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Estimation of Chlorophyll from *Enteromorpha intestinalis* and Evaluation of Its Role in Human Health Promotion

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Abstract

Background: The green alga *Enteromorpha intestinalis* (now classified as *Ulva intestinalis*) is widely studied for its ecological importance and potential health benefits. Chlorophyll, a key pigment in photosynthetic organisms, is known for its role in cellular detoxification, immune support, and anti-inflammatory effects. This study aims to estimate the chlorophyll content in *Ulva intestinalis* and evaluate its potential as a functional food component due to its antioxidant and therapeutic properties.

Methods: Chlorophyll concentration in *Ulva intestinalis* was quantified using spectrophotometric methods. Both aqueous and acetone extraction techniques were employed to isolate chlorophyll pigments. The study analyzed chlorophyll content across different wavelengths to assess the efficiency and reliability of various estimation methods. The results were correlated with documented health benefits, focusing on detoxification, immune enhancement, and anti-inflammatory effects. Statistical comparisons were made between the extraction methods and wavelengths to determine optimal conditions.

Results: Preliminary findings indicate that *Ulva intestinalis* contains substantial levels of chlorophyll, particularly Chlorophyll-a and Chlorophyll-b. The spectrophotometric analysis demonstrated that certain wavelengths were more effective in detecting higher chlorophyll concentrations. These findings support the alga's antioxidant potential and highlight its functional health properties.

Conclusion: *Ulva intestinalis* shows promise as a functional food and dietary supplement due to its rich chlorophyll content and associated health benefits. The study underscores the importance of selecting effective wavelengths for accurate chlorophyll estimation and emphasizes the alga's potential in promoting human health and environmental sustainability. Further research is recommended to explore its application in sustainable nutrition and pharmaceuticals.

Keywords: *Enteromorpha intestinalis*, Chlorophyll estimation, spectrophotometric determination, bioactive compounds, functional food.

Introduction

Algae have been recognized as a critical component in both ecological systems (Nunes C. P., & Oliveira R. P., 2022) and human health due to their diverse range of bioactive compounds (Carvalho & Silva, 2023). Among various types of algae, green algae—particularly specie such as *Enteromorpha intestinalis* (now classified as *Ulva intestinalis*)—have attracted significant attention due to their high nutritional value (Thao D. T. *et.al.,* 2020), potential medicinal properties, and environmental benefits (Lee & Kim, 2019).

Enteromorpha intestinalis, a marine algae from the Ulvaceae family, is found in coastal regions worldwide and is especially noted for its chlorophyll content, which plays a central role in photosynthesis (Zhang & Li, 2021). This alga provides a wide array of bioactive compounds that have been suggested to contribute to human health (Bartley, D., & Váradi, M., 2021) making it a valuable subject of study for its potential applications in functional foods and supplements (Khan and Ahmad, 2020).

Chlorophyll, the green pigment responsible for absorbing light in photosynthetic organisms, is not only crucial for plant metabolism but also has significant implications for human health. Its antioxidant potential (Markham J. W. & Williams R. M., 2020), (Kumar R. & Sharma R., 2020) and detoxifying properties are well-documented, offering a range of potential benefits, such as promoting cellular repair, reducing inflammation (Kim H. S. & Park S. H., 2021) and improving digestive health (Khan and Ahmad, 2020). The ability of chlorophyll to enhance overall health has been explored in numerous studies, particularly its role in detoxification and supporting the immune system (Lee & Kim, 2019) and chronic disease (Saleh F. M. & Herath G., 2021) such as cardiovascular problems (Lee et al., 2018). As chlorophyll is present in varying quantities across different algae species, measuring its content in *Enteromorpha intestinalis* can offer insights into its potential benefits for human consumption.

