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Original Article

GC-MS profiling and antioxidant activity of ethanol extracts from tissue-cultured *Kappaphycus alvarezii* and their effect on Nile Tilapia (*Oreochromis niloticus*) growth performance and hematological parameters

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Abstract: This study aimed to identify the bioactive compounds present in *Kappaphycus alvarezii* ethanol extracts using various solvent ratios, through GC-MS analysis and antioxidant activity assays. The ultimate goal is to provide critical insights supporting the application of *K. alvarezii* extract as a feed additive in the diet of Nile tilapia (*Oreochromis niloticus*). The research was conducted in two experimental phases. Phase 1 involved the preparation of ethanol extracts using three different solvent ratios: 1:3 (A), 1:4 (B), and 1:5 (C). Each extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to profile the chemical constituents, followed by evaluation of their antioxidant activity. Phase 2 assessed the application of these ethanol extracts in commercial fish feed over a 40-day rearing period. Four dietary treatments were tested: commercial feed only (P1), and commercial feed supplemented with *K. alvarezii* extract at ratios of 1:3 (P2), 1:4 (P3), and 1:5 (P4). A Completely Randomized Design (CRD) with three replicates per treatment was employed, resulting in a total of 12 experimental units. Growth performance and survival rate of Nile tilapia were evaluated. GC-MS profiling revealed that the ethanol extracts were dominated by palmitic acid (hexadecanoic acid), a compound known for its antioxidant potential. The extract with a 1:3 solvent ratio exhibited the strongest antioxidant activity, with an IC_{50} value of 34.66 ppm. Application of this extract in fish feed significantly influenced growth and survival performance. The 1:3 treatment yielded the best results, enhancing absolute weight gain (4.51±0.47 g), specific growth rate (1±0.07%/day), and achieving a 100% survival rate. Additionally, this treatment improved the fish's health status, as indicated by elevated erythrocyte counts (306,000 cells μL^{-1}) and hematocrit levels (25.7%).

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Introduction

The use of tissue-cultured seedlings in seaweed cultivation offers numerous advantages, one of which is accelerated growth, enabling large-scale production (Reddy et al., 2017; Rama et al., 2018; Budiyanto et al., 2019; Cahyani et al., 2020). Reddy et al. (2017) reported that tissue-cultured seaweed can grow 1.5 to 1.8 times faster than naturally grown seaweed. This increase in productivity expands the potential applications of tissue-cultured seaweed across food and non-food industries.

Seaweed is widely recognized for its utility as a source of carrageenan, agar, and alginate—key ingredients in various industries, including pharmaceuticals, nutraceuticals, cosmetics, and functional foods (Chandimali et al., 2023). In addition, seaweed contains a diverse range of bioactive compounds exhibiting extensive biological activities, such as phenolic compounds with anti-inflammatory, antioxidant, antibacterial, antifungal, anticancer, antiviral, neurotrophic, and antihypertensive properties (Ganesan et al., 2019; Biris-Dorhoi et al., 2020; Lopes et al., 2021; Pereira and Valado, 2021). The phytochemical properties and bioactive components of seaweed have also been utilized in aquaculture, where they have been shown to enhance both the quantity and quality of aquaculture yields (Pratama et al., 2022). One such application is as a

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feed additive. In aquaculture, feed additives can enhance growth performance and feed efficiency. One promising species for use as a natural feed additive is *Kappaphycus alvarezii*, a red seaweed (Rhodophyceae) propagated through tissue culture. Ariano et al. (2021) found that *K. alvarezii* has greater potential as a feed additive compared to *K. striatum*, due to its superior chemical and nutritional composition.

Previous work by El-Beltagi et al. (2022) identified several bioactive compounds in *K. alvarezii*, including flavonoids, alkaloids, triterpenoids, proteins, carbohydrates, and lipids. Phytochemical screening has also revealed the presence of alkaloids, steroids, triterpenoids, and flavonoids in the ethanol extracts, as well as triterpenoids and flavonoids in the ethyl acetate extracts of *K. alvarezii* (Lumbessy et al., 2024). Nearly all bioactive compounds found in red algae have been shown to possess antioxidant properties (Valente et al., 2015; Araújo et al., 2015; Azeez et al., 2020; Morais et al., 2020; Vazirzadeh et al., 2020). Antioxidants are substances that inhibit or prevent oxidative damage to cells. They function by donating electrons to stabilize free radicals, thereby protecting cells and enhancing the innate immune response in aquatic organisms (Tripathy, 2016).

The bioactive compounds in *K. alvarezii* can be extracted through solvent extraction. This process involves separating target compounds from the raw material using an appropriate solvent. Standard extraction techniques include maceration, reflux, Soxhlet, percolation, and ultrasound-assisted extraction, with maceration being the most frequently used method. The choice of solvent plays a crucial role, as it affects the extraction of chemical constituents based on their polarity and toxicity. Solvent polarity affects not only the rate of extraction but also the type and yield of active compounds recovered (Santos et al., 2019; Anova and Yeni, 2020).

