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INFLUENCE OF SEAWEED LIQUID EXTRACTS ON THE GROWTH AND NUTRITIONAL PROFILE OF THE MICROALGA, *NANNOCHLOROPSIS OCULATA* D. J. HIBBERD 1981

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ABSTRACT
This study investigates the potential of seaweed extracts as nutrient sources for the growth of microalga, Nannochloropsis oculata. The proximate composition analysis of seaweeds revealed that the presence of significant levels of macronutrients, micronutrients, and trace elements essential for microalgal cultivation. The growth performance of N. oculata was evaluated in culture media supplemented with varying concentrations of seaweed extracts. The highest cell density (9.22 × 10^6 cells/mL) was observed in the 5% U. lactuca extract-supplemented flask on the 8^{th} day. Biochemical analysis indicated that the maximum protein (44.37 ± 4.3%), lipid (25.1 ± 2.5%), and carbohydrate (19.7 ± 2%) contents were recorded in cultures supplemented with seaweed extracts. Pigment analysis further demonstrated increased chlorophyll-a levels in microalgae grown in Ulva-based media. These findings suggest that seaweed extracts, particularly from U. lactuca, can serve as sustainable, natural alternatives to conventional microalgal culture media, promoting growth and biochemical enrichment.
KEY WORDS*: Seaweed, microalgae, growth rate, nutritional profile, culture media*

INTRODUCTION

Microalgae are widely distributed in nature and have adapted to diverse environments, exhibiting significant variations in size, morphology, life cycle, pigments, and metabolites (Matsunaga *et al*., 2009). Recently, microalgae have gained global attention due to their extensive applications in renewable energy, biopharmaceuticals, and nutraceutical industries (Khan *et al*., 2018). They also play a crucial role in mariculture, as the larval stages of many aquatic organisms rely directly on microalgae for nutrition (Carioca, 2010; Abarna *et al*., 2022). The growth and proximate composition of microalgae are significantly influenced by environmental factors such as temperature, salinity, light intensity, and nutrient availability in the culture medium (Thompson, 1996; Brown *et al*., 2002). Large-scale microalgal cultivation requires substantial amounts of nutrients and additives, leading to high operational costs. The

nutrient composition of the culture medium directly impacts microalgal growth and metabolite accumulation (Benavente Valdés *et al*., 2016). Therefore, selecting an optimal culture medium that meets the nutritional needs of microalgae is essential for efficient biomass production (Abarna *et al*., 2022). Using cost-effective raw materials and by-products as nutrient sources presents a sustainable alternative for microalgal cultivation, reducing production expenses and promoting environmental sustainability (Lam and Lee, 2012).

Nannochloropsis species are unicellular microalgae characterized by coccoid cells with polysaccharide cell walls. They belong to the Eustigmatophyceae class, containing only chlorophyll-*a* while lacking chlorophyll-*b* and *c* (Antia and Cheng, 1982). *Nannochloropsis oculata* is considered a promising candidate for industrial applications due to its ability to accumulate high concentrations of eicosapentaenoic acid (EPA) and monounsaturated fatty acids. Additionally, *N. oculata* has demonstrated significant immunomodulatory properties. Its sterol-rich fraction exhibits anti-inflammatory and anticancer activities, while its water-soluble polysaccharides possess in vitro immunostimulatory effects, making it a valuable resource for biomedical applications (Sanjeewa *et al.*, 2016; Zahran *et al*., 2023).

Seaweeds, or macroalgae, are rich in macronutrients, micronutrients, amino acids, vitamins, and growth-promoting hormones, making them an excellent natural fertilizer for various crops (Kaliaperumal *et al*., 1987; Spinelli *et al*., *2010*; Chojnacka *et al*., 2015). Seaweed extracts enhance plant growth, improve stress tolerance, boost antioxidant content, and increase nutrient uptake from the soil. Research has also demonstrated that seaweed extracts can serve as beneficial additives for microalgal cultures (Rathore *et al*., 2009). These extracts are biodegradable, non-polluting, and non-hazardous, making them an environmentally friendly alternative to synthetic fertilizers (Dhargalkar and Pereira, 2005). Although seaweed liquid fertilizers (SLFs) have been widely studied as fertilizers for angiosperms, their application in microalgal cultivation remains limited (Gireesh, 2009; Raja *et al*., 2015; Abarna *et al*., 2022). Utilizing seaweed-based media not only provides essential nutrients for microalgal growth but also promotes sustainability by mitigating environmental pollution. The present study aims to explore sustainable nutritional supplements as an alternative to commercial culture media for microalgal cultivation. Specifically, this study investigates the effects of aqueous extracts from two seaweed species, *Ulva lactuca* and *Ulva reticulata*, on the growth and biochemical composition of the microalga *N. oculata*.