The study of chlorophyll content in *Enteromorpha intestinalis* serves as a key step in understanding how this alga can contribute to human health. By quantifying chlorophyll concentrations using spectrophotometric methods, researchers can determine the alga's efficiency in delivering health-promoting benefits. Previous studies have shown that algae, including *Enteromorpha intestinalis*, are rich in bioactive compounds (Wu, H. X., and Zhang J.L., 2021) such as vitamins, minerals, and antioxidants, which may be useful in alleviating chronic diseases and promoting overall well-being (Zhang & Li, 2021) along with its efficacy in an anti-microbial (Cho, Y. S., & Lee, H. M., 2019) properties. Chlorophyll is a crucial element in the metabolism of photosynthetic organisms, including algae, plants, and some bacteria. It absorbs light primarily in the red and blue regions of the electromagnetic spectrum, which it then converts into chemical energy to fuel the process of photosynthesis (Zhang & Li, 2021). However, chlorophyll does not only play a role in plant and algae metabolism. It also has significant implications for human health, particularly due to its functional properties (Silva D. & Ribeiro M., 2020) such as scavenging of free radicals and cleansing properties. Chlorophyll has been suggested to offer a range of clinical benefits (Velmurugan A., & Lee, S. J., 2022) including promoting of cellular repair, reducing inflammation, and improving digestive health (Khan and Ahmad, 2020). This research aims to estimate the chlorophyll content of *Enteromorpha intestinalis* and explore its potential contributions to human health, focusing on the antioxidant and anti-inflammatory properties of chlorophyll, among other mechanisms. These are widely categorized under functional food (Fernandes, A. J., & Almeida, D. R., 2021) systems and bioactive compounds (Dufossé, L., & Santos, A., 2020).

The primary objective of the study is to investigate the chlorophyll content in *Enteromorpha intestinalis* and analyze its potential effects on human health. Specifically, the hypothesis driving this research is that higher chlorophyll content in *Enteromorpha intestinalis* correlates with enhanced antioxidant and anti-inflammatory properties, which can be beneficial for human health (Rao, P. V., & Reddy, P. V., 2022) as a valuable functional food (Zhang et al., 2020). This research experiment also compare various chlorophyll extraction methods, such as acetone and aqueous extraction, followed by spectrophotometric estimation, to determine the most reliable technique of quantifying chlorophyll in marine algae (Carvalho & Silva, 2023).

The research contains thorough study of wide-ranging review of the literature on the role of algae in human health, particularly *Enteromorpha intestinalis*, and the significant benefits of chlorophyll. It also describes the method used to extract and quantify chlorophyll in the alga, followed by an analysis of the results and their relevance to human health. However, experimental research design concludes with the discussions on implications of the findings and potential uses of *Enteromorpha intestinalis* in nutritional science, functional foods and recommendations for future research.
Through the study of chlorophyll content in *Enteromorpha intestinalis*, this research experiment contribute to a growing body of potential algae as a sustainable and health-promoting food source. The findings of this study leads to the development of understanding the specific wavelengths optimal for the growth of *Enteromorpha intestinalis* species and also as a novel dietary supplements effective in improving human health and promoting sustainable consumption practices.

Material and Methods

The fresh alga was collected through the streams of Taluka: Alibaug located at Saral, District of Raigad. After collection of algal specimens; the sample was experimented for morphological identification of species followed by extract preparation in two solvents such as Aqueous and Acetone. These extracts were further experimented to observe the amount of chlorophyll-a & Chlorophyll-b content at various wavelength of 400nm, 420nm, 470nm, 500nm, 530nm, 620nm, 660nm and 700nm was analyzed followed by its efficacy to understand the importance of chlorophyll in environment and human health. The comparative study of each chlorophyll- a; in different solvent system was used to analyze the effectivity rate of estimation of chlorophylls in the *Enteromorpha* alga.

