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NUTRITIONAL CONTENT AND CHEMICAL ANALYSIS OF SEAWEED RESIDUES EXTRACTED THROUGH VARIOUS CARRAGEENAN PROCESSING METHODS AS POTENTIAL PLANT BIOSTIMULANT

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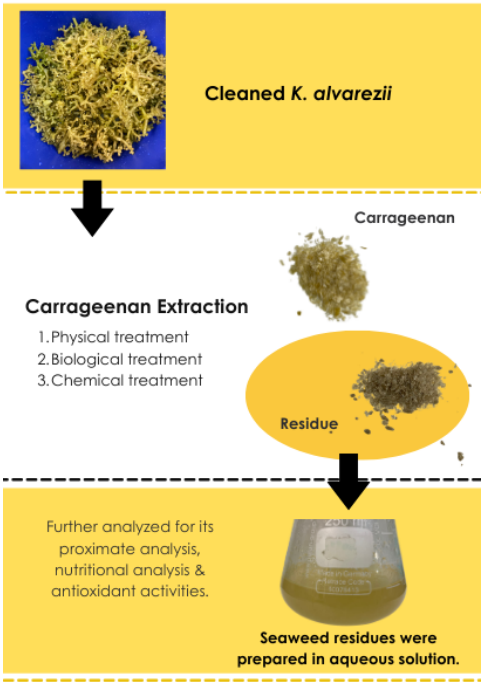
Graphical abstract



Abstract

The rising interest in plant biostimulants as eco-friendly alternatives to chemical fertilizers has led to significant attention toward *Kappaphycus alvarezii*. This seaweed species is rich in nutrients, phytohormones, and bioactive compounds, making it a promising candidate for plant biostimulant. However, the circumstances appear to be different when considering seaweed residues. In this study, the nutritional and biochemical compounds of the seaweed residues which generated through various carrageenan extraction methods such as physical, biological and chemical treatments on *Kappaphycus alvarezii* were determined. Proximate analysis conducted on all residues using the AOAC method revealed moisture content at 99% (w/w), protein levels of less than 0.1% (w/w), fat content between 2.1% and 3.1% (w/w), and ash levels ranging from 0.01% to 0..1% (w/w). No detectable levels of total carbohydrates were found in any of the residues. Furthermore, nutritional analysis via inductively coupled plasma–optical emission spectroscopy (ICP-OES) identified macronutrient concentrations in the residues, including nitrogen (<0.1% (w/w)), phosphorus (675–840 mg/kg), potassium (1325 – 1470 mg/kg), magnesium (52.5 – 85 mg/kg), calcium (310 – 625 mg/kg) and sodium (525 – 745 mg/kg), as well as trace micronutrients (iron, manganese, boron, and molybdenum) at specific concentrations. Remarkably, the DPPH assay showed that all the residues exhibited antioxidant activities, ranging from 47% until 56%. This study highlights the potential of seaweed residues as plant biostimulants, offering a sustainable addition to agricultural practices while contributing to effective zero-waste management in the seaweed industry.

Keywords: *Kappaphycus alvarezii*, carrageenan extraction, seaweed waste, nutritional content, chemical analysis

Abstrak

Minat yang semakin meningkat dalam biostimulan tumbuhan sebagai alternatif yang mesra alam kepada baja kimia telah menarik perhatian yang signifikan terhadap penggunaan *Kappaphycus alvarezii*. Spesies rumpai laut ini berpotensi sebagai biostimulan tumbuhan yang efektif kerana ianya kaya dengan nutrien, fitohormon, dan sebatian bioaktif. Namun begitu, melihat kepada sisa rumpai laut pula, ianya masih kurang dalam penyelidikan dan potensinya sebagai biostimulan tumbuhan belum dikaji dengan lebih mendalam. Oleh itu, dalam kajian ini, kandungan nutrisi serta sebatian bioaktif sisa rumpai laut yang diperoleh daripada pelbagai kaedah pengekstrakan karagenan seperti rawatan fizikal, biologi dan kimia yang dilakukan pada *K. alvarezii* telah ditentukan. Melalui analisis proksimat yang dilakukan menggunakan kaedah AOAC, terdapat kandungan lembapan pada 99% (w/w), tahap protein kurang daripada 0.1% (w/w), kandungan lemak antara 2.1% hingga 3.1% (w/w), dan kandungan abu antara 0.01% hingga 0.1% (w/w) pada semua jenis sisa. Bagi kandungan karbohidrat, ianya tidak dikesan pada mana-mana sisa rumpai laut. Tambahan pula, analisis pemakanan melalui spektroskopi pelepasan optik plasma (ICP-OES) yang digabungkan secara induktif mendedahkan kehadiran makronutrien termasuk nitrogen (<0.1% (w/w)), fosforus (675–840 mg/kg), kalium (1325–1470 mg/kg), magnesium (52.5–85 mg/kg), kalsium (310–625 mg/kg), dan natrium (525–745 mg/kg), serta mikronutrien (besi, mangan, boron, dan molibdenum) pada kepekatan tertentu. Selain itu, ujian DPPH menunjukkan bahawa semua sisa menunjukkan aktiviti antioksidan yang kuat, iaitu di antara 47% hingga 56%. Kajian ini menonjolkan potensi sisa rumpai laut sebagai biostimulan tumbuhan serta menyumbang kepada pembangunan pertanian lestari dan pengurusan sisa sifar yang berkesan dalam industri rumpai laut pada masa yang sama.

