industry.

According to Baweja et al. (2009), tissue culture is a sustainable, scalable, and efficient method to increase seed supply and grow uniform seedlings for mass production in a shorter time. It is one of the advancements of *in vitro* culture techniques of various seaweed species wherein explants can be cultivated axenically in enriched or artificial seawater culture media that may lead to callus formation and direct shoot regeneration (Baweja et al. 2009; Jiksing et al. 2022). In addition, seaweed tissue culture maximizes the add-on value of seaweed resources and is beneficial for a wide range of biotechnological applications (Reddy et al. 2008; Baweja et al. 2009). At the same time, the supplementation of biostimulants and plant growth regulators in tissue culture media

improves seedling quality by enhancing their growth and survival (Crouch and van Staden 1993; Jiksing et al. 2022). Using seaweed extracts as biostimulants is a sustainable alternative for improving growth and crop yield for terrestrial crops (Khan et al. 2009; Shukla et al. 2019). Benítez García et al. (2020) confirmed that aqueous seaweed extracts contain diverse compounds known as plant growth regulators (PGRs), which have been utilized in agricultural practices to increase crop productivity. Divya et al. (2015) also stated that the presence of phytohormones such as indole-3-acetic acid (IAA), kinetin, zeatin, gibberellins, auxins, and cytokinins, as well as macro- and micronutrients in seaweeds, prove that they can be used as phycobiostimulants or -bioeffectors.

The green seaweed species (Chlorophyta) under the genus *Ulva* are exploited and utilized for a wide range of applications. Several studies revealed the potential of *Ulva* seaweed liquid extract in improving the yield and growth of various terrestrial crops. For instance, Latique et al. (2021) reported that seaweed liquid extract from *Ulva rigida* enhanced growth parameters in wheat plants. The aqueous seaweed extract derived from *Ulva lactuca* also promoted seedling growth of cowpea (*Vigna unguiculata*) in terms of shoot length, root length, fresh and dry weight, and chlorophyll and carotenoids content (Gireesh et al. 2011). However, its growth-promoting potential and effects on seaweed species are not yet fully assessed and are still subject to further studies.

Hence, the present study aimed to determine the efficacy of *Ulva* seaweed liquid extract (SLE) as phycobiostimulant based on its nutrient composition and physicochemical properties and its effects on the survival (%), direct axes formation (%), shoot length, and chlorophyll-*a* (chl-*a*) content of *E. denticulatum* after 45 days of tissue culture.

2. MATERIALS AND METHODS

2.1. Preparation of *Ulva* SLE

The powdered *Ulva* was obtained from Eco Life G1, a dried mixture of various *Ulva* species, gathered from wild stocks in Cebu, Bohol, Zamboanga, and Tawi-Tawi (Eco Life 2023). While primarily sold as a seaweed meal for animal feed, Eco Life G1 contains amino acids, vitamins, minerals, and sulfated polysaccharide (e.g., ulvan), which has many uses for industrial applications. The biochemical profile of Eco Life G1 powdered seaweed is shown in Table 1. Eco Life G1 is a product of a privately owned Filipino company, Trading Room, Inc., which is based

Table 1. Specifications of Eco Life G1 powdered *Ulva* spp. (Eco Life 2023)

Chemical Composition	
Dry Matter	89.4%
Gross Energy	1,892 kcal kg–1
Crude Protein	9.98%
Crude fat	0.32%
Ash	48.35%
Crude fiber	4.19%
Neutral Detergent Fiber	23.52%
Acid Detergent Fiber	9.03%
Xanthophyll	15.32 ppm
Amino acids (9.98%)	
Lysine	0.29%
Methionine	0.10%
Cystine	0.32%
Threonine	0.41%
Valine	0.32%
Isoleucine	0.27%
Phenylalanine	0.35%
Tyrosine	0.34%
Histidine	0.05%
Arginine	0.42%
Leucine	0.44%
Vitamins	
E (α-tocopherol)	3.42 ppm
B5 (as Ca-pantothenate)	1.64 ppm
B2 (Riboflavin)	3.29 ppm
B6 (Pyridoxine)	63.71 ppm
B3 (Niacinamide)	7.07 ppm
Minerals	
Iron	3.14 x 103 ppm
Zinc	18.08 ppm
Manganese	28.83 ppm
Magnesium	2.08 x 104 ppm
Copper	6.98 ppm
Potassium	1.49 x 103 ppm
Selenium	0.78 ppm
Cobalt	1.04 ppm
Sodium Chloride	5.67%
Ca	8.22%
Total P	0.37%
Digestible P	0.056%
Heavy metals	
Arsenic	0.81 ppm
Cadmium	0.04 ppm
Lead	0.09 ppm
Mercury	< 0.03 ppm

in Malolos City, Bulacan, Philippines.

