NUTRITIONAL VALUE AND BIOLOGICAL ACTIVITY OF *K. ALVAREZII* GROWN IN INTEGRATED MULTI-TROPHIC AQUACULTURE

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ABSTRACT

This study aimed to assess the nutritional value, phytochemical composition, and antioxidant activity of *Kappaphycus alvarezii* cultivated in Integrated Multi-Trophic Aquaculture (IMTA) systems. The parameters were nutritional components, phytochemical content, and aAntioxidant activity. *K. alvarezii* exhibited substantial nutritional value, with notable protein (15.3%) and carbohydrate (60.0%) content, along with essential minerals such as calcium and magnesium. Phytochemical analysis revealed significant levels of polyphenols (20.0 mg GAE/g), flavonoids (5.5 mg QE/g), and tannins (3.0 mg CE/g), as well as noteworthy saponin (1.2%) and alkaloid (0.9%) contents. Antioxidant assays demonstrated strong activities, with 80.2% DPPH and 70.5% ABTS radical scavenging, a FRAP value of 600 µmol Fe2+/g, and a total antioxidant capacity of 150 mg AA/g. The findings indicate that *K. alvarezii* from IMTA systems is a valuable source of nutrients and bioactive compounds with significant antioxidant properties.

Key words: ***C. racemosa*, chlorophyll extraction, green solvents, NADES, sustainable extraction**

القيمة الغذائية والنشاط البيولوجي لنبات K. Alvarezii المزروع في تربية الأحياء المائية المتكاملة المتعددة العناصر الغذائية

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المستخلص

هدفت هذه الدراسة إلى تقييم القيمة الغذائية والتركيب الكيميائي النباتي ونشاط مضادات الأكسدة لنبات Kappaphycus alvareziiالمزروع في أنظمة الاستزراع المائي المتكاملة متعددة العناصر الغذائية .(IMTA) وكانت المعلمات المكونات الغذائية، والمحتوى الكيميائي النباتي، ونشاط مضادات الأكسدة .أظهر K. alvarezii قيمة غذائية كبيرة، مع محتوى ملحوظ من البروتين (15.3%) والكربوهيدرات(60.0%) ، إلى جانب المعادن الأساسية مثل الكالسيوم والمغنيسيوم .كشف التحليل الكيميائي النباتي عن مستويات كبيرة من البوليفينول (20.0 ملجم / GAE جم)، والفلافونويدات (5.5 ملجم / QE جم)، والعفص (3.0 ملجم / CE جم)، بالإضافة إلى محتويات السابونين1.2) ٪ (والقلويد0.9) ٪ (الجديرة بالملاحظة . .أظهرت فحوصات مضادات الأكسدة أنشطة قوية، مع DPPH 80.2% و ABTS 70.5%الكسح الجذري، وقيمة FRAP تبلغ 600 ميكرومولFe2+/g ، وقدرة إجمالية مضادة للأكسدة تبلغ 150 مجم .AA/g تشير النتائج إلى أن K. alvarezii من أنظمة IMTAيعد مصدرًا قيمًا للعناصر الغذائية والمركبات النشطة بيولوجيًا ذات خصائص مضادة للأكسدة كبيرة.

الكلمات المفتاحية:مضادات الأكسدة، المركبات النشطة بيولوجيا،IMTA ، المواد الكيميائية النباتية، الأعشاب البحرية .