MATERIALS AND METHODS

Microalgal culture

Marine microalgae, *N. oculata*, stock culture was obtained from the Rajiv Gandhi Centre for Aquaculture (RGCA) in Sirkazhi, Tamil Nadu. It was subcultured in Conway's medium, maintained at a temperature of 22°C, with a photoperiod of 12:12 hours (light: dark). The culture flasks were shaken three times a day to keep the algal cells suspended.

Seaweed extract preparation

Fresh seaweeds were collected from the Thengapattanam coast in Tamil Nadu, located on the southwest coast of India. The seaweeds were identified as *Ulva lactuca* and *Ulva reticulata* using an identification manual (Manisseri *et al*., 2012). The collected seaweeds were thoroughly washed to remove dust, salt, and epiphytes, shade-dried for 24 hours, and powdered using a blender. Fifty grams of dried seaweed powder was placed in a beaker with 150 mL of distilled water and 20% sodium carbonate, then heated at 60°C for 3 hours in a water bath. After fermentation, the pH of the solution was adjusted to 7.5 by adding 20% hydrochloric acid. The fermented solution was filtered using a sieve and centrifuged at 5000 rpm for 20 minutes. The resultant supernatant was used as a nutrient supplement for microalgal culture media.

Estimation of proximate composition in seaweed

The proximate composition of seaweed powder (*U. lactuca* and *U. reticulata*), including total proteins, lipids, carbohydrates, fiber, and ash, was determined using standard methods (AOAC, 2005). Phosphorus content was estimated using the Amino Naphthol Sulphonic method (Allen, 1940). Sodium and potassium levels were measured using a flame photometer (Hald, 1947), while other essential nutrients such as calcium, magnesium, iron, zinc, manganese, and copper were analyzed using atomic absorption spectroscopy (AAS).

Experimental setup

All experiments were conducted in 250 mL conical flasks in triplicate. Filtered seawater was sterilized and inoculated with 10% microalgal inoculum. Different concentrations of seaweed extract (1%, 3%, and 5%) were added as nutritional supplements, while Conway's medium served as the control (without seaweed extract).

Microalgal growth analysis

Microalgal growth was monitored over nine days. One mL of sample from each treatment was taken daily, and the number of cells was counted using a hemocytometer under a light microscope.

Estimation of nutritional profile in microalgae

The total protein, carbohydrate, and lipid content in both experimental and control microalgal samples were analyzed using the methods of Lowry *et al.* (1951), Dubois *et al.* (1956), and Folch *et al*. (1957), respectively.

Pigment analysis in microalgae

Chlorophyll content in experimental and control microalgal samples was analyzed following the standard procedure of Strickland and Parson (1969). A 5 mL algal sample was taken from each treatment and control setup, then centrifuged in a refrigerated centrifuge (Eppendorf) at 5000 rpm for 5 minutes at 4°C. The supernatant was discarded, and 5 mL of N,N-dimethylformamide was added to the pellet and incubated for 24 hours. After incubation, the sample was centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected separately. Optical density (OD) was measured at 664 nm using a spectrophotometer. Chlorophyll-a concentration was calculated using the formula: Chlorophyll-*a* (µg/mL) = OD value × 11.92.

Statistical analysis

All experiments were conducted in triplicates, and the average value was considered for analysis. The mean and standard deviation of the triplicates were calculated using MS Excel 10 software. One-way ANOVA was performed to determine the differences between treatments and the control. Duncan's multiple range test was used to assess mean differences at a significance level of 0.05.

RESULTS AND DISCUSSIONS

Proximate composition of seaweed

The two seaweed species analyzed contained abundant levels of macronutrients, micronutrients, and trace elements essential for microalgal growth, as shown in Table 1. The dominant nutrients observed were sodium (Na), potassium (K), calcium (Ca), phosphorus (P), and magnesium (Mg), while iron (Fe) and zinc (Zn) were also present in significant amounts. Manganese (Mn) and copper (Cu) were detected at trace levels in both *U. lactuca* and *U. reticulata*.