Kata kunci: *Kappaphycus alvarezii*, pengestrakan karagenan, sisa rumpai laut, kandungan nutrisi, analisis kimia

1.0 INTRODUCTION

Over the past decade, global seaweed production has nearly tripled, as reported by the Food and Agriculture Organization (FAO) [1], contributing to food security, carbon capture, and global sustainability efforts. Seaweed, a fast-growing plant that requires minimal fertilizers, is rich in nutrients and bioactive compounds. Based on their photosynthetic pigments, seaweeds are classified into red (*Rhodophyceae*), brown (*Phaeophyceae*), and green (*Chlorophyta*) groups [2]. Their biochemical compounds make them valuable for use in food, agriculture, and renewable energy industries [3].

However, seaweed residue from hydrocolloid extraction and the disposal of blooms from marine eutrophication, such as the massive Sargassum bloom in the central Atlantic, raise environmental concerns [4]. Yun *et al*. (2022) reported that refined carrageenan production generates over 75% residual biomass, containing up to 44.2% (w/w) total carbohydrates [5, 6]. Therefore, effective waste management is essential in the seaweed industry to address these issues.

Seaweed extracts have shown significant potential in agriculture, particularly as biostimulants. Biostimulants are defined as substances which are functioning to increase the efficiency of nutrition, tolerance to abiotic stress or qualitative characteristics of crops regardless of their nutrient content [7]. Numerous studies highlight the positive effects of seaweed extracts on plant growth, including better germination, root development, leaf area, tiller count, plant weight, and fruit quality [8]. This is attributed to the rich content of major and minor nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid-like growth-promoting compounds found in seaweed extracts [9, 10]. Integrating biostimulants into agriculture is known to be a cost-effective, eco-friendly approach that reduces dependence on chemical fertilizers, which can harm human health and the environment [11, 12].

Basically, plants require three essential macronutrients—nitrogen, phosphorus, and potassium—for critical physiological functions. Nitrogen is key to chlorophyll synthesis, photosynthesis, and vegetative growth [13, 14]. Phosphorus supports cell division, root development, and flower initiation, particularly in young cells [15, 16]. Meanwhile, potassium regulates metabolic processes like turgor-driven movements, osmoregulation, protein synthesis, and enzyme activation [15].

Seaweed residues also hold promise as agricultural plant biostimulants, as indicated by several studies [13,

14, 17]. This positive impact can be attributed to the presence of remaining nutrients within seaweed residual. Yudiati *et al*., (2021) reported the presence of essential macro-and micronutrients like nitrogen, phosphate, magnesium, and iron in alginate waste fertilizer. Additionally, composts composed of seaweed wracks and *Undaria pinnatifida*, collected from the coast of Puerto Madryn, also exhibited distinct carbon and nitrogen ratios [18]. However, in contrast to research on raw seaweed extracts, there is still little information on the properties and application of seaweed residues as a plant biostimulant. The majority of research studies have utilized the fermentation process to develop a plant biostimulant from seaweed wastes, necessitating extended time and additional resources. Limited investigations have been undertaken to explore the potential of *K. alvarezii* residues as a plant biostimulant, hence the purpose of this study.

Seaweeds are commonly extracted for carrageenan production using chemical, physical, and biological methods. Chemical extraction, typically using alkaline reagents like potassium hydroxide, where the presence hydroxide (OH^-) in the alkaline reagent led to removal of the sulphate group in carrageenan through desulphation at the 6-position of galactose unit and then forming recurring 3,6 anhydrous-D-galactose. The anion (K^+) in the alkaline reagent acts to stabilize the charges of the removed sulphate [19]. The addition of potassium chloride was performed for the next step to precipitate the extracted carrageenan. Physical extraction, such as microwave-assisted extraction (MAE), employed microwave energy to disrupt algal cells for easy release of carrageenan into the solvent, making it more environmentally friendly due to reduced solvent use and energy consumption [20]. In addition, the natural moisture content of algae is high which makes it susceptible to microwave radiation [21]. The use of distilled water as a solvent also enhanced the efficacy of MAE due to its ability to absorb electromagnetic energy consequently generating rapid internal heat in the seaweed and increasing the cell pressure, leading to cell wall disruption [22,23]. As for biological extraction, it employs cellulase enzymes to break down seaweed cell walls, but yields can be lower due to factors like temperature and time taken for extraction process [24,25].