A ratio of 1:20 (weight/volume) of powdered *Ulva* and distilled water was prepared for the *Ulva* seaweed liquid extract (Divya et al. 2015). Initially, 50 g of powdered *Ulva* was weighed using an analytical balance and transferred to a 2-L glass reagent bottle using a funnel. One liter (1 L) of distilled water was added to the glass reagent bottle. The algal powder suspension was then mixed manually for five minutes and autoclaved at standard program and conditions (i.e., 15 psi at 121°C for 30 minutes).

After autoclaving, the *Ulva* SLE was filtered using a cheesecloth to separate the aqueous phase from the solid residues; the filtrate was then allowed to stand overnight at room temperature (28±1°C). The liquid extract was further centrifuged at 9,000 rpm at 4°C for 30 minutes. The supernatant was then carefully transferred to 50-mL Falcon tubes with proper labels, including the name of the extract, date of extraction, batch number, and tube number. The *Ulva* SLE was kept at room temperature, not exceeding 30°C, until further use.

Samples of *Ulva* SLE were also submitted to the Department of Agriculture Region VI Analytical Laboratory, Iloilo City and the Sugar Regulatory Administration (SRA), Bacolod, for proximate composition and mineral analyses, respectively.

2.2. Preparation of *Eucheuma denticulatum* explants and tissue culture experiment

Approximately ten kilograms (10 kg) of fresh *Eucheuma denticulatum* were obtained from a seaweed farm in Sorsogon, Philippines, on 17 June 2022. These seaweeds are cultivated using a fixed off-bottom line method (Hurtado et al. 2014). The collected algae were kept cool in a Styrofoam box lined with banana leaves and were transported by airplane to the Institute of Aquaculture Hatchery Complex, University of the Philippines (UP) Visayas, Miagao, Iloilo, within 12 hours. Upon arrival, the Styrofoam box was left open for 30 minutes to allow heat dissipation before the collected samples were transferred to 300-L fiberglass tanks. The seaweeds were acclimatized for 14 days before the tissue culture experiment. During acclimatization, *E. denticulatum* were placed in fiberglass tanks with UV-filtered seawater under ambient conditions (i.e., at 28 ± 2°C, 30 ± 3 psu, pH 8 ± 0.2, 130 ± 5 µmol photons m^{-2} s^{-1}) and provided with moderate aeration. The renewal of the UV-filtered seawater was done every seven days.

The protocol for seaweed tissue culture was based on Hurtado et al. (2009) and Luhan and Mateo (2017). Healthy and epiphyte-free, apical 2-cm segments of *E. denticulatum* were initially cut using a sterile scalpel blade, brushed with 0.05% povidone-iodine solution, and rinsed three times with UV-filtered seawater. The segments were then cut into 3–5 mm-thick cross sections and again rinsed three to four times with UV-filtered seawater. Approximately 2,400 sections of *E. denticulatum* were prepared for the tissue culture experiment.

One hundred sections of *E. denticulatum* were stocked in 1-L culture vessels (1 section:10 mL), each containing *Ulva* SLE-seawater solution at different concentrations (i.e., 0.01, 0.1, 1, 2, and 3 mL L^{-1}) and one control group (i.e., UV-filtered seawater). Each treatment concentration and control had four replicates. One milliliter (1 mL) each of indole-3-acetic acid (IAA) and kinetin (plant growth regulators as priming agents to stimulate cell division) (Tibubos et al. 2017; Ali et al. 2018; Jiksing et al. 2022) were added to all the treatment and control groups at the start of culture experiment (i.e., Day 0) only.