Results

The absorbance value at different wavelengths of aqueous extract and acetone extract for Chlorophyll-a and Chlorophyll-b is mentioned in Table 1. While, the individual solvent system amount of Chlorophyll-a and Chlorophyll-b content in mg/gram is presented in Table 2 and Table 3 Followed by the graphical presentation in Figure 1 and Figure 2. However, the comparative graphical presentation of Chlorophyll-a in two different solvent (between the group) is presented in Figure 3 with the values mentioned in Table 4 followed by Figure 4 with its values mentioned in Table 5 for Chlorophyll-b (between the group) of plant sample.

Sr. No.	Plant extract	Absorbance (OD) nm							
		400	**420**	**470**	**500**	**530**	**620**	**660**	**700**
1	Aqueous (Aq.)	0.09	0.10	0.12	0.14	0.12	0.16	0.11	0.07
2	Acetone (At.)	0.36	0.62	0.47	0.32	0.28	0.24	0.25	0.14

Table 1

	Absorbance (nm)	**400**	**420**	**470**	**500**	**530**	**620**	**660**	**700**
Aq.	Chlorophyll-A (mg/gm)	6.46	6.2	5.66	5.21	5.66	4.58	5.93	7.00

	Chlorophyll –B (mg/gm)	17.33	19.62	24.20	28.78	24.20	33.36	21.91	0.0127

Table 2

	Absorbance (nm)	400	420	470	500	530	620	660	700
At.	Chlorophyll-A (mg/gm)	8.09	1.10	5.13	9.17	10.24	11.32	11.10	14.11
	Chlorophyll –B (mg/gm)	75.88	135.42	101.07	66.72	57.56	48.40	50.69	0.0255