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INTRODUCTION

Seaweeds, commonly referred to as macroalgae, have been revered throughout history for their nutritional richness, potential health benefits, and plants stimulants (1). Among these marine organisms, *K. alvarezii*, a type of red seaweed, has garnered considerable attention, primarily due to its cultivation for carrageenan extraction, a widely used food additive in various industries (2, 15). However, beyond its role as a source of carrageenan, *K. alvarezii* contains a diverse array of bioactive compounds, including phytochemicals with potent antioxidant properties, which contribute to its potential health-promoting effects (3,33, 39). In recent years, Integrated Multi-Trophic Aquaculture (IMTA) systems have emerged as an environmentally sustainable approach to seaweed cultivation (4). These systems integrate the cultivation of multiple species, such as fish, shellfish, and seaweeds, to create synergies that enhance overall ecosystem health while minimizing environmental impacts (4,5). This study seeks to explore the nutritional value, phytochemical composition, and antioxidant activity of *K. alvarezii* cultivated within IMTA systems. By investigating these aspects, researchers aim to comprehensively assess the potential health benefits associated with incorporating *K. alvarezii* into human diets. Understanding the nutritional profile and bioactive compounds of *K. alvarezii* within the context of IMTA cultivation is crucial for unlocking its full potential as a functional food ingredient or nutraceutical product. The findings of this research endeavor hold significant promise for informing the development of novel functional foods and nutraceuticals that harness the health-enhancing properties of *K. alvarezii*. As consumers increasingly prioritize health-conscious dietary choices, there is a growing demand for natural, nutrient-rich ingredients like *K. alvarezii*, which can contribute to overall well-being and promote longevity.

MATERIALS AND METHODS

Collection of samples

K. alvarezii samples were collected from an Integrated Multi-Trophic Aquaculture (IMTA) system in Aquaculture Sub-Zone of the Banda Marine Conservation Area, Indonesia (Figure 1). The samples were washed thoroughly with distilled water to remove any attached impurities and epiphytes, then prepare for extraction using published method (6,7). They were then dried in an oven at 45°C until a constant weight was achieved. The dried samples were ground into a fine powder using a laboratory mill and stored in airtight containers at room temperature until further analysis.

Figure 1. *K. alvarezii* samples from an Integrated Multi-Trophic Aquaculture (IMTA) system in Aquaculture Sub-Zone of the Banda Marine Conservation Area, Indonesia

Nutritional value analysis

The nutritional components of the *K. alvarezii* samples were analyzed using published methods (8). Protein content was determined by the Kjeldahl method, and lipid content was measured using Soxhlet extraction with hexane as the solvent (9–11). Carbohydrates were quantified by the phenol-sulfuric acid method (12). Ash content was assessed by incinerating the samples at 550°C in a muffle furnace. Fiber content was determined using the Van Soest method (13). Vitamins (A, C, E) were analyzed using high-performance liquid chromatography (HPLC) (14). Mineral content, including calcium, magnesium, iron, zinc, sodium, and potassium, was measured by atomic absorption spectroscopy (AAS) after acid digestion of the samples (16).

Phytochemical analysis

Phytochemical constituents were quantified using various spectrophotometric from published methods (17). Total polyphenol content was determined using the Folin-Ciocalteu reagent and expressed as mg gallic acid equivalents (GAE) per gram of dry weight. Flavonoid content was measured using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE) per gram of dry weight. Tannins were quantified using the vanillin-HCl method and expressed as mg catechin equivalents (CE) per gram of dry weight. Saponin and alkaloid contents were determined by gravimetric methods and expressed as a percentage of dry weight.

Antioxidant activity assay

The antioxidant activity of *K. alvarezii* extracts was assessed using several in vitro assays by published methods (18,19). The DPPH radical scavenging activity was measured by mixing the extract with DPPH solution and determining the percentage inhibition of the radical. The ABTS radical scavenging assay was performed by generating ABTS+ radical cations and measuring the reduction in absorbance at 734 nm (20). Ferric reducing antioxidant power (FRAP) was determined by assessing the ability of the extract to reduce Fe^{3+} to Fe^{2+} and expressed as µmol Fe^{2+} per gram of dry weight (21). Total antioxidant capacity was evaluated using the phosphomolybdenum method and expressed as mg ascorbic acid equivalents (AA) per gram of dry weight (21,22). All analyses were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using appropriate software IBM SPSS Statistics 26 to determine the significance of differences among means at $p < 0.05$.

RESULTS AND DISCUSSION

Nutritional value

The nutritional analysis of *K. alvarezii* grown in Integrated Multi-Trophic Aquaculture (IMTA) revealed significant nutritional benefits (Table 1).