However, there is still limited comprehensive research on the use of *K. alvarezii* extracts as feed additives in fish diets. Therefore, this study aims to identify bioactive compounds in *K. alvarezii* extracts obtained with ethanol as the solvent, using GC-MS analysis and antioxidant activity assays. The findings are expected to support the potential application of *K. alvarezii* extract as a functional feed additive for aquaculture, particularly in Nile tilapia (*Oreochromis niloticus*) farming.

Materials and Methods

The research was conducted from May to October 2024. The fresh *K. alvarezii* used in this study was cultivated using tissue-cultured seedlings for 45 days in Teluk Ekas, East Lombok. This experimental research was performed in two main stages:

Stage 1: Extraction, GC-MS analysis, and antioxidant assay: The first stage involved preparing of ethanol extracts from *K. alvarezii*, followed by GC-MS analysis and testing for antioxidant activity. The variable tested was the ethanol-to-seaweed ratio, with three extraction treatments: A: *K. alvarezii* extract: Ethanol (1:3), B: *K. alvarezii* extract: Ethanol (1:4), and C: *K. alvarezii* extract: Ethanol (1:5).

Stage 2: Application of *K. alvarezii* ethanol extract in Nile tilapia feed: The second stage involved evaluating the application of *K. alvarezii* ethanol extract in Nile tilapia feed during a 40-day culture period. Four dietary treatments were tested: P1: Commercial feed (control), P2: Commercial feed + *K. alvarezii* ethanol extract (1:3 solvent ratio), P3: Commercial feed + *K. alvarezii* ethanol extract (1:4 solvent ratio), and P4: Commercial feed + *K. alvarezii* ethanol extract (1:5 solvent ratio). A Completely Randomized Design (CRD) was employed, consisting of four treatments.

Preparation and extraction of *K. alvarezii* powder: Fresh *K. alvarezii* cultivated for 42 days was air-dried in the shade (without direct sunlight) until completely dehydrated. The dried seaweed was then cut into small pieces, ground using a blender, and sieved to obtain a fine powder. The extraction was performed using the maceration method, in which the seaweed powder was soaked in ethanol at varying solvent ratios, following the protocols of Putra et al. (2016) and Podungge et al. (2017). A total of 100 g of *K. alvarezii* powder was placed in an Erlenmeyer flask and mixed with 96%

ethanol, following the treatment groups: 1:3 solvent ratio, 300 mL of ethanol was added, 1:4 solvent ratio, 400 mL of ethanol was added, and A 1:5 solvent ratio was used, with 500 mL of ethanol added.

The flasks were covered with aluminum foil and macerated for 30 hours at 30°C, stirring every 5 hours to ensure uniform mixing. After maceration, the mixture was filtered, and the filtrate was concentrated using a rotary vacuum evaporator (EYELA N-1100). The resulting extract was stored in aluminum foil-covered containers. Extraction yield was calculated for each treatment using the following formula (Astika et al., 2022) of: Extraction yield = (Weight of extract) / (Weight of sample) X 100% of Extraction yield = (Weight of extract) / (Weight of sample) x 100%.

Chemical profiling of *K. alvarezii* extract via GC-MS: A 1 μL aliquot of the *K. alvarezii* ethanol extract was injected into a Shimadzu QP 2010 SE Gas Chromatography-Mass Spectrometry (GC-MS) instrument. The system was equipped with an Rtx-5MS capillary column (5% diphenyl/95% dimethylpolysiloxane) and a Carbowax (polyethylene glycol) column. The identification of chemical constituents was based on the interpretation of mass spectral fragmentation patterns. These patterns were matched against reference spectra from the National Institute of Standards and Technology (NIST) library and the Wiley7 compound database.

Antioxidant activity assay of *K. alvarezii* extract: The antioxidant activity of the extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, as described by Subri and Zin (2020). A total of 25 mg of *K. alvarezii* extract was dissolved in 125 mL of ethanol, yielding a stock concentration of 200 μg mL^{-1}. Serial dilutions of the extract were prepared, and each concentration was mixed with 2 mL of 50 μM DPPH solution. The mixtures were homogenized and incubated in the dark at room temperature for 30 minutes. Following incubation, the absorbance of each solution was measured at 517 nm using a UV-Vis spectrophotometer to determine DPPH radical scavenging activity. The absorbance values were used to calculate the IC_{50} (half-maximal inhibitory concentration). Vitamin C was used as a positive control. The percentage inhibition for each sample was plotted on the y-axis and extract concentration on the x-axis to derive a linear regression equation (y = a + bx). The IC_{50} value is the concentration required to scavenge 50% of the DPPH radicals. Each sample was tested in triplicate, and results were reported as standard deviation (SD). The percentage of DPPH radical scavenging activity was calculated using the following formula (Wu et al., 2022): DPPH inhibitor activity (%) = (absorbent control-absorbing sample) / (absorbent control) x 100%.