Table 3

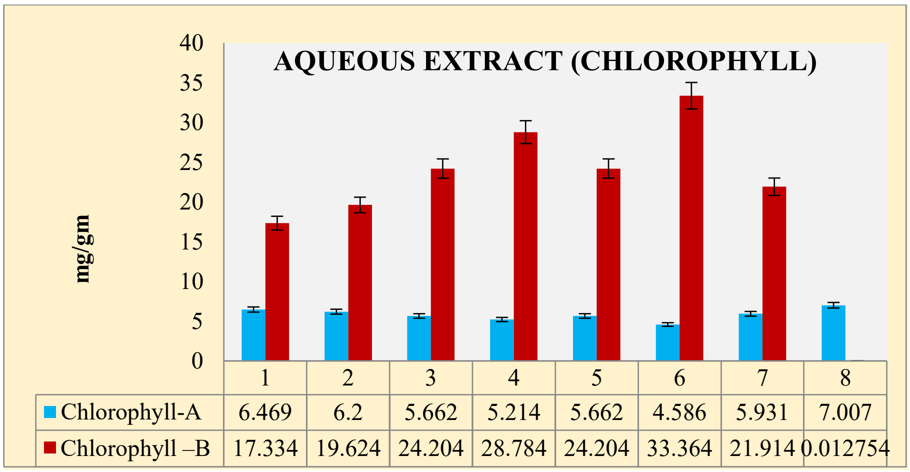


Figure 1

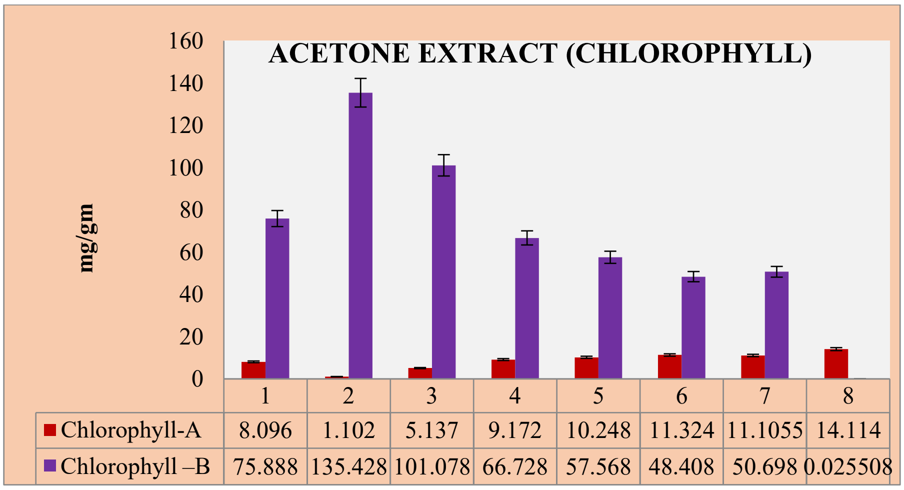


Figure 2

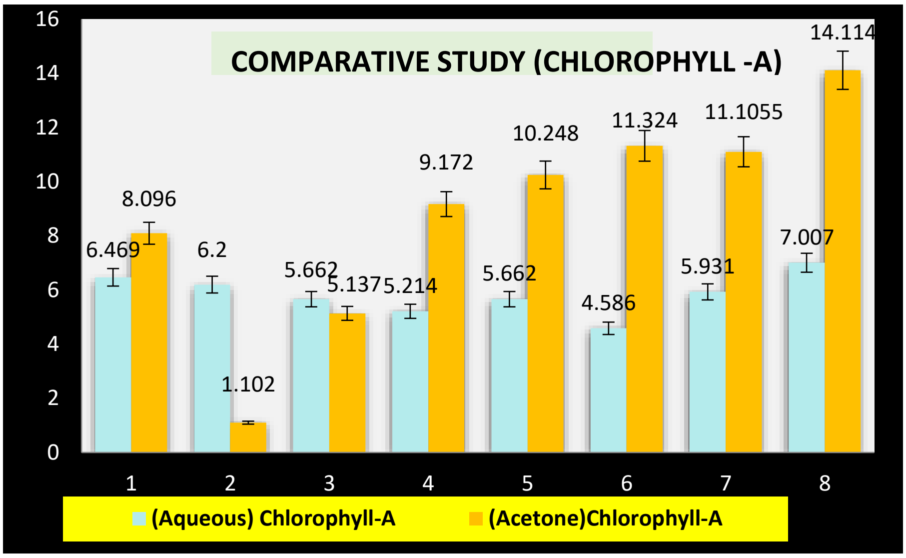


Figure 3

orbance (nm)		400	420	470	500	530	620	660	700
Aqueous extract	Chlorophyll-A	**6.469**	6.2	5.662	5.214	5.662	4.586	5.931	**7.007**
Acetone extract	Chlorophyll-A	8.096	1.102	5.137	9.172	10.25	**11.32**	**11.106**	**14.11**