The cultures were incubated for 45 days in a walk-in culture room of the Seaweed Laboratory, UP Visayas, under controlled conditions: 23–24°C, 110 ± 5 µmol photons m^{-2} s^{-1} incident irradiance (LED tubes) at 13:11h L:D photoperiod, and with moderate aeration. Sampling and renewal of the culture media were done every seven days.

At the end of the 45-day culture period, the following parameters were determined: survival (%); direct axes formation (%); shoot length (mm), and chl-*a* content.

The percent survival of the seaweed explants was calculated using the following equation:

$$\%\, Survival = \frac{final\ number\ of\ algal\ sections\ at\ day\ 45}{initial\ number\ of\ algal\ sections} \times 100$$

The percentage of direct axes formation was determined using the following equation:

$$\%\, direct\ axes\ formation = \frac{number\ of\ shoots\ formed\ at\ day\ 45}{initial\ number\ of\ algal\ sections} \times 100$$

Shoot length of the seaweed explants was likewise measured using a Vernier caliper.

For chl-*a* content determination, ten percent (10%) of the viable seaweed segments per treatment replicate were randomly selected for pigment extraction. Chl-*a* was extracted using 10 mL of N, N-dimethylformamide under dark refrigeration (10°C) for 24 hours. The absorbance of the extracts was then measured using a UV-VIS spectrophotometer.

Chl-*a* (μg g_{fw}^{-1}) was calculated using the following equation (Porra et al. 1989):

$$chl-a = \frac{[12.00\,(Abs_{664} - Abs_{750}) - 3.11\,(Abs_{647} - Abs_{750})] \times V}{FW}$$

where Abs_{664}, Abs_{647}, and Abs750 are the absorbances at 664, 647, and 750 nm, respectively, V is the volume of extraction solvent (i.e., 10 mL N, N-dimethylformamide), and FW is the fresh weight of the algal sample extracted in grams.

2.3. Statistical analyses

Statistical analyses were performed using R version 4.3.1 (R Development Core Team 2023). Assumptions for the normality and homogeneity of experimental data were tested using Shapiro-Wilk's test and Levene's test, respectively. Data were log-transformed to meet these assumptions if necessary. One-way analysis of variance (ANOVA, at $p = 0.05$ significance level) and Tukey's HSD test were used to determine the significant difference among the treatments.

3. RESULTS

3.1. Physicochemical properties of *Ulva* SLE

Elements such as phosphorus, nitrogen, carbon, sodium, iron, copper, manganese, zinc, and calcium, and compounds like potassium oxide, sodium chloride, and crude protein were found in the *Ulva* liquid extract. However, magnesium was not detected. Table 2 presents the results of the proximate composition and mineral analyses of the *Ulva* SLE.

3.2. Tissue culture of *Eucheuma denticulatum*

3.2.1. Survival of *Eucheuma denticulatum*

The results of the percent survival showed that 0.01, 0.1, 1, and 3 mL L^{-1} *Ulva* SLE concentrations obtained the highest value of 100 ± 0% (mean ± standard error of mean, SEM; Figure 1). This was followed by 2 mL L^{-1} concentration with a value of 99.8 ± 0.2%, and the control (UV-filtered seawater), which obtained a value of 99.5 ± 0.5%. Statistical analysis revealed no significant difference among all treatments.

3.2.2. Direct axes formation of *Eucheuma denticulatum*

A significant difference in the direct axes formation of *E. denticulatum* among the different concentrations of the *Ulva* SLE and control was found ($p < 0.001$; Figure 2). The highest percentage was observed in the 1 and 2 mL L^{-1} *Ulva* SLE (99.8–100%), followed by 0.1 and 3 mL L^{-1} (95–95.2%), and 0.01 mL L^{-1} (91.8 ± 1.9%). The control group had the lowest direct axes formation of 88.5 ± 0.6%. While the emergence of shoots was observed in all treatments as

Table 2. Physicochemical profile of *Ulva* SLE.