Application of *K. alvarezii* extract to Nile tilapia commercial feed: The extract was applied to tilapia feed by spraying *the K. alvarezii ethanol extract at* 2 g/kg, according to the designated treatment groups. The commercial feed used was Hi-Pro Vite (28-30% protein, 6-8% lipid, 4-6% fibre, 10-13% ash, and 11-13% moisture). The extract was diluted with 10 mL of distilled water before application. The treated feed was air-dried at room temperature to remove excess moisture. Fish were reared in 45 L containers for 30 days, with a stocking density of 10 fish per container. Feed was administered according to the treatment groups at a feeding rate of 5% of total biomass, divided into three daily feedings. Throughout the experimental period, fish growth performance and water quality parameters were monitored at 10-day intervals. Dead fish were recorded daily, and uneaten feed and waste were siphoned regularly to maintain water quality.

The growth performance parameters analyzed included absolute weight gain, specific growth rate (SGR), and survival rate (SR) (Lugert et al., 2016). Additionally, hematological parameters (erythrocyte count and hematocrit value) (Witeska et al., 2022) and water quality indicators (temperature, pH, and dissolved oxygen) were measured.

Data analysis: Extraction yield, GC-MS results, antioxidant activity, hematological parameters, and water quality data were analyzed descriptively. Growth performance and survival data were analyzed using analysis of variance (ANOVA) at a 95% confidence level. If significant differences were

detected (P<0.05), the means were further compared using Duncan's Multiple Range Test (DMRT).

Results

Yield of *K. alvarezii* extract: The results showed that the ethanol extract yield from *K. alvarezii* powder across all solvent-ratio treatments ranged from 3 to 6% (Table 1). The highest extract yield was obtained from the 1:5 solvent-to-sample ratio treatment (P4), followed by the 1:4 ratio (P3). The lowest yield was observed in the 1:3 ratio treatment (P2).

Gas Chromatography–Mass Spectrometry (GC-MS) analysis of *K. alvarezii* ethanol extract: GC-MS analysis revealed the presence of 17 chromatographic peaks across all ethanol extract treatments of *K. alvarezii*, using different solvent-to-sample ratios (Table 2; Fig. 1). Nine compounds were identified in the extract with a 1:3 solvent ratio (A), while seven compounds were detected in both 1:4 (B) and 1:5 (C). The chromatograms revealed multiple compounds with distinct retention times (Fig. 1). Each compound was further characterized by its molecular formula, molecular weight, retention time, similarity index, and relative abundance in the extract. The detailed compound profiles for each solvent ratio treatment are presented in Table 2.

Among the detected compounds, five exhibited similarity indices below 90%, indicating a moderate match with compounds in the Wiley7 Library database. These included: 9-Octadecenoic acid (Z)- (85%) and Adipinsaeure, Di(Oct-4-Yl-Ester) (81%) in Treatment A, Hexadecanoic acid, ethyl ester (83%) and 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl- (81%) in Treatment B, and 9-Octadecenoic acid (Z)-, ethyl ester (86%) in Treatment C. The remaining eleven compounds demonstrated high similarity indices, ranging from 91 to 97%, suggesting strong spectral matches with entries in the *Wiley7* Library (Bhuyar et al., 2019).

Antioxidant activity assay: The antioxidant activity of *K. alvarezii* ethanol extracts obtained using different solvent ratios is presented in Table 3, which indicates that each treatment exhibited varying capacities to scavenge free radicals. The DPPH radical

Table 1. Yield of *Kappaphycus alvarezii* ethanol extract at different solvent ratios.