Table 4

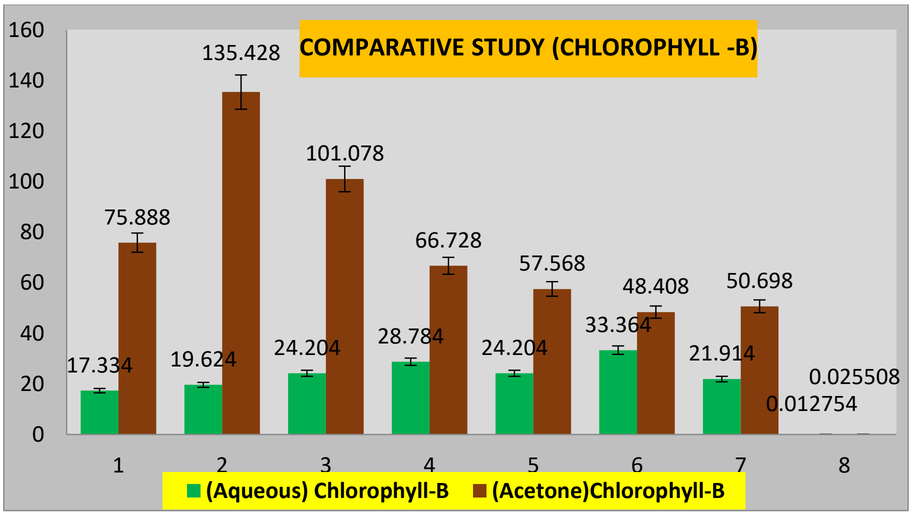


Figure 4

bsorbance (nm)		00	20	70	00	30	20	60	00
Aqueous extract	Chlorophyll-B	17.334	19.624	24.204	28.784	24.204	33.364	21.914	0.0128
Acetone extract	Chlorophyll-B	75.888	135.43	101.08	66.728	57.568	48.408	50.698	0.0255

Table 5

Discussion

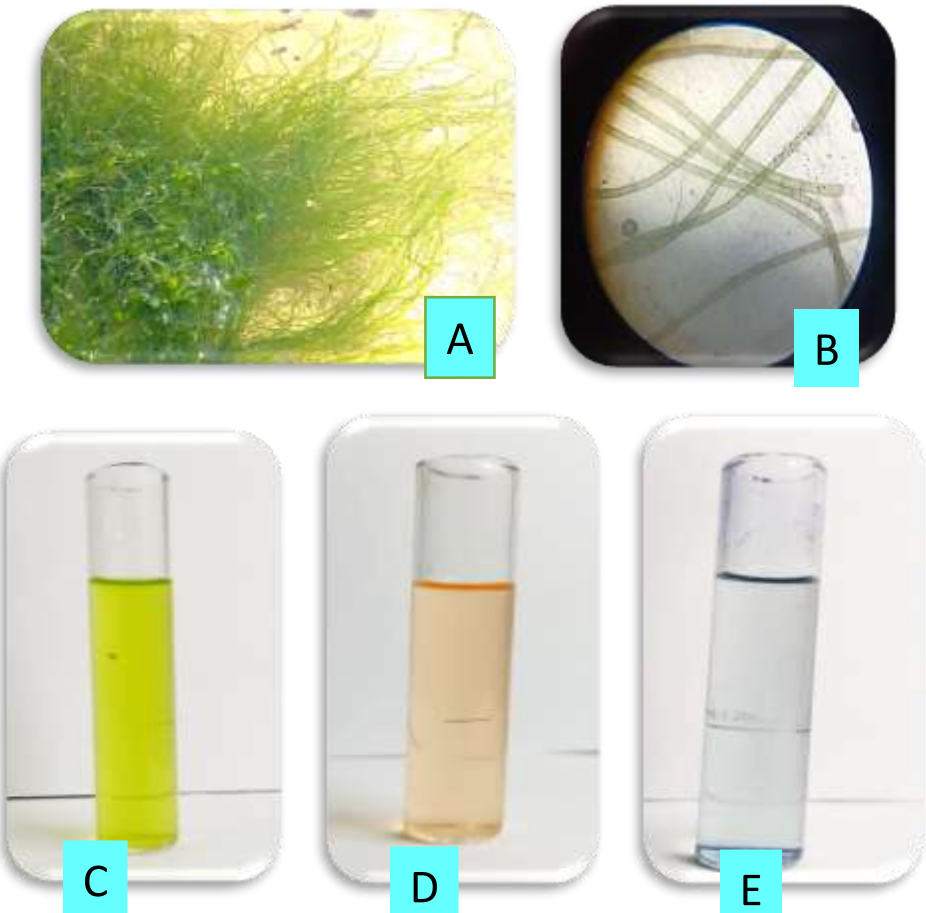
Aqueous extract:

Chlorophyll-A was observed to be highest at an absorbance of 700nm > 2^{nd} highest at 400nm > 3^{rd} highest at 420nm (7.00 > 6.46> 6.2 {mg/gm}). While, at 660 nm> 530nm=470nm > 500nm > 620nm is the sequence of the amount of chlorophyll-A (such as 5.93 > 5.66=5.66> 5.21> 4.58 {mg/gm}) was observed. ***Chlorophyll –B*** was observed to be highest at an absorbance of 620nm> 2^{nd} highest at 500nm> 3rd highest at 530nm/470nm (33.34 > 28.78> 24.20 {mg/gm}). While, at 660nm > 420nm > 400nm is the sequence of the amount of chlorophyll-B (such as 21.91 >19.62 > 400 {mg/gm}) was observed.