In this study, *K. alvarezii* was subjected to biological, physical, and chemical extraction methods. The yield of the resulting carrageenan and seaweed residues across all extraction methods were determined. The nutritional content and biochemical compounds in all residues were determined to assess their potential as plant biostimulants. The findings of this research offer valuable insights into the production of high-value products from seaweed residues that can contribute to sustainable agriculture and environmentally friendly waste management practices.

2.0 METHODOLOGY

2.1 Materials

Kappaphycus alvarezii was obtained from Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia. The potassium hydroxide and 2-propanol were products from EMSURE. The potassium chloride, cellulase enzyme and ethanol were purchased from Merck, SIGMA and Systerm respectively. Throughout this study, Millipore deionized water was utilized.

2.2 Carrageenan Extraction

Selection of fresh *Kappaphycus alvarezii* was performed prior to washing to remove the impurities. After fresh seaweed was selected, it was cleaned using tap water and distilled water subsequently. Then, it was further air-dried using the oven at 40°C for about 4 days. The dried seaweed was washed again by soaking in distilled water for 45 minutes to remove excess salts and air-dried again in the oven for 24 hours before proceeding with carrageenan extraction [26].

2.2.1 Chemical Extraction

For carrageenan extraction through chemical treatment method, it was carried out as described by Solorzano-Chavez *et al.,* (2019) and Liu *et al*., (2022) with some modifications [27,28]. Approximately 35 g of dried seaweed were pretreated with 6% of potassium hydroxide for 24 hours at 25°C. Afterwards, the dried seaweed was sieved to separate it from the alkaline solution and dried in the oven overnight. Next, in order to remove excess alkaline solution on the dried seaweed, it was washed twice in 1 L of distilled water for 10 minutes and further dried again at 60°C.

Five grams of the dried seaweed were milled using a crusher and then added to an Erlenmeyer flask which was filled with 400mL of distilled water. Shaking of the flask was done for 2 hours at 65°C and 120 rpm in a shaker incubator. The next step was a filtration stage where a strainer was used to separate the residue and filtrate. The filtrate was further precipitated using 5% of potassium chloride with one time the volume of the filtrate. After that, obtained carrageenan which was in gel form was separated from the solvent using filter cloth. Both carrageenan and residue were dehydrated in the oven. Residue was washed with distilled water beforehand to remove excess potassium hydroxide (KOH) on it.

2.2.2 Physical Extraction

Microwave assisted extraction was used for physical carrageenan extraction methods [29]. For the recovery of carrageenan, the milled dried seaweed was processed by a commercial microwave (SHARP Digital Microwave Oven, R607EK). Firstly, dried milled

algae and distilled water were introduced at a solid liquid ratio of 1:30. (w:v) in the beaker. Then, the seaweed was heated for 6 minutes with power of 50P in the microwave. As the sample was chilled to 55°C, the liquid fraction which consists of the carrageenan was separated from the solid fraction (residue) using a strainer. Next, the liquid fraction was taken for precipitation using ethanol with a ratio of 1:1 (v:v). Subsequently, the mixture was separated using a strainer to obtain the carrageenan.

2.2.3 *Biological Extraction*

Biological extraction method was performed as described by Tarman *et al.,* (2020) and Varadarajan *et al*., (2009) with a few modifications [24,25]. The dried seaweed (20 g) was heated first at 60°C for 20 minutes to expand the algal cells and break the cell wall for easy carrageenan extraction. Next, 0.1g of cellulase was added into the mixture and boiled in a water bath with a shaker at 60°C for 1 hour. Centrifugation of the suspension was made at 4°C and 5000 rpm for 15 minutes which results in separation of filtrate and residue. 2-propanol was poured into the filtrate at ratio of 1:1 for precipitation of carrageenan and then centrifuged at 12000 rpm and 4°C for 30 minutes into order to separate the carrageenan and solvent.