Solvent-to-Sample Ratio	Extract yield (%)
(A) 1: 3	3
(B) 1: 4	4
(C) 1: 5	6

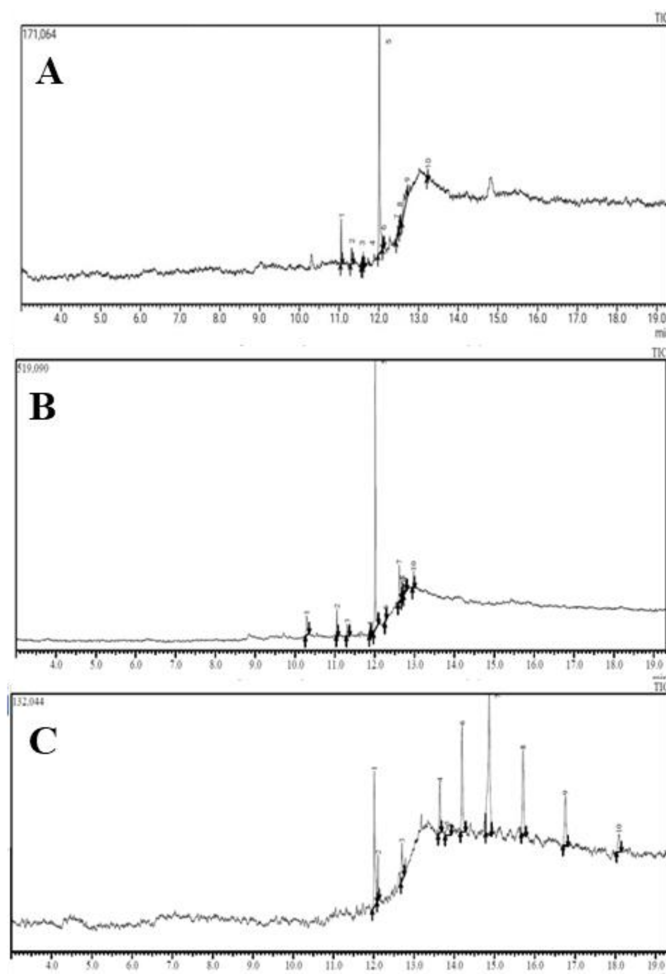

Figure 1. GC-MS chromatograms of *Kappaphycus alvarezii* ethanol extracts at different solvent-to-sample ratios: (A) 1:3, (B) 1:4, and (C) 1:5.

scavenging activity of the ethanol extracts of *K. alvarezii* was also evaluated to determine IC_{50} values across different solvent-to-sample ratios (Table 3).

Growth performance and survival rate of Nile tilapia: The results showed that dietary supplementation of *K. alvarezii* ethanol extracts at different ethanol-to-sample ratios significantly influenced the growth performance and survival rate of *O. niloticus* during the 40-day culture period. The results revealed that all treatments with *K. alvarezii* extract (P2, P3, and P4) resulted in a significantly

Table 2. GC-MS-based chemical profile of *Kappaphycus alvarezii* ethanol extracts at different solvent ratios.

No.	Compound Name	Molecular Formula	BM	*K. alvarezii* Ethanol Extracts								
				1:3 (A)			1:4 (B)			1:5 (C)		
				Retention Time (min)	Area (%)	SI (%)	Retention Time (min)	Area (%)	SI (%)	Retention Time (min)	Area (%)	SI (%)
1	Benzoic acid, 4-(1,1-dimethylethyl)	$C_{11}H_{14}O_2$	178	10.291	6.81	91	-	-	-	-	-	-
2	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	11.307	3.78	95	11.315	4.35	91	-	-	-
3	Hexadecanoic acid, Palmitic acid	$C_{16}H_{32}O_2$	256	12.010	62.32	95	12.012	55.32	95	12.015	15.40	95
4	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	11.880	1.52	94	-	-	-	-	-	-
5	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282	12.272	2.08	85	-	-	-	-	-	-
6	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	-	-	-	11.610	2.44	91	-	-	-
7	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	12.686	5.55	91	12.110	2.53	83	12.017	3.32	95
8	9-Octadecenoic acid (Z)-, ethyl ester	$C_{20}H_{38}O_2$	310	-	-	-	-	-	-	12.702	5.28	86
9	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-	$C_{20}H_{40}O$	296	-	-	-	11.578	1.62	81	-	-	-
10	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl			-	-	-	12.533	2.77	84	-	-	-
11	Adipinsaeure, Di(Oct-4-Yl-Ester)	$C_{22}H_{42}O_4$	239	12.777	2.75	81	-	-	-	-	-	-
12	Di-(9-Octadecenoyl)-Glycerol	$C_{39}H_{72}O_5$	621	12.613	14.63	90	-	-	-	-	-	-
13	Heptadecane	$C_{17}H_{36}$	240	11.050	7.13	97	11.054	4.32	97	-	-	-
14	Pentacosane	$C_{25}H_{52}$	352	-	-	-	-	-	-	13.642	4.78	96
15	Pentacosane	$C_{25}H_{52}$	352	-	-	-	-	-	-	14.195	12.76	97
16	Pentacosane	$C_{25}H_{52}$	352	-	-	-	-	-	-	15.709	12.85	97
17	Pentacosane	$C_{25}H_{52}$	352	-	-		-	-	-	18.092	3.62	95
	Total Compound			9			7			7		

Table 3. Antioxidant activity of *Kappaphycus alvarezii* ethanol extracts at different solvent ratios using the DPPH method.