Acetone extract:

Chlorophyll-A was observed to be highest at an absorbance of 700nm > 2^{nd} highest at 620nm > 3^{rd} highest at 660nm (14.11 > 11.32 > 11.10 {mg/gm}).While, at 530 nm> 500nm > 400nm > 470nm > 420nm is the sequence of the amount of chlorophyll-A (such as 10.25 > 9.17> 8.09> 5.13> 1.10 {mg/gm}) was observed. ***Chlorophyll –B*** was observed to be highest at an absorbance of 420nm> 2^{nd} highest at 470nm> 3^{rd} highest at 400nm (135.43 > 101.08> 75.88 {mg/gm }). While, at 500nm > 530nm > 660nm> 620nm > 700nm is the sequence of the amount of chlorophyll-B (such as 66.72 >56.56 > 50.69> 48.40> 0.025 {mg/gm}) was observed.

Photoplate



A: Sample collection of algal species, **B:** Microscopic identification of algal sample **C, D, E:** Extracts of *Enteromorpha intestinalis* species in different solvent system.

Conclusion

Acetone is observed to be an excellent solvent system as compared to aqueous solvent system due to its high polarity to dissolve the components of chlorophyll-A & chlorophyll-B. Kingdom plantae consist algae as one of the most primitive in classification system. However, algal species provide much of earth's oxygen; producing green pigment which acts as a photoreceptor (absorb energy from light) and supports the biochemical process. *Enteromorpha intestinalis* algae serves as a food base for almost all aquatic life since easily grown in aquatic substrates. *Enteromorpha intestinalis* species is processed and widely used to obtain green lavers which are lightly roasted, crushed and used as topping or coating on foods or in soups. Chlorophylls are useful for health: chlorophylls boost red blood cells, helps in weight loss, heal damaged skin, neutralize toxins, anti-inflammatory and prevents cancer. However, chlorophylls acts as an excellent anti-oxidant and is high in vitamin C, A and E which refers in reducing signs of ageing. Studies have shown the use of topical creams infused with chlorophylls provided the results of anti-inflammatory and anti-bacterial properties. Hence, an opportunistic scope in a sector of topical cream production forms an excellent in cosmetic industry through in-depth research.

The experimental research conducted on the estimation of chlorophyll content in *Enteromorpha intestinalis* (now reclassified as *Ulva intestinalis*) has yielded significant insights into how controlled environmental conditions can optimize chlorophyll production in this green alga. The key to maximizing chlorophyll yields lies in establishing the right environmental parameters that influence photosynthesis, particularly light conditions, which play a crucial role in the synthesis of chlorophyll. The research outlined an innovative approach in which artificial culturing systems can be used to manipulate light wavelength, duration, and intensity to enhance chlorophyll content.

In these culturing systems, it is important to understand that chlorophyll synthesis is highly dependent on the specific wavelengths of light that the algae are exposed to. Algae, including *Enteromorpha intestinalis*, utilize light in the blue and red regions of the light spectrum, with chlorophyll a and chlorophyll b absorbing these wavelengths most efficiently. The optimal light conditions for chlorophyll production was observed to occur when the algae are exposed to specific light wavelengths, particularly in the range of 400-500 nm (blue light) and 620-700 nm (red light).The experimental research completed on estimation of chlorophyll content of *Enteromorpha intestinalis* successfully provides an framework for obtaining good amount of chlorophylls through artificial culturing system in which one can use a light source of specific Nano-meters of wavelength and achieve highest volume of chlorophyll.

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