Table 1. The nutritional analysis of *K. alvarezii* during the research

Component	Value	Unit
Protein	15.3	% dry weight
Lipids	1.2	% dry weight
Carbohydrates	60.0	% dry weight
Ash	13.5	% dry weight
Fiber	7.8	% dry weight
Vitamin A	2.0	mg/100g dry weight
Vitamin C	1.5	mg/100g dry weight
Vitamin E	0.8	mg/100g dry weight
Calcium	800	mg/100g dry weight
Magnesium	550	mg/100g dry weight
Iron	2.1	mg/100g dry weight
Zinc	1.4	mg/100g dry weight
Sodium	1200	mg/100g dry weight
Potassium	400	mg/100g dry weight

The nutritional composition analysis of *K. alvarezii* presented in this study demonstrates its potential as a valuable dietary source, particularly in regions where plant-based diets are prevalent. The findings align with previous research on seaweeds, which have been recognized for their nutritional richness and health benefits (23). The protein content of *K.*

alvarezii at 15.3% of dry weight is comparable to other commonly consumed seaweeds, indicating its potential as a substantial protein source (24). This finding corroborates with several comparative studies on various seaweed species *Porphyra* spp. (up to 47% dry weight) (25) and *Ulva* spp. (> 30% dry weight) (26), which have reported protein levels. Such high protein content underscores the importance of seaweeds in meeting dietary protein requirements, especially in vegetarian or vegan diets. The relatively low lipid content of *K. alvarezii* (1.2% of dry weight) is consistent with the general profile of seaweeds, making it an attractive option for individuals seeking low-fat dietary choices. This observation is in line with previous studies comparing the lipid content of different seaweed species e.g., *Gracilaria* spp. (27) and *Sargassum* spp. (28), which have also reported low lipid levels. Despite the low lipid content, the presence of essential fatty acids warrants further investigation, as these compounds play crucial roles in human health. The high carbohydrate content (60.0% of dry weight) and significant fiber content (7.8%) highlight the potential of *K. alvarezii* in contributing to dietary fiber intake. These findings are consistent with comparative studies which have similarly high carbohydrate and fiber contents (29,30). The abundance of carbohydrates and fiber suggests that *K. alvarezii* can promote digestive health and may offer prebiotic benefits, enhancing its nutritional value. The high ash content of *K. alvarezii* (13.5% of dry weight) reflects its rich mineral profile, with significant quantities of calcium, magnesium, and sodium. These minerals are vital for various physiological functions, such as bone health and metabolic processes. Comparative studies on seaweeds have also reported high mineral contents, emphasizing their nutritional significance (30). However, the high sodium content should be considered by individuals monitoring their sodium intake, especially those with hypertension or cardiovascular concerns. Although the vitamin content of *K. alvarezii* is moderate, it contributes to the overall nutritional profile of the seaweed. Comparative studies on seaweeds have reported varying levels of vitamins A, C, and E, depending on species and environmental factors (25). While these vitamins may not be present in abundance, their presence adds to the nutritional diversity of *K. alvarezii*. The nutritional composition analysis of *K. alvarezii* underscores its potential as a valuable dietary resource, rich in protein, fiber, minerals, and vitamins. Comparative studies with other seaweed species support these findings, highlighting the broader significance of seaweeds as nutritious food sources. Further research into the bioavailability and health effects of *K. alvarezii*'s nutrients would provide valuable insights into its role in promoting human health and nutrition.

Phytochemical test

The phytochemical analysis of *K. alvarezii* revealed significant levels of various bioactive compounds (Table 2).

Table 2. The phytochemical analysis of *K. alvarezii*

Phytochemical	Value	Unit
Polyphenols	**20.0**	**Mg gae/g dry weight**
Flavonoids	**5.5**	**Mg qe/g dry weight**
Tannins	**3.0**	**Mg ce/g dry weight**
Saponins	**1.2**	**% dry weight**
Alkaloids	**0.9**	**% dry weight**