Treatments	Concentration (ppm)	Absorbance	Inhibition (%)	Regression Equations	IC_{50} (ppm)	Categories
K. alvarezii ethanol extract at 1:3 (A)	4	0.709	18.60	y = 1.0499x + 13.613	34.66	Very Strong
	8	0.677	22.27	R^2 = 0.9768		
	16	0.631	27.55			
	24	0.514	40.99			
	32	0.463	46.84			
K. alvarezii ethanol extract at 1:4 (B)	4	0.671	22.96	y = 0.1969x + 22.754	138.37	Moderate
	8	0.656	24.68	R^2 = 0.9634		
	16	0.641	26.41			
	24	0.632	27.44			
	32	0.62	28.82			
K. alvarezii ethanol extract at 1:5 (C)	4	0.715	17.91	y = 0.2347x + 17.389	138.95	Moderate
	8	0.699	19.75	R^2 = 0.9675		
	16	0.684	21.47			
	24	0.676	22.39			
	32	0.652	25.14			
Vitamin C	5	0.401	9.07	y = 3.1746x - 5.941	17.62	Very Strong
	10	0.333	24.49	R^2 = 0.9916		
	15	0.246	44.22			
	20	0.177	59.86			
	25	0.129	70.75			

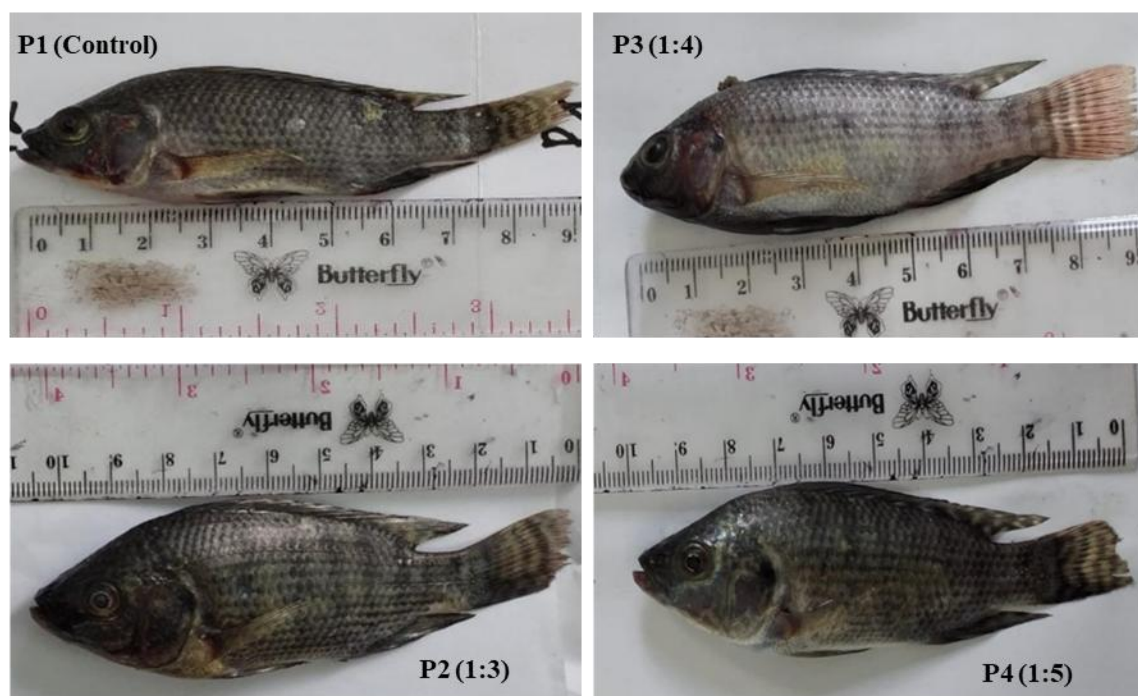

Figure 2. Nile tilapia fed diets supplemented with *Kappaphycus alvarezii* ethanol extracts at different solvent ratios.

higher final body weight than the control group (P1) (Fig. 2). Moreover, the inclusion of *K. alvarezii* ethanol extract at ratios of 1:3 (P2) and 1:5 (P4) notably enhanced the specific growth rate (SGR) and higher survival rates (Figs. 3, 4, 5).

Hematological profile (erythrocytes and

Table 4. Erythrocyte count and hematocrit levels of Nile tilapia fed diets supplemented with *Kappaphycus alvarezii* ethanol extracts.

Treatments	Erythrocyte Count (cells µL⁻¹)	Hematocrit (%)
Control (P1)	187,000	18.57
K. alvarezii Ethanol Extract 1:3 (P2)	306,000	25.71
K. alvarezii Ethanol Extract 1:4 (P3)	281,000	24.28
K. alvarezii Ethanol Extract 1:5 (P4)	293,000	24.28

Table 5. Water quality parameters during the rearing period.