Both carrageenan and residue obtained from all of these extraction methods were further dried in the oven. The extraction yield for carrageenan and residue was calculated using Equation 1.

$$R\ (\%) = \left(\frac{weight\ of\ driedcarrageenan/residue(g)}{weight\ of\ dried\ seaweed\ powder\ (g)}\right) \times 100\% \quad (1)$$

2.3 Sample Preparation

Prior proximate analysis and nutrient content analysis, the seaweed residues were prepared in aqueous solution as described by Fayzi *et al*., (2020) with some modifications [30]. 1 g of the dried seaweed waste were dissolved in 100 ml of distilled water. The mixture was incubated for 3 days in the shaker incubator at room temperature. The seaweed waste extract was further filtered using Whatman paper and the filtrate stored in the centrifuge tube at 4°C until further analysis.

2.4 Determination of Physicochemical and Proximate Analysis of Seaweed Waste

Physicochemical analysis included parameters such as pH and colour of the seaweed waste extract. The measurements of pH of the extracts were conducted by using a pH meter (Cyberscan pH Meter 6000). Meanwhile, the colours of the extracts were determined directly through eye observation. For proximate analysis, the samples were sent to the Institute of Bioproduct Development, Universiti Teknologi Malaysia, an ISO/IEC 17025 accredited laboratory. The proximate composition of the extracts including moisture, ash, protein, fat, total carbohydrate and energy value were determined using the Association of Official Analytical Chemists (AOAC, 2000) procedure [31].

2.5 Determination of Nutrient Content of Seaweed Waste

Similar to proximate analysis, the samples were sent to the Institute of Bioproduct Development, Universiti Teknologi Malaysia (ISO/IEC 17025 accredited laboratory) for determination of the nutritional composition. Macronutrients namely phosphorus, potassium, magnesium, sodium and calcium and micronutrients such as iron, manganese, boron and molybdenum were detected and quantified through inductively coupled plasma–optical emission spectroscopy (ICP-OES)[32]. Total nitrogen (macronutrient) was analyzed by referring to Malaysian Standard MS 1120 (1998).

2.6 Determination of Antioxidant Properties of Seaweed Waste

Prior to assessing the antioxidant properties of seaweed waste, a preliminary step involved extracting the waste using methanol as a solvent. In this process, the sample extracts were combined with the solvent at a ratio of 1:10 (w/v). This step was followed by continuous shaking of the samples for 24 hours using an orbital shaker at room temperature. Next, the samples were filtered using Whatman No. 1 filter paper and further concentrated using a hot air oven.

Antioxidant activity was evaluated using the DPPH free radical scavenging assay, following a protocol based on Leelavathi & Prasad (2014) method with minor modifications [33]. Initially, 1 mL of a 0.1 mM DPPH solution was mixed with 1 mL of residue extracts at varying concentrations: 2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL. This mixture was thoroughly vortexed and left in darkness at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm using spectrophotometer, with methanol serving as a blank. Below is the formula (Equation 2) used to calculate the percentage of DPPH free radicals scavenging activity:

$$I = \left(\frac{A0-A1}{A0}\right) \times 100\% \quad (2)$$

A0: control absorbance (0.1 mM of DPPH solution + methanol)
A1: absorbance of residue extract

All reactions were conducted in duplicate, and the degree of purple colour production and decolourization shows the algal extracts' free radical scavenging activity. The antioxidant activity of the extracts was then assessed in comparison to that of ascorbic acid, employed as a standard antioxidant reference.

2.7 Data Analysis

Quantitative data including yield of carrageenan and residue obtained from different extraction methods as well as the antioxidant activities were statistically analyzed using SPSS version 16 in one-way ANOVA LSD.

3.0 RESULTS AND DISCUSSION

3.1 Carrageenan Extraction

After drying of the carrageenan and residue in the oven, their yield was calculated according to Equation 1. Based on Figure 1, the chemical extraction method produced the highest carrageenan yield (57.64%), followed by the physical extraction method (52.28%) and biological extraction method (12.26%). However, no significant difference was observed in carrageenan yield between the chemical and physical extraction methods. In contrast, the biological extraction method yielded the highest amount of residue at 68.92%, which was significantly different from the residue yields of both the chemical extraction method (27.79%) and the physical extraction method (18.22%).