Physical Data	
Color / Appearance	Yellow green
Odor	Mild odor
pH	6.37
Typical Analysis	
Crude protein	0.06%
Total Phosphorus	0.001%
Total Organic Carbon	0.29%
Nitrogen	9,300 ppm
Iron	260 ppm
Zinc	113.2 ppm
Manganese	56.9 ppm
Magnesium	Not detected
Copper	48.3 ppm
Potassium oxide	6,600 ppm
Sodium	28.7 ppm
Calcium	0.14%
NaCl	0.49%

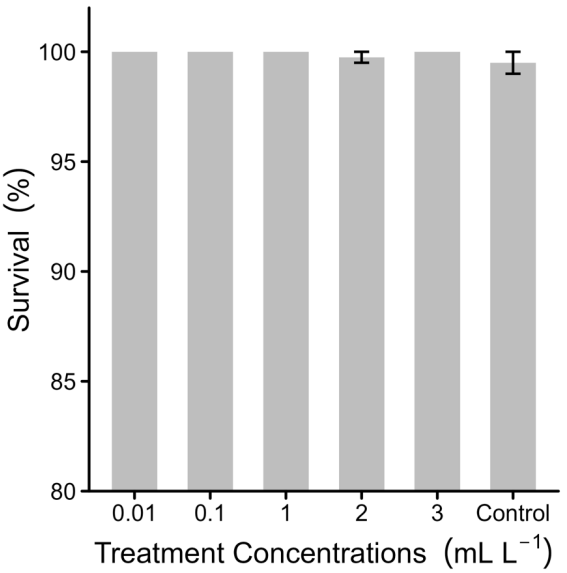

Figure 1. Survival (%; mean ± standard error of mean, SEM; n = 4) in *Eucheuma denticulatum* after 45 days of culture at varying concentrations of *Ulva* seaweed liquid extract (0.01, 0.1, 1, 2, and 3 mL L^{-1}) and control (UV-filtered seawater). The absence of error bars indicates a standard error of zero per treatment. The starting value of the y-axis was set to 80% for better visualization of results.

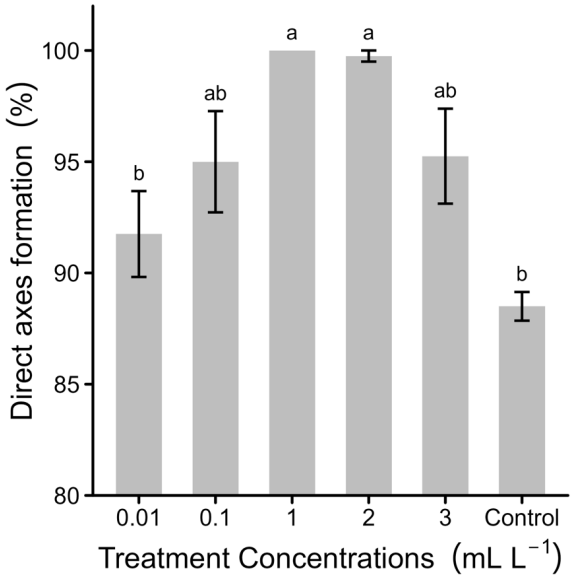

Figure 2. Direct axes formation (%; mean ± SEM, $n = 4$) in *Eucheuma denticulatum* after 45 days of culture at varying concentrations of *Ulva* seaweed liquid extract (0.01, 0.1, 1, 2, and 3 mL L^{-1}) and control (UV-filtered seawater). Means with different letter(s) are statistically different at the 5% confidence level based on the post-hoc Tukey test. The starting value of the y-axis was set to 80% for better visualization of results.

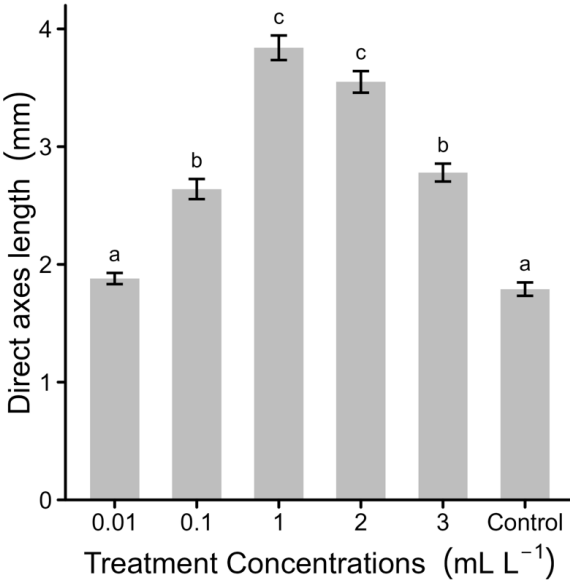

Figure 3. Direct axes length (mm; mean ± SEM) in *Eucheuma denticulatum* after 45 days of culture at varying concentrations of *Ulva* seaweed liquid extract (0.01, 0.1, 1, 2, and 3 mL L^{-1}) and control (UV-filtered seawater). Means with different letter(s) are statistically different at the 5% confidence level based on the post-hoc Tukey test.