Growth of *N. oculata*

Seaweeds accumulate high levels of nutrients from surrounding water, and extracts derived from them can serve as fertilizers for algal growth. In addition to macronutrients, seaweed extracts also contain trace elements and growth promoters (Fornes *et al*., 1993). In this study, the growth of *N. oculata* in terms of cell density was highest (9.22×10^6 cells/mL) in the 5% *U. lactuca* extract-supplemented culture flask on the 8th day. In contrast, the cell density in the 1% *U. reticulata* extract-supplemented

flask was 8.02×10^6 cells/mL. These results indicate that 5% *U. lactuca* extract provides sufficient nutrients for optimal *N. oculata* growth. The growth pattern of *N. oculata* in different culture setups is illustrated in Figure 1.

Nutritional composition in *N. oculata*

The biochemical composition of microalgal biomass varies depending on the nutrient concentration of the culture medium (Herrero *et al*., 1991). Therefore, biochemical analysis helps determine the most suitable media for microalgal culture. Nutritional profile results of *N. oculata* grown in two seaweed extracts at various concentrations are given in Figure 2. In this study, the maximum protein content (44.37 ± 4.3%) was recorded in the 5% *U. lactuca* extract-supplemented flask, while the minimum protein content (29.91 ± 3 %) was observed in the 1% *U. reticulata* extract-supplemented flask. The maximum lipid production (25.1 ± 2.5%) was found in the 5% *U. lactuca* extract-supplemented flask, whereas the minimum lipid production (14.5 ± 1.5%) was observed in the 1% *U. reticulata* extract-supplemented flask. The highest carbohydrate content (19.7 ± 2%) was observed in the 3% *U. reticulata* extract-supplemented flask, while the lowest (16.53 ± 1.6%) was recorded in the 1% *U. lactuca* extract-supplemented flask.

Pigment profile in *N. oculata*

The maximum chlorophyll-*a* content (1.449 ± 0.15 µg/mL) was recorded in the 5% *U. lactuca* extract-supplemented flask, while the minimum (1.321 ± 0.13 µg/mL) was observed in the 1% *U. reticulata* extract-supplemented flask. Chlorophyll- *a* content in *N. oculata* grown in two seaweed extracts at various concentrations is shown in Figure 3.

Generally, potassium and other nutrients in seaweed extracts are water-soluble and readily available for plant absorption, effectively addressing nutrient deficiencies (Mohanty *et al*., 2013; Abarna *et al*., 2022). Previous studies have confirmed that seaweeds contain substantial quantities of macronutrients, micronutrients, and trace elements (Villares *et al*., 2007). Karemore *et al*. (2013) emphasized the crucial role of nutrients such as nitrogen (N), P, K, Ca, and Mg in microalgal biomass production. In this study, an extract was prepared from seaweed containing all essential nutrients required for microalgal growth, potentially acting as a biostimulant for enhancing the growth of *N. oculata*. Higher nutrient concentrations were observed in *Kappaphycus alvarezii* extract compared to *Turbinaria conoides* extract (Raja *et al*., 2015; Abarna *et al*., 2022). The findings of Reddy *et al*. (2022) align with this study, as they also reported that *Ulva* species contain the highest protein content among various seaweed species.

Consequently, this study supports the use of green seaweeds as viable candidates for preparing media supplements that meet the nutritional requirements of microalgae.

Observations revealed that the exponential phase of *N. oculata* growth commenced on the 3rd day, while the stationary phase was reached after the 8th day, with no further cell division observed. Similar results were documented by Abarna *et al*. (2022) in *N. oculata* and *Dunaliella salina* when using seaweed-based liquid fertilizers. Alvarado *et al*. (2008) reported that *Chaetoceros mulleri* cultured in seaweed extract compost exhibited comparable algal growth and biochemical composition to those cultured in commercial media such as Walne's and agricultural fertilizers. The enhanced growth performance observed in this study may be attributed to the presence of growth-promoting substances such as auxins, gibberellins, and cytokinins in liquid fertilizers derived from *Ulva* species (Rosyida *et al*., 2021). Additionally, Rohani-Ghadikolaei *et al*. (2012) reported that *Isochrysis galbana* cultivated in F/2 media supplemented with seaweed extracts (*U. lactuca, Enteromorpha intestinalis,* and *Gracilaria corticata*) exhibited increased cell density.