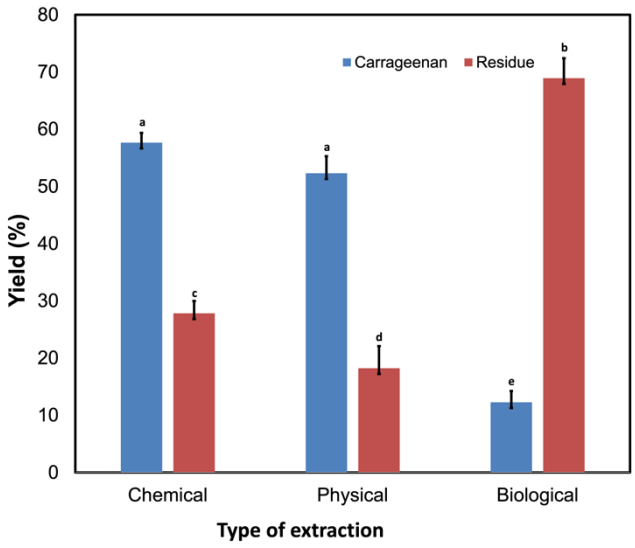


Figure 1 Yield of the carrageenan and residue which obtained through chemical, physical and biological extraction methods. Columns marked with different letters indicate significant differences at $P \leq 0.05$. The values shown represent the mean (n=3), with bars indicating the standard error.

By referring to other researchers' findings on the alkaline extraction process, the yield of obtained carrageenan ranged from about 23% until 36% only after extraction for a few hours (2 hours until 8 hours) [19,28,34,35]. However, in this study, the carrageenan yield was doubled due to 24 hours of pre-treatment using KOH. This outcome was slightly similar with a study conducted by Solorzano-Chavez *et al.*, (2019), where approximately 60% of carrageenan yield was obtained [27]. This showed that longer extraction period causes higher carrageenan yield. In addition, a study conducted by Ilias *et al.*, (2017) showed that the longest duration for carrageenan extraction (5 hours) produced the greatest yield (44.5%) [19]. The comparison between the carrageenan yields from the physical and alkaline extraction methods is notable, demonstrating slight similarity. However, in contrast to the findings by Ponthier *et al.*, (2020), the carrageenan yield in this study is slightly lower [29]. This discrepancy may be attributed to variations in extraction temperature. Ponthier *et al.*, (2020) maintained the extraction temperature at 150 °C for carrageenan recovery [29], whereas in this study, extraction was carried out at a lower temperature (< 100 °C).

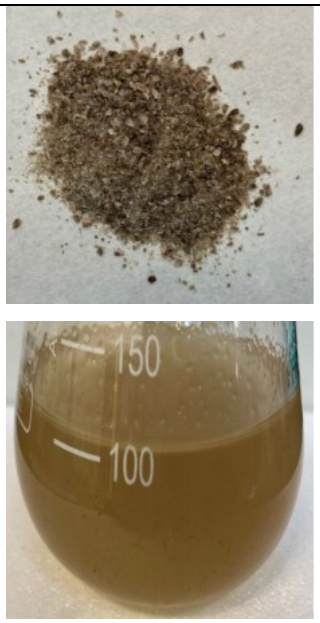
In contrast, the biological extraction method resulted in a lower carrageenan yield significantly, leaving a high amount of seaweed residue compared to other methods. The study by Tarman *et al.*, (2020) reported a higher carrageenan yield (71.28%), likely attributed to the consecutive pre-treatments before enzymatic treatment, involving heating at 60°C for 20 minutes and subsequent delignification through autoclaving at 121°C for 2 hours [24]. However, the enzyme-assisted extraction method is deemed economically unfeasible on an industrial scale due to the high cost of enzymes [36]. The subsequent sections further explore the proximate analysis and nutritional content of the seaweed residues, which are the by-products of the extraction processes. This evaluation aims to assess their potential as plant biostimulants for agricultural applications.

3.2 Determination of Physicochemical and Proximate Analysis of Seaweed Waste

3.2.1 Physicochemical Analysis of Seaweed Waste

As shown in Table 1, all residues exhibited a neutral or nearly neutral pH. In the chemical extraction method, an alkaline solution, potassium hydroxide (KOH), was used for carrageenan extraction. However, the final pH remained neutral because of thorough washing of the residue after the extraction process. On the other hand, the physical and biological methods utilized both neutral pH of solvents for the carrageenan extraction and precipitation. Notably, residues from these methods did not undergo alkaline treatment and thus did not require additional washing, unlike those from the chemical extraction method.

Table 1 Physicochemical analysis of the seaweed residue generated from the chemical, physical and biological extraction methods

	Chemical extraction	Physical extraction	Biological extraction
pH	7.00	7.22	6.83
Colour			

In terms of colour, the residue from chemical extraction exhibited a significantly lighter shade compared to other residues and raw *K. alvarezzii*. This disparity is attributed to the alkaline treatment during chemical extraction, which effectively removes colouring matter and certain proteins. Studies by Pozo *et al.*, (2020) and Xiao *et al.*, (2021) emphasize that alkaline treatment and cell wall breakdown can lead to the removal or degradation of pigments like chlorophyll and carotenoids [37,38]. Furthermore, thermal treatment, a common step in carrageenan extraction, indirectly contributes to colour alterations due to the susceptibility of chlorophyll to heat [39].