early as Day 7, significantly higher shoot formation of the E. denticulatum segments cultured in 1 and 2 mL L–1 concentrations (p = 0.001) was observed on Day 14 (Supplementary Figure 1).

3.2.3. Direct axes length of *Eucheuma denticulatum*

A significant difference was also observed in the direct axes length of *E. denticulatum* segments among treatment and control groups ($p < 0.001$; Figure 3). The longest direct shoot measurement was observed in the 1 and 2 mL L^{-1} concentrations (3.6–3.8 mm). It was then followed by seaweed segments cultured in 0.1 and 3 mL L^{-1} *Ulva* SLE (2.6–2.8 mm). The shortest shoot measurements, on one hand, were observed in the 0.01 mL L^{-1} treatment concentration and the control (1.8–1.9 mm).

3.2.4. Chlorophyll-*a* content of *Eucheuma denticulatum*

The chl-*a* content of *E. denticulatum* explants by the end of the 45-day culture period was apparently influenced by *Ulva* SLE ($p < 0.001$; Figure 4). Algal sections cultured in 2 and 3 mL L^{-1} *Ulva* SLE had the highest pigment concentration, with values of 93.5 ± 10.1 and 90.1 ± 3.0 µg g_{fw}^{-1}, respectively. Low chl-*a* content, on one hand, was observed in 1, 0.1, and 0.01 mL L^{-1} treatment concentrations, with corresponding values of 58.4 ± 2.7, 47.5 ± 2.5, and 47.5 ± 4.0 µg g_{fw}^{-1}. The lowest chl-*a* measurement was observed in the control group (35.2 ± 4.0 µg g_{fw}^{-1}).

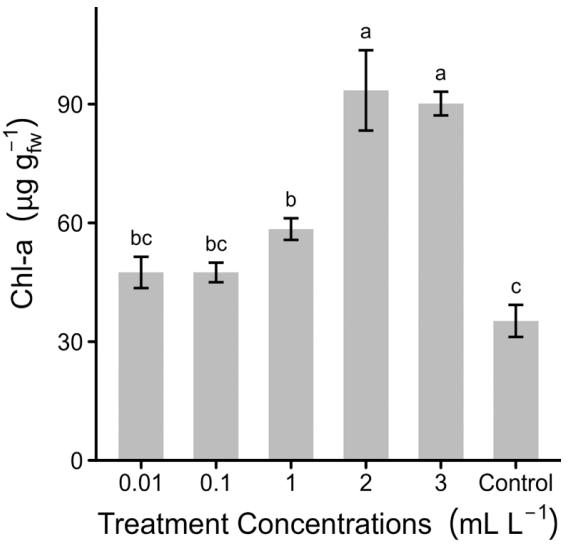

Figure 4. Chlorophyll-*a* (µg g_{fw}^{-1}; mean ± SEM, $n = 4$) in *Eucheuma denticulatum* after 45 days of culture at varying concentrations of *Ulva* seaweed liquid extract (0.01, 0.1, 1, 2, and 3 mL L^{-1}) and control (UV-filtered seawater). Means with different letter(s) are statistically different at the 5% confidence level based on the post-hoc Tukey test.

4. DISCUSSION

Seaweed liquid extracts are of great interest because of their wide range of uses, specifically in improving yield, elevating resistance to stress and diseases, and enhancing the post-harvest shelf life of various agricultural crops (Khan et al. 2009; Shukla et al. 2019; Hurtado et al. 2021). Such activities are often linked to the bioactive compounds in seaweeds, such as micro- and macronutrients, carotenoids, phenolics, and plant growth regulators (Benítez García et al. 2020). Ali et al. (2021) also asserted that algal extracts are often referred to as biostimulants rather than fertilizers because they could stimulate plant growth and defense responses even when applied in small quantities.