Parameters	Unit	Results	Source: Fujaya et al. (2022)
Temperature	˚C	28.3-29.3	27.4-29.6
pH	-	7.9-7.9	6.2-8
DO	mg L^{-1}	5.9-6.9	≥ 3

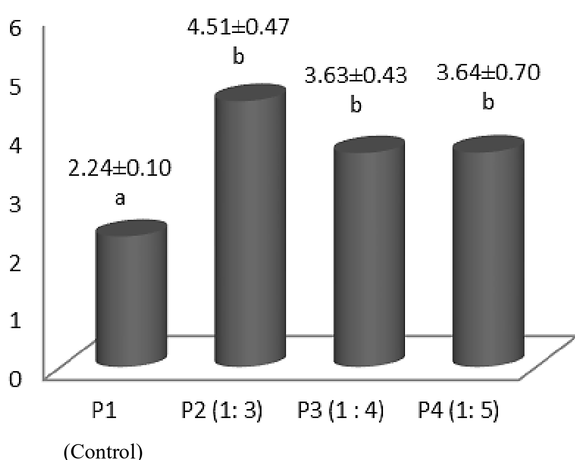

Figure 3. Absolute weight gain of Nile tilapia fed diets supplemented with *Kappaphycus alvarezii* ethanol extracts at different solvent ratios.

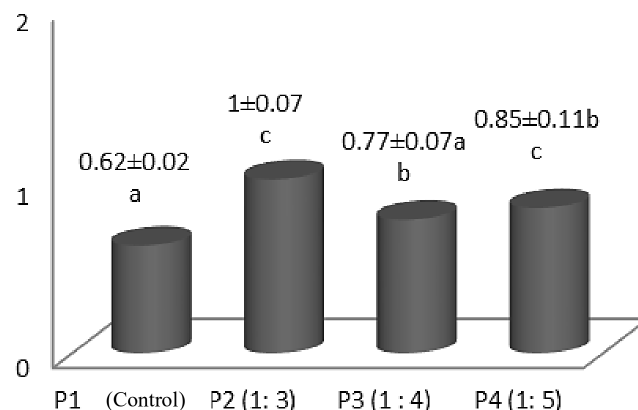

Figure 4. Specific growth rate of Nile tilapia fed diets supplemented with *Kappaphycus alvarezii* ethanol extracts at different solvent ratios.

hematocrit): The hematological analysis revealed that erythrocyte counts ranged from 187,000 to 306,000 cells μL^{-1}, and hematocrit values ranged from 18.57 to 25.71%. The highest values for both erythrocytes and hematocrit were observed in the group fed *K. alvarezii* extract at a 1:3 ratio (P2), with erythrocyte levels of 306,000 cells μL^{-1} and hematocrit values of 25.71%, respectively (Table 4).

Water quality during the experimental period: Water quality measurements across all treatments remained within optimal ranges. They were sufficient to support the growth and survival of Nile tilapia throughout the 40-day culture period (Table 5).

Discussions

The use of ethanol as a solvent in the extraction process of *K. alvarezii* powder in this study was based on its polar nature. Ethanol can penetrate cell walls, accelerate diffusion, and effectively extract bioactive compounds, increasing extraction yield (Tiwari et al., 2011). Our findings are consistent with those of Fayaz et al. (2005), who reported that ethanol extraction yields more polyphenolic compounds than other extraction methods. According to Noviyanty et al. (2019), the higher the solvent polarity, the greater the extraction yield. This is because more polar solvents exhibit superior extraction efficiency. The findings of this study indicate that the highest yield was achieved at a solvent-to-sample ratio of 1:5, with a yield value of 6%. Variations in solvent concentration significantly affected the extraction yield, as increasing the solvent volume enhances the solubility and mass transfer of extractable compounds into the solvent phase. Putra et al. (2020) reported that the solvent-to-sample ratio during extraction plays a critical role in determining the final yield. Moreover, Djaeni et al. (2017) explained that a higher solvent

Table 6. Antioxidant activity strength categories (Badarinath et al., 2010).

IC_{50} Value	Categories
50 ppm	Very strong
50-100 ppm	Strong
100-150 ppm	Moderate
150-200 ppm	Weak
> 200 ppm	Very weak

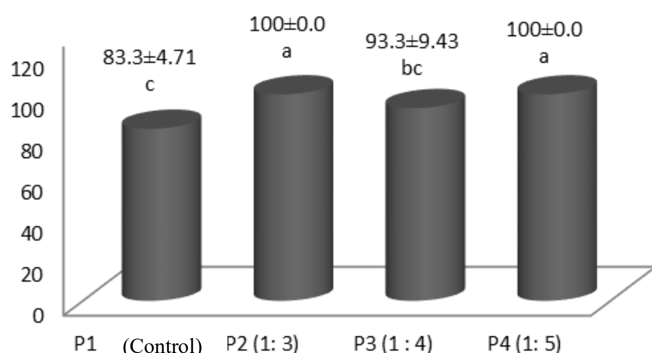

Figure 5. Survival rate of Nile tilapia fed diets supplemented with *Kappaphycus alvarezii* ethanol extracts at different solvent ratios.

ratio implies a larger solvent volume, increasing the contact surface area between the solvent and the sample. This, in turn, facilitates greater diffusion activity, resulting in the extraction of more bioactive compounds. In addition, multiple factors affect the extraction yield, including the type of raw material, drying temperature, extraction temperature and duration, and the concentration of active compounds present in the raw material.