3.2.2 *Proximate Analysis of Seaweed Waste*

Table 2 shows the result of proximate analysis generated from the chemical, physical and biological extraction methods which were performed using the AOAC method, in accordance with the study conducted by Xiren G. K. & Aminah A (2017) [31]. All samples demonstrated moisture content about 99%. Moisture content represents the water amount present in the sample [40]. In contrast, ash and protein for all residues were 0.1% or below. Solorzano-Chavez *et al.*, (2019) reported protein content in carrageenan residue extracted from different *K. alvarezii* strains ranging from 1% to 2% (g/100 g on a dry basis) [27]. This might be due to protein dissolution in polar solvents during carrageenan extraction [41]. The chemical extraction residue had the highest fat content (3.1%), followed by the biological extraction residue (2.8%) and the physical extraction residue (2.1%). Mandalka *et al.*, (2022) noted that seaweed is not a common source of lipids due to its low lipid content, which typically ranges up to 4% in dry matter [42]. There was no total carbohydrate detected in this study. Some studies however reported a considerable amount of galactan and glucan remaining in the residue after carrageenan extraction through alkaline treatment [28,42,45]. Despite the negligible carbohydrate content, this may not significantly impact the residue's potential as a plant biostimulant since carbohydrate content is not a primary factor in plant biostimulant specifications.

3.3 Primary Macronutrients of Seaweed Waste

Raw seaweed is rich in organic and inorganic components which are beneficial for the plant's quantity and quality by enhancing plant growth, protection and immune stimulation [44]. After the extraction processes, the remaining nutritional content of the seaweed residue are presented in Table 3. The seaweed residues analyzed in this study revealed the presence of phosphorus, and potassium as the most abundant nutrients. The trend in macronutrient content across the extraction methods shows that potassium content was highest in the physical extraction method (1470 mg/kg), followed by biological (1335 mg/kg) and chemical (1325 mg/kg) methods. On the other hand, phosphorus content was highest in the chemical extraction method (840 mg/kg), followed by physical (750 mg/kg) and biological (675 mg/kg) methods.

Table 2 Proximate analysis of the seaweed residue generated from the chemical, physical and biological extraction methods

Parameters	Chemical Residue	Physical Residue	Biological Residue
Moisture (% w/w)	99	99	98.8
Ash (% w/w)	0.1	0.01	0.1
Protein (% w/w)	<0.1	<0.1	<0.1
Fat (% w/w)	3.1	2.1	2.8
Total Carbohydrate (% w/w)	0	0	0
Energy value of food (kcal/100g)	27.6	19.2	25.6

Table 3 Macronutrients content of the seaweed residue generated from the chemical, physical and biological extraction methods

Nutrient Content	Physical Residue	Biological Residue	Chemical Residue
Total Nitrogen (% w/w)	<0.1	<0.1	<0.1
Phosphorus (mg/kg)	750	675	840
Potassium (mg/kg)	1470	1335	1325

This indicates that the physical extraction method yielded the highest potassium content, while the chemical extraction method yielded the highest phosphorus content. The differences in nutrient content among the methods can be attributed to the varying extraction processes and their ability to effectively capture specific nutrients present in the seaweed residues. Nevertheless, nitrogen was not detected in all residues.

3.4 Secondary Macronutrients and Micronutrient of Seaweed Waste

As shown in Table 4, the secondary macronutrients present in significant amount across all seaweed residues are sodium, calcium, and magnesium. The finding is an agreement with Vaghela *et al.*, (2022) where sodium, calcium and magnesium are available in *Kappaphycus alvarezii* sap extract [45]. A large amount of sodium is unnecessary and can be harmful to plant growth due to the potential detrimental effects of excess Na^+ on plants. Despite that, its presence proved to be beneficial to plant growth during low-potassium-input conditions [46,47]. The highest calcium content was obtained through the physical extraction method, reaching 625 mg/kg, followed by chemical method (410 mg/kg) and biological method at 310 mg/kg. This suggests that the physical extraction method was more effective in extracting calcium from the seaweed residues compared to other methods. Calcium is a critical element for plants, forming a major component of the middle lamella in the cell wall. Additionally, calcium plays a crucial role in activating various enzymes, especially during periods of environmental stress [16, 48]. Magnesium content in the residues varied, ranging from 53 mg/kg to 85 mg/kg. Magnesium serves as the central atom in chlorophyll molecules and is a structural component of ribosomes, aiding in maintaining their configuration during protein synthesis [16, 49]. Calcium and magnesium are secondary nutrients, essential to supplement in addition to primary macronutrients (N, P, and K). Deficiency in these nutrients can hinder plant growth and development, as investigated by Veazie *et al.*, (2022) in their study on the impact of macronutrient deficiency on the growth of *Lactuca sativa* [50].