The present study revealed the efficacy of a seaweed liquid extract from *Ulva* in improving the growth and chl-*a* content of *E. denticulatum* after 45 days of tissue culture. In fact, the direct axes formation and shoot length of *E. denticulatum* segments of the treatment groups were relatively higher than in the control group (i.e., UV-filtered seawater only). Among all the treatments, the 1 and 2 mL L^{-1} *Ulva* SLE had the highest direct axes formation and longest shoot measurements. A decline in these growth parameters was also observed in *Ulva* SLE treatments below and above such concentrations, suggesting a dose-dependent effect. A similar pattern was observed in the study conducted by Gireesh et al. (2011) in determining the effects of seaweed liquid extract from *Ulva lactuca* on the growth and germination of *Vigna unguiculata* seedlings. Results of their study revealed that seedlings soaked at lower concentrations (i.e., 0 and 5%) resulted in lower germination, shoot length, and root length compared to SLE concentrations of 10 and 20%; higher concentrations of SLE (i.e., 30, 40, and 50%) also inhibited germination, shoot length, and root length of *Vigna unguiculata* seedlings. Hence, testing dose-response curves is necessary to determine optimal ranges for maximum plant growth performance while minimizing the adverse effects of excessive application.

The chl-*a* content of *E. denticulatum* micropropagules likewise increased with the addition of *Ulva* SLE in the culture medium. However, the results of the chlorophyll pigment analysis were inconsistent with those of direct axes morphogenesis and growth. The 2 and 3 mL L^{-1} concentrations were observed to have the highest chl-*a* content. These insights into the optimum concentration of *Ulva* SLE for the tissue culture of *E. denticulatum* should be interpreted cautiously, considering the induction of direct shoots as the primary index for growth (Hurtado et al. 2009; Tibubos et al. 2017; Ali et al. 2018). Growth integrates all metabolic processes, including nutrient uptake and assimilation, biosynthesis, and homeostasis. Nonetheless, chlorophyll is a vital pigment for photoautotrophic organisms, playing a significant role in regulating their photosynthetic capacity. Primarily, its role is to absorb light energy to initiate photosynthetic electron transport, photophosphorylation, and carbon assimilation in the process of photosynthesis (Li et al. 2018). Thus, determining the chlorophyll content of *E. denticulatum* explants in the present study is essential as it indirectly elucidates the influence of the *Ulva* SLE in pigment synthesis, overall photosynthetic performance, and so the growth of the cultured seaweed. The application of *Ulva* SLE improved the chl-*a* of *E. denticulatum* by perhaps increasing the biogenesis of chloroplasts and reducing chlorophyll degradation (Jannin et al. 2012). Moreover, the presence of nutrients, especially nitrogen, could have influenced pigment synthesis (Carvalho et al. 2011) in *E. denticulatum*, which corresponds to the other studies that involved land-based crops which were also treated with seaweed liquid extracts (Gireesh et al. 2011; Castellanos-Barriga et al. 2017; Yao et al. 2020; Paulert et al. 2021).

Indeed, the results of the proximate composition and mineral analyses of the *Ulva* SLE showed that there is a noticeable amount of nitrogen among all elements detected. Nitrogen is one of the limiting macronutrients in seaweed growth and development, and it is involved in the synthesis of carbohydrates, proteins, and lipids (Yodsuwan et al. 2017; Zarrinmehr et al. 2020; Yaakob et al. 2021). Its presence in the *Ulva* SLE could also be one of the reasons why it improved the growth and direct regeneration of *E. denticulatum*. Aside from nitrogen, other trace elements like calcium, sodium, iron, copper, manganese, and zinc, and compounds like crude protein, sodium chloride, and potassium oxide were also detected in the *Ulva* SLE. Such observations coincided with numerous studies showing the presence of mineral nutrients in extracts from various species of *Ulva* (Gireesh et al. 2011; Divya et al. 2015; Castellanos-Barriga et al. 2017; Latique et al. 2021). It is, however, difficult to compare the proximate and nutrient composition results of previous studies to the present study because of the differences in the algal biomass (including spatio-temporal variations) and extraction methods used for these seaweed-derived biostimulants. The multiple bioactive compounds in

the seaweed extracts may either be naturally present in the seaweeds or may be novel products from the hydrolytic processes during extraction (Vaghela et al., 2023). Nevertheless, *Ulva* SLE in the present study contains active metabolites in physiologically relevant concentration, as evidenced by the improved growth performance of *E. denticulatum* in tissue culture.