Rohani-Ghadikolaei *et al*. (2012) documented that the protein content of *I. galbana* increased when seaweed extract was supplemented with F/2 medium. Comparisons with previous studies by Lakshmi and Sheeja (2021) suggest that red algal seaweed liquid fertilizers derived from *Kappaphycus alvarezii* and *Gracilaria acerosa* effectively enhance both growth and protein content in microalgae cultures. Alvarado *et al*. (2008) reported that *C. muelleri* cultivated in brown seaweed compost with disodium silicate amendments exhibited increased lipid accumulation. Similarly, Gireesh *et al*. (2009) found that *D. salina* grown in seaweed extract had a higher lipid content than controls using Conway or Walne's media. Rohani-Ghadikolaei *et al*. (2012) reported comparable results for *I. galbana*, which showed similar lipid production levels when grown in seaweed extract-supplemented media and F/2 medium. Liang *et al*. (2010) suggested that the increased lipid production observed in microalgae treated with seaweed extracts is due to the presence of convertible carbon sources that stimulate lipid accumulation. Rohani-Ghadikolaei *et al*. (2012) reported a significant increase in carbohydrate levels in *I. galbana* grown in F/2 media supplemented with seaweed extracts from *U. lactuca, E. intestinalis,* and *G. corticata*. Lakshmi and Sheeja (2021) also observed a similar increase in carbohydrate levels in *Chlorella vulgaris* cultured in BBM media supplemented with seaweed extracts. Studies by Bharathi *et al*. (2021) showed that *Picochlorum maculatum* cultured with *Sargassum wightii, S. muticum,* and *Turbinaria ornata* extracts exhibited higher protein, carbohydrate, and lipid levels compared to controls.

TABLE 1. Biochemical profile in dried *Ulva lactuca* and *Ulva reticulata*

S. No.	Parameters	*Ulva lactuca*	*Ulva reticulata*
1.	Protein (%)	10.75 ± 0.12	11.64 ±0.11
2.	Carbohydrate (%)	53.40 ±0.52	51.62 ±0.64
3.	Lipid (%)	3.51 ±0.03	2.85 ±0.03
4.	Ash (%)	11.50 ±0.19	10.72 ±0.15
5.	Fiber (%)	31.26 ±0.82	28.21 ±0.67
6.	Sodium (mg/100gm)	461± 6	430 ±5
7.	Calcium (mg/100gm)	2128 ±17	1980 ±23
8.	Potassium (mg/100gm)	565 ±8	583 ±7
9.	Phosphorous (mg/100gm)	84.00 ±1.50	87.20 ±1.57
10.	Magnesium (mg/100gm)	2358 ±21	2260 ±14
11.	Iron (mg/100gm)	23.10 ±0.20	21.74 ±0.23
12.	Zinc (mg/100gm)	4.64 ±0.02	4.82 ±0.02
13.	Manganese (mg/100gm)	1.32 ±0.02	1.27 ±0.02
14.	Copper (mg/100gm)	0.73 ± 0.01	0.81 ±0.01

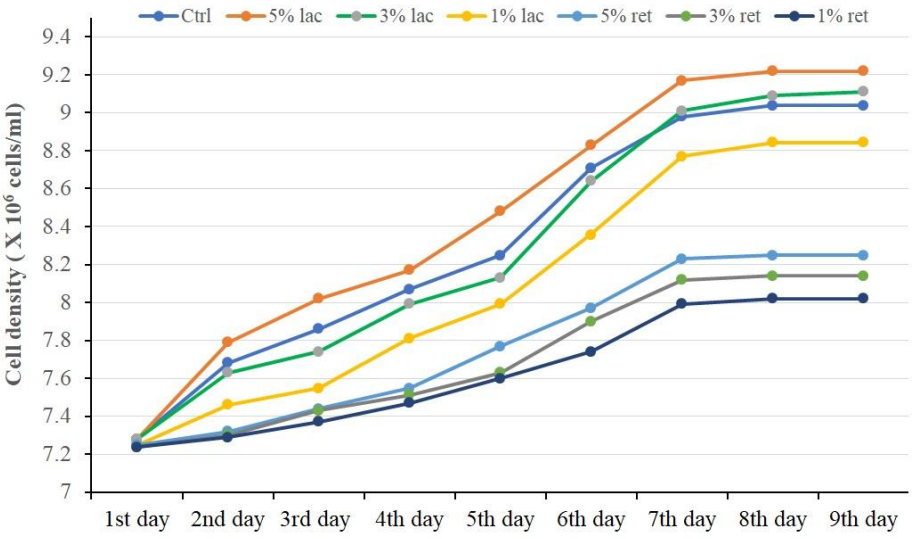

FIG 1. Growth rate of *N. oculata* in different concentrations of seaweed extracts