In addition to secondary macronutrients, Table 4 also indicates the presence of essential micronutrients in the seaweed residues, including iron, manganese, boron, and molybdenum, albeit in lower concentrations compared to other macronutrients. Notably, a significant amount of iron was detected only in residues derived from the physical extraction method, while boron was consistently more abundant than iron across all seaweed residues. These micronutrients play vital roles in specific biological processes crucial for plant growth and development [51, 52].

3.5 Antioxidant Properties of Seaweed Waste

In plants, synthesis of reactive oxygen species (ROS) occurred in most of the cellular compartments, such as chloroplasts, mitochondria, peroxisomes, apoplast, plasma membrane, cell wall and endoplasmic

reticulum. Nonetheless, the primary locations for ROS generation are the chloroplasts, peroxisomes, and the mitochondrial respiratory electron transport system [53]. Notably, even minor environmental changes can disrupt the redox balance in plants, leading to sudden surges in ROS levels [54].

Table 4 Micronutrients content of the seaweed residue generated from the chemical, physical and biological extraction methods

Nutrient Content	Physical Residue	Biological Residue	Chemical Residue
Magnesium (mg/kg)	81.5	53.5	85
Calcium (mg/kg)	625	310	410
Iron (mg/kg)	3.1	<0.5	<0.5
Manganese (mg/kg)	<0.5	0.6	<0.5
Boron (mg/kg)	1.2	1.0	3.0
Molybdenum (mg/kg)	<0.5	<0.5	<0.5
Sodium (mg/kg)	745	525	615

For example, during periods of drought, the photosynthetic electron transport system encounters an increased load of electrons due to intense light absorption, coupled with stomatal closure as a protective measure to prevent water loss. Consequently, when plants are exposed to prolonged adverse conditions, the cumulative buildup of ROS can have detrimental impacts on cellular metabolism [53]. While plants possess intricate internal antioxidant systems to maintain redox balance, the use of biostimulants with antioxidant properties will help to improve the defense mechanisms especially during abiotic and biotic stress.

Figure 2 illustrates the DPPH radical scavenging activity of seaweed residues, demonstrating their capability to neutralize this stable free radical. The scavenging activities of these residues ranged from 49% to 52%, with no significant differences detected among them. Notably, a significant difference in scavenging activity was observed between the biological and physical residues at a concentration of 1000 µg/mL. While the radical scavenging activities of the residues were nearly comparable to that of raw *Kappaphycus alvarezii* (56%) across all tested concentrations, a significant difference was still evident between the residues and the raw seaweed. Both the residues and raw seaweed exhibited significantly lower scavenging activities compared to the control, ascorbic acid, which consistently showed over 80% activity.

Additionally, non-linear trends were observed in the results shown in Figure 2. Statistical analysis revealed no significant differences in scavenging activity across the tested concentrations within the same sample, suggesting that the control group, raw seaweed, and residues may have reached a plateau at 125 µg/mL. However, significant differences in the biological residue at 125 µg/mL and 250 µg/mL suggest potential measurement inconsistencies. These inconsistencies could be attributed to various factors, including experimental precision, preparation of the DPPH solution, preparation of extract and final mixture solutions, and the use of micropipettes and volumetric pipettes [55].

Moreover, Vaghela *et al*., (2022) suggest that the antioxidant properties can be attributed to the presence of bioactive compounds like flavonoids, phenolics, phlorotannins, diterpenes, phytosterols, and quinones in the seaweed extracts [45]. These compounds can serve as defensive and signaling molecules, inducing nod genes in plants. Furthermore, their research demonstrates that *Kappaphycus alvarezii* extracts exhibit antioxidant activity, and phytochemical screening confirms the presence of flavonoids, phenolics, steroids, and quinones. Nevertheless, the potency of antioxidant activities is influenced by various factors, including the choice of solvents, extraction duration, seaweed species, and the extraction method itself. For instance, a study by Diyana et al. (2015) revealed that the highest DPPH scavenging activity for *K. alvarezii* was observed in the 50% acetone extract (35.63%), surpassing the 100% methanol extract (19.35%) [56]. Conversely, Leelavathi & Prasad (2014) indicate that the 1000 µg/ml petroleum ether extract of *K. alvarezii* (42%) slightly outperformed the 1000µg/ml methanol extract (32%) [33]. However, in the study by Papitha *et al*., (2020), the 1000µg/ml methanol extract *of K. alvarezii* (74.24%) exhibited superior antioxidant activity compared to the 1000µg/ml petroleum ether extract (62.34%), whereby Soxhlet apparatus was used for extract preparation [57]. Consequently, it is evident that multiple variables influence the outcomes of seaweed extract antioxidant activities.