The Eco Life G1 powdered *Ulva* had a higher mineral composition when compared to the seaweed liquid extract, and magnesium was not detected in the *Ulva* SLE. The disparities in their nutrient content could be attributed to the process of producing the seaweed liquid extract. According to Kidgell et al. (2019), it is important to understand the extraction, isolation, and purification processes of polysaccharides and other minerals to attain the maximum yield needed for their biological activities. Pappou et al. (2022) also claimed that the extraction yield of bioactive compounds in *Ulva* depends on various factors such as solvent type, solvent-to-solid ratio, temperature, and extraction time. Thus, it is recommended to optimize the extraction process further, utilizing different solvents and techniques to ensure a high yield of nutrients and minerals found in *Ulva*.

Additionally, the *Ulva* SLE is believed to contain growth-promoting phytohormones that also contributed to its efficacy in stimulating meristematic cell growth and direct axes formation in *E. denticulatum*. Several studies revealed the presence of phytohormones in *Ulva*; for instance, in the study conducted by Divya et al. (2015), the seaweed liquid extract from *Ulva lactuca* contained plant growth hormones like cytokinin (370.86 µg g^{-1} dry weight) and auxin (290.52 µg g^{-1} dry weight). The study of Benítez García et al. (2020) reported the presence of plant growth regulators in *Ulva lactuca* extract such as salicylic acid (67.6 ± 4.2 ng g^{-1}), indole acetic acid (49.3 ± 5.2 ng g^{-1}), abscisic acid (17.8 ± 5.2 ng g^{-1}), and gibberellins (1.51 ± 0.64 ng g^{-1}). The Eco Life G1 powdered *Ulva* likewise contains α-tocopherol and other phenolic compounds associated with biological activities such as antioxidant properties (Benítez García et al. 2020; Latique et al. 2021), which could elicit stress response and protective mechanisms in *E. denticulatum*. Polysaccharides such as ulvans from these green seaweed species were also regarded as biological protection agents against plant diseases (Vera et al. 2011). Alternatively, the beneficial effects observed in plants following seaweed extract application were associated with the activation and up-regulation of specific photosynthesis, hormone signaling, and defense and growth-related genes, as argued by Omidbakhshfard et al. (2020) and Wally et al. (2013), as well as with the stimulation of enzymatic activities in the metabolic pathways of the seaweed extract-treated plants (Paulert et al. 2021). Extensive analysis of the nutrient availability, plant growth regulators, and bioactive metabolites in the *Ulva* SLE is necessary to provide robust evidence of their effects on the growth and direct regeneration of eucheumatoid explants. Examining the underlying mechanisms of how the *Ulva* SLE improves the growth and photosynthesis of cultured seaweed species and agricultural crops would also be an interesting topic for future studies.

5. CONCLUSION

The study presented promising results on the efficacy of seaweed liquid extract from *Ulva* spp. in improving the growth and chl-*a* content of *Eucheuma denticulatum* in tissue culture. *Ulva* SLE developed in this study contained mineral nutrients that are crucial for the growth and direct regeneration of *E. denticulatum*, hence its success rates for tissue culture. Thus, it can be a potential alternative to existing nutrient or biostimulant enrichment techniques, which could help address phyconomic issues concerning the availability of good quality and high-yielding eucheumatoid cultivars for large-scale production.

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SUPPLEMENTARY MATERIAL

Below is the link to the electronic supplementary material. Supplementary file

AUTHOR CONTRIBUTIONS

Borlongan IA: Conceptualization, Supervision, Formal analysis, Resources, Writing – Original draft preparation, Writing – Review and Editing, Project administration, Funding acquisition. **Toroy LA:** Methodology, Formal analysis, Resources, Writing – Original draft preparation, Writing – Review and Editing, Funding Acquisition. **Roco O:** Resources, Writing – Review and Editing.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

No animal or human trials were carried out by the authors.

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