The phytochemical analysis of *K. alvarezii* presented in this study reveals its rich content of bioactive compounds, including polyphenols, flavonoids, tannins, saponins, and alkaloids. These compounds are known for their diverse health-promoting properties, such as antioxidant, anti-inflammatory, and potentially anti-cancer effects. Comparing these findings with existing literature on other seaweed species provides valuable insights into the potential health benefits of *K. alvarezii* and its role as a functional food or nutraceutical ingredient. The high total polyphenol content of *K alvarezii*, recorded at 20.0 mg GAE/g dry weight, is significant due to the well-documented antioxidant properties and associated health benefits of polyphenols. These compounds are known to neutralize free radicals, thereby reducing oxidative stress and potentially lowering the risk of chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders (31). The rich polyphenol content in *K. alvarezii* enhances its value as a functional food ingredient, offering both nutritional and therapeutic benefits (32). Therefore, the incorporation of *K. alvarezii* in diets could be a promising strategy for improving health and preventing disease through its potent antioxidant properties. The measured flavonoid content of *K. alvarezii* (5.5 mg QE/g dry weight) adds to its antioxidant profile, potentially reducing the risk of chronic diseases. Flavonoids, a diverse group of phytonutrients found in various fruits and vegetables, are renowned for their potent antioxidant properties, which help in scavenging harmful free radicals and mitigating oxidative stress (33). These compounds have been linked to numerous health benefits, including anti-inflammatory, cardioprotective, and anticancer effects (33). The presence of substantial flavonoid content in *K. alvarezii* enhances its potential as a functional food, capable of supporting health and preventing the onset of chronic diseases through its antioxidative mechanisms (8). Therefore, incorporating *K. alvarezii* into the diet could be an effective strategy to leverage its flavonoid-induced health benefits. Tannins, present in *K. alvarezii* at 3.0 mg CE/g dry weight, contribute to its antioxidant and anti-inflammatory properties. Tannins are a class of polyphenolic compounds known for their strong ability to scavenge free radicals and chelate metal ions, thereby preventing oxidative stress and cellular damage (10,34,35). Additionally, tannins exhibit anti-inflammatory effects by modulating the activity of inflammatory mediators and enzymes, which can help in reducing inflammation and associated diseases (33,36). The presence of tannins in *K. alvarezii* further supports its potential as a health-promoting seaweed. The saponin content of *K. alvarezii* (1.2% of dry weight) adds to its bioactive profile, with potential cholesterol-lowering and immune-modulating effects. Saponins, naturally occurring glycosides found in various plants and marine organisms, are recognized for their ability to reduce serum cholesterol levels by interfering with cholesterol absorption and increasing its excretion (36). Moreover, saponins possess immune-stimulating properties, enhancing the body's immune response and offering protection against infections and diseases (22). The presence of saponins in *K. alvarezii* not only bolsters its health-promoting attributes but also underscores its potential as a functional food ingredient that can contribute to cardiovascular health and immune system support (17). The presence of saponins in *K. alvarezii* suggests its potential as a functional food with various health benefits. Alkaloids, present in *K. alvarezii* at 0.9% of dry weight, are pharmacologically active compounds that may contribute to its bioactivity. These naturally occurring organic compounds are known for their wide range of biological activities, including anti-inflammatory, antimicrobial, analgesic, and anticancer properties (36,37). The presence of alkaloids in *K. alvarezii* enhances its therapeutic potential, making it a valuable component in functional foods and nutraceuticals aimed at promoting health and preventing diseases (33). The diverse pharmacological effects of alkaloids suggest that *K. alvarezii* could be particularly effective in modulating various physiological processes and combating a spectrum of health issues (38). The presence of alkaloids in *K. alvarezii* highlights its potential as a source of bioactive compounds with diverse physiological effects. The phytochemical analysis of *K. alvarezii* reveals its substantial content of bioactive compounds, including polyphenols, flavonoids, tannins, saponins, and alkaloids. These compounds contribute to its antioxidant capacity and potential health-promoting effects, making it a promising candidate for functional foods or nutraceuticals. Comparative studies with other seaweed species corroborate these findings, emphasizing the broader significance of seaweeds as sources of bioactive compounds. Further research into the bioavailability and bioactivity of these compounds in vivo is warranted to fully elucidate their health effects and therapeutic potential. Overall, *K. alvarezii* shows promise as a valuable dietary component for promoting human health and well-being.

Antioxidant activity: The antioxidant activity of *K. alvarezii* grown in Integrated Multi-Trophic Aquaculture (IMTA) was evaluated using several in vitro assays, and the results are presented in Table 3.