GC-MS analysis revealed 17 chromatographic peaks across all ethanol extract treatments of *K. alvarezii*, using different solvent-to-sample ratios. Our results are consistent with those reported by Natarajan et al. (2023), who identified nine compounds in the ethanolic extract of *K. alvarezii* using GC-MS and subsequently evaluated their hepatoprotective and nephroprotective effects in albino rats. In the 1:3 ratio (A), the nine identified compounds showed retention times ranging from 11.050 to 12.777 minutes. The major constituents were Palmitic acid (Hexadecanoic acid), representing 62.32% of the total area at a retention time of 12.010 minutes, and Di-(9-Octadecenoyl)-Glycerol (14.63%) at 12.613 minutes. For the 1:4 ratio (B), seven compounds were identified, with retention times ranging from 11.054 to 12.533 minutes. Palmitic acid again dominated the profile, accounting for 55.32% of the total area at a retention time of 12.012 minutes. In the 1:5 ratio (C), seven compounds were also identified, with retention times ranging from 12.015 to 18.092 minutes. Palmitic acid remained the predominant compound (15.40%) at 12.015 minutes, followed by Pentacosane (12.76% and 12.85%), which was detected at two retention times of 14.195 and 15.709 minutes, respectively.

Palmitic acid (Hexadecanoic acid) was the most abundant compound across all ethanolic extract treatments. Based on the detected chromatographic peaks, this acid was the predominant compound in the extracts of the 1:3 (A) and 1:4 (B) solvent-to-sample ratios. However, its relative abundance decreased as the solvent volume increased, suggesting dilution effects or competitive extraction by other compounds at higher solvent concentrations. Palmitic acid is a saturated fatty acid with antioxidant properties, particularly its ability to neutralize free radicals and mitigate oxidative stress (Rashad et al., 2025). Additionally, it exhibits various biological activities, including cholesterol-lowering effects, antioxidant potential, and inhibition of hemolysis (Uma et al., 2011; Siswadi and Saragih, 2021).

The high percentage of Palmitic acid detected in the current study aligns with previous findings. Young et al. (2014) reported Palmitic acid as a dominant fatty acid in *K. alvarezii* propagated through micropropagule regeneration (36.65%) and sea cultivation (36.36%). Similarly, Muralidhar et al. (2010) identified Palmitic acid as the principal fatty acid in *K. alvarezii* collected from Indian waters (49.72%), while Jayasinghe et al. (2018) reported a concentration of 40.33% in samples from Sri Lankan waters. In the 1:5 extract (Treatment C), Pentacosane was also detected in multiple peaks in addition to Palmitic acid. Pentacosane is an alkane compound and a plant-derived secondary metabolite commonly found in essential oils. It is known for its antibacterial and antimicrobial properties (Sunita and Meena, 2018; Carev et al., 2023), further highlighting the bioactive potential of *K. alvarezii* ethanol extracts.

The antioxidant activity assay demonstrated that each treatment exhibited varying capacities in scavenging free radicals. A lower absorbance value indicates higher antioxidant activity. This inverse relationship is attributed to the fact that, at higher concentrations, antioxidant compounds are more effective at neutralizing free radicals. As a result, the concentration of unreacted DPPH decreases, thereby reducing absorbance (Ika et al., 2023). Antioxidant activity is quantitatively expressed by the IC_{50} value, which is inversely proportional to antioxidant strength. Variations in IC_{50} values can be attributed to differences in the concentration of active antioxidant compounds within each extract. A higher IC_{50} value indicates weaker antioxidant activity; conversely, a lower IC_{50} value indicates greater antioxidant activity. The IC_{50} results from the current study showed that the ethanol extract of K. alvarezii at a 1:3 ratio (Treatment A) exhibited strong antioxidant activity (IC_{50} < 50 ppm). In contrast, the extracts at ratios of 1:4 (B) and 1:5 (C) demonstrated moderate antioxidant activity (100 < IC_{50} < 250 ppm). As expected, vitamin C showed very strong antioxidant activity (IC_{50} < 50 ppm). Notably, the antioxidant activity of the 1:3 ethanol extract of *K. alvarezii* (A) was comparable to that of vitamin C, suggesting that this extract holds significant potential as a natural antioxidant agent, particularly in its efficacy to neutralize free radicals.