When it comes to research on antioxidant activities, there is still a relative scarcity of studies focused on seaweed residues compared to raw *K. alvarezii*. However, Harb *et* al., (2023) have conducted research on the antioxidant activities of Brazilian beach-cast seaweeds using the ABTS assay [58]. Their findings indicate that the antioxidant activities were notably higher in extracts obtained from brown and red beach-cast seaweeds compared to those from green seaweeds.

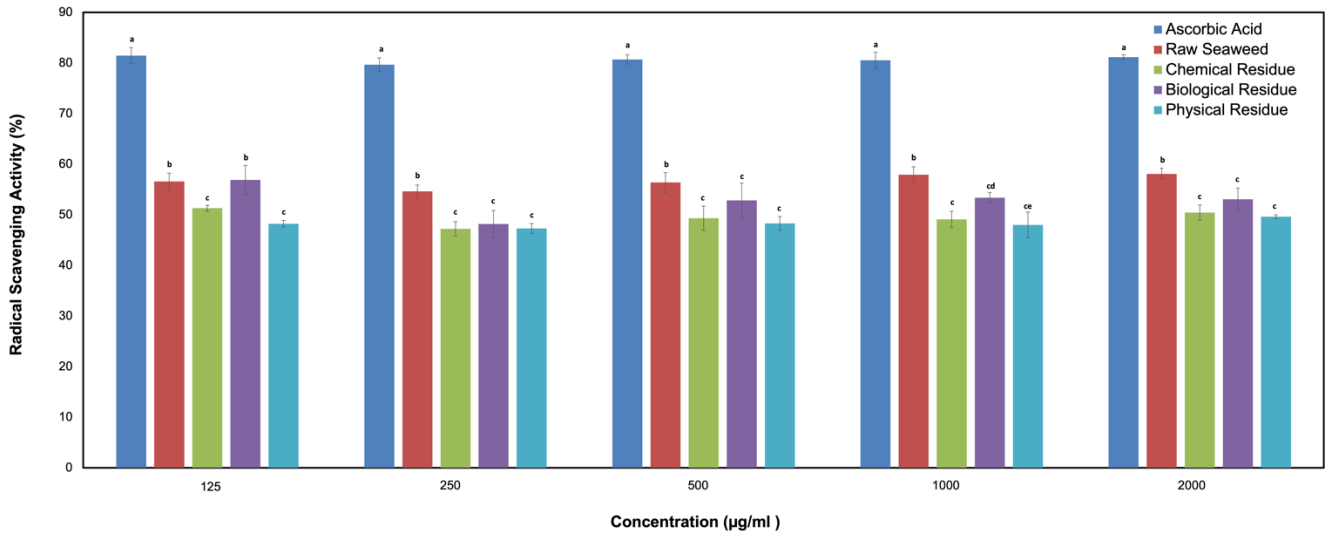


Figure 2 Scavenging activity of DPPH by the residues obtained through chemical, physical and biological extraction methods. Columns marked with different letters indicate significant differences at P ≤ 0.05 within the same concentration across different samples. The values represent the mean (n=3), with bars indicating the standard error

Furthermore, the levels of phenolic compounds were most abundant in brown seaweed species. For future investigations, it is advisable to conduct screenings of the bioactive compounds present in seaweed residues resulting from carrageenan processing and assessing the concentrations of these compounds in order to provide valuable insights and enhance the correlation with antioxidant activities.

4.0 CONCLUSION

The carrageenan extraction methods, whether physical, biological, or chemical, generated notable quantities of residues as by-products. Following the extraction, these residues retained certain levels of protein, fat, and ash. Regardless of the extraction method, potassium, phosphorus, sodium, and calcium were the major nutrients consistently found in all seaweed residues. The extraction methods showed slight variations in yield, with the physical extraction method excelling in extracting potassium, sodium, and calcium, while the chemical extraction method was superior in extracting phosphorus and magnesium. Additionally, essential micronutrients such as iron, manganese, boron, and molybdenum were also present in all seaweed residues. Notably, the antioxidant assays revealed that the seaweed residues exhibited significant antioxidant properties. These findings indicate the valuable presence of vital macro and micronutrients as well as antioxidant properties in seaweed residues, highlighting their potential as effective plant biostimulants. Future studies should prioritize evaluating their effectiveness in enhancing crop growth, nutrient uptake, and overall plant health.

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Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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