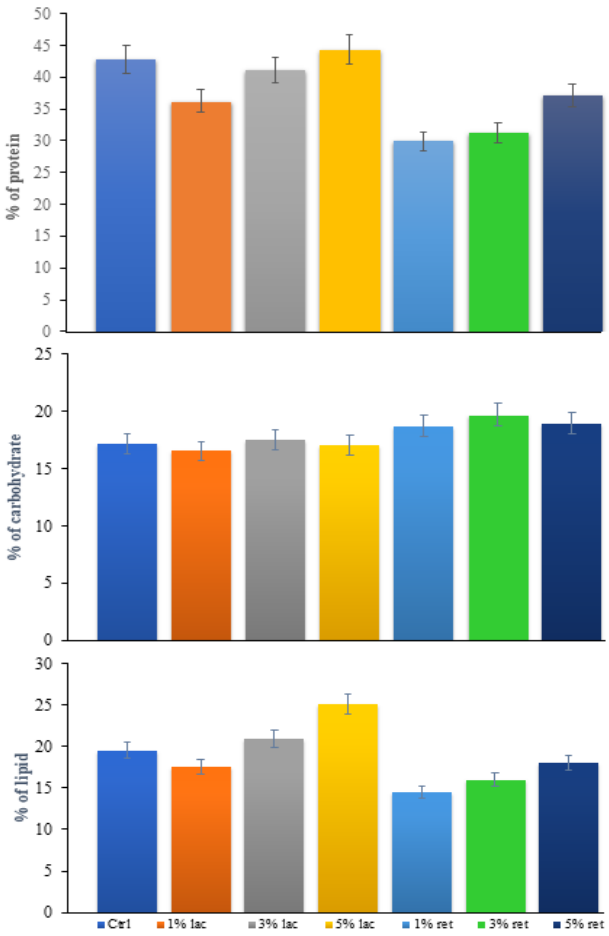

FIG. 2. Nutritional profile of *N. oculata* grown in two seaweed extracts at various concentrations

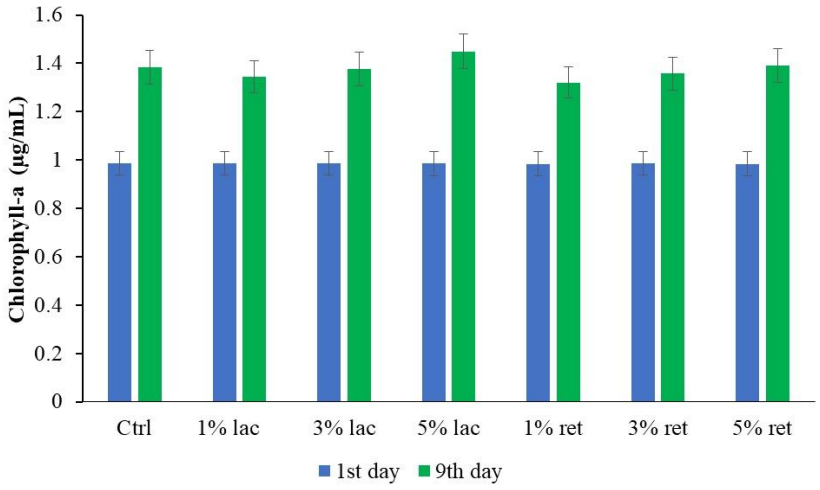

FIG. 3. Chlorophyll-*a* content in *N. oculata* grown in two seaweed extracts at various concentrations

Lakshmi and Sheeja (2021) reported a similar increase in pigment synthesis in *Chlorella vulgaris* cultured in BBM medium supplemented with seaweed extracts from *G. corticata* and *Grateuloupia lithophila*. *Chlorella variabilis* exhibited enhanced pigment synthesis when cultured in Zarrouk's medium with *K. alvarezii* liquid extract (Sati *et al*., 2021). The increase in pigment synthesis in microalgae treated with seaweed extract is likely due to the presence of phytohormones, particularly cytokinins, which enhance nutrient uptake and stimulate pigment production (Zhang *et al*., 2008).

CONCLUSIONS

This study demonstrates that seaweed extracts, particularly from *Ulva lactuca*, serve as effective nutrient sources for enhancing the growth and biochemical composition of *N. oculata*. Given the ease of cultivation of *U. lactuca*, it presents a sustainable alternative to relying on wild seaweed resources. The cost-effectiveness and simplicity of seaweed extract preparation make it a viable option for large-scale industrial applications, benefiting sectors such as aquaculture and agriculture. The continued exploration of seaweed-based microalgal culture media holds promise for improving economic viability and promoting environmentally sustainable practices.

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