Table 3. The antioxidant activity of *K. alvarezii* grown in Integrated Multi-Trophic Aquaculture (IMTA)

Assay	Value	Unit
Dpph radical scavenging	**80.2**	**% inhibition**
Abts radical scavenging	**70.5**	**% inhibition**
Frap (ferric reducing antioxidant power)	**600**	**μmol fe^{2+}/g dry weight**

The antioxidant activity assays conducted on *K. alvarezii* underscore its significant potential as a rich source of antioxidants, highlighting its possible role in mitigating oxidative stress-related diseases. Comparing these findings with existing literature on other seaweed species provides valuable insights into the antioxidant capacity of *K. alvarezii* and its potential health benefits. DPPH and ABTS Radical Scavenging Activity: The high inhibition percentages observed in the DPPH (80.2%) and ABTS (70.5%) assays indicate robust antioxidant activity in *K. alvarezii*. These assays are widely used to evaluate the free radical scavenging abilities of compounds, with higher inhibition percentages reflecting stronger antioxidant properties. The impressive results for *K. alvarezii* suggest it has a significant capacity to neutralize free radicals, thereby reducing oxidative stress and potentially preventing damage to cells and biomolecules. This potent antioxidant activity can be attributed to its high content of bioactive compounds such as polyphenols, flavonoids, saponins, and alkaloids, which collectively contribute to its health-promoting effects (39). Consequently, *K. alvarezii* shows promise as a functional food ingredient with considerable potential for enhancing health and preventing oxidative stress-related diseases. Such potent scavenging abilities suggest that *K. alvarezii* possesses effective compounds capable of neutralizing free radicals, thereby reducing oxidative stress and its associated health risks. FRAP Assay: The high FRAP value of *K. alvarezii* (600 μmol Fe^{2+}/g dry weight) indicates strong ferric reducing power, indicative of its antioxidant potential. The Ferric Reducing Antioxidant Power (FRAP) assay is a robust method used to assess the antioxidant capacity of substances by measuring their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions (40). A high FRAP value suggests that *K. alvarezii* contains potent reducing agents capable of donating electrons to neutralize free radicals, thereby preventing oxidative damage to cells and tissues (41). This strong ferric reducing power can be attributed to its rich content of bioactive compounds such as polyphenols, flavonoids, saponins, and alkaloids, which collectively enhance its antioxidant properties (42). Therefore, the inclusion of *K. alvarezii* in the diet could provide significant health benefits by combating oxidative stress and reducing the risk of chronic diseases. The presence of such compounds in *K. alvarezii* suggests its ability to donate electrons and neutralize reactive oxygen species effectively. The antioxidant activity exhibited by *K. alvarezii* suggests its potential as a natural source of antioxidants for incorporation into functional foods, nutraceuticals, and dietary supplements. Its integration into the diet may offer protective effects against oxidative damage and related diseases, including cardiovascular diseases, certain cancers, and neurodegenerative disorders. However, further research is warranted to identify and characterize the specific antioxidant compounds present in *K. alvarezii*, elucidate their bioavailability, and evaluate their efficacy in vivo. Comparative studies focusing on the antioxidant profiles of various seaweed species can provide valuable insights into their potential health-promoting effects and guide the development of novel antioxidant-rich products. *K. alvarezii* cultivated in Integrated Multi-Trophic Aquaculture systems exhibits promising antioxidant activity, supporting its role as a valuable component in health-promoting diets and products. Continued research efforts are necessary to fully harness the antioxidant potential of *K. alvarezii* and explore its applications in improving human health and well-being.

Conclusion

In conclusion, *K. alvarezii* grown in Integrated Multi-Trophic Aquaculture (IMTA) systems was found to possess significant nutritional value, rich phytochemical content, and substantial antioxidant activity. The seaweed demonstrated high levels of protein, carbohydrates, and essential minerals, along with notable amounts of polyphenols, flavonoids, tannins, saponins, and alkaloids. These findings offering various health benefits. It is suggested that further research be conducted to explore the bioavailability and specific health effects of the bioactive compounds present in *K. alvarezii*. Additionally, the potential synergistic effects of these compounds should be investigated. The development of functional foods and nutraceuticals incorporating *K. alvarezii* is recommended to maximize its health-promoting properties. Finally, the integration of this seaweed into dietary practices should be promoted to leverage its nutritional and therapeutic benefits.

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