The antioxidant activity of the ethanol extract of *K. alvarezii* at a 1:3 ratio was the highest among the 1:4 and 1:5 extracts. This finding is supported by the GC-MS analysis, which revealed that the bioactive compound palmitic acid (hexadecanoic acid)—a known antioxidant candidate—was present in a higher concentration in Treatment A. Additionally, two other potential antioxidant compounds were identified in Treatment A: 9-octadecenoic acid (Z)- (oleic acid) at 2.08%, and heptadecane at 7.13%, both of which are classified as potent antioxidants. Heptadecane, in particular, has been reported to possess strong anticancer, antioxidant, and antibacterial properties (Kim et al., 2013; Kumaresan et al., 2015; Vijayalingam and Rajesh, 2019). Oleic acid has also been recognized for its significant antioxidant activity (Wei et al., 2016). Notably, heptadecane and oleic acid were not detected in the 1:4 (B) and 1:5 (C). The moderate antioxidant activity observed in the 1:4 (B) and 1:5 (C) ethanol extracts of *K. alvarezii* is likely due to the presence of interfering substances such as salts, minerals, and other nutrients. These substances, likely introduced by the use of larger solvent volumes, may interfere with or inhibit the activity of antioxidant compounds.

The characterization of bioactive compounds in the ethanol extract of *K. alvarezii*, as determined through GC-MS and antioxidant assays, highlights its potential application, particularly in the aquafeed industry as a feed additive to enhance metabolism and improve the health status of Nile tilapia. Nile tilapia were fed diets supplemented with *K. alvarezii* ethanol extracts at different solvent ratios in the present study. A 40-day feeding trial showed that supplementing commercial feed with *K. alvarezii* extract significantly affected absolute weight gain, specific growth rate (SGR), and survival rate in Nile tilapia. Based on the results, all treatments involving commercial feed supplemented with ethanol extract of *K. alvarezii* at different solvent ratios (P2, P3, and P4) significantly increased the absolute weight gain. Meanwhile, treatments with the ethanol extract of *K. alvarezii* at 1:3 (P2) and 1:5 (P4) ratios yielded higher specific growth rates and survival rates in Nile tilapia. These findings suggest that both P2 and P4 are comparable in enhancing metabolic performance and overall health status in Nile tilapia. This effect is likely attributed not only to the antioxidant activity of bioactive compounds, such as palmitic acid (hexadecanoic acid), present in both treatments, but also to the presence of pentacosane, which appears in several peaks in the GC-MS chromatogram of the 1:5 ethanol extract (P4). Lumbessy et al. (2024) also demonstrated that supplementing commercial feed with *E. cottonii* seaweed extract, prepared using various organic solvents (ethanol, ethyl acetate, or a combination of both) at a 1:5 ratio, significantly improved Nile tilapia growth.

The effective application of ethanol extract from *K. alvarezii* in Nile tilapia aquaculture is likely related

to the presence of bioactive compounds. These compounds include antioxidant agents such as aromatic hydrocarbons, phenolic compounds, and fatty acids. According to Oke and Hamburger (2002), aromatic compounds containing hydroxyl groups, particularly ortho-dihydroxy or trihydroxy substitutions, can form relatively stable radicals. The presence of hydroxyl groups is a key structural feature required for a compound to exhibit antioxidant activity. In addition to aromatic hydrocarbons, phenolic compounds and their derivatives, and fatty acids are recognized as effective natural antioxidants. Phenolic compounds, characterized by hydroxyl groups attached to an aromatic ring, can neutralize free radicals by donating a hydrogen atom (proton donor) from their hydroxyl group, thereby stabilizing the radical species (Tamat et al., 2007). Furthermore, Fratianni et al. (2021) reported that fatty acids also possess significant antioxidant potential. Based on our findings, the ethanol extract of *K. alvarezii* may function as a natural antioxidant and can be utilized as a feed additive in Nile tilapia diets.

The use of ethanol extract of *K. alvarezii* as a feed additive in Nile tilapia diets also appears to support improved physiological conditions and overall fish health. This is evidenced by the elevated erythrocyte and hematocrit values observed in all treatment groups that received commercial feed supplemented with the ethanol extract of *K. alvarezii*. Among the treatments, the 1:3 extract ratio (P2) produced the highest erythrocyte and hematocrit values. Erythrocyte counts in Nile tilapia in this study ranged from 187,000 to 306,000 cells μL^{-1}, which falls within a favorable physiological range across all treatments. Hartika et al. (2014) reported that the normal erythrocyte range in teleosts is 20,000-3,000,000 cells μL^{-1}. Erythrocytes function to deliver oxygen to body tissues with the assistance of hemoglobin (Hb). A high erythrocyte count indicates that homeostatic processes in the fish are functioning effectively (Ambarwati et al., 2022). Meanwhile, hematocrit values ranged from 18.57 to 25.71% across the treatments, and values for all groups receiving the ethanol extract (P2, P3, and P4) remained within acceptable physiological limits. According to Sudirman et al. (2021), the normal hematocrit range for fish is 21-41%. In line with the increase in erythrocyte count, hematocrit values also tended to rise (Tanbiyaskur et al., 2022).

Erythrocyte and hematocrit levels can indicate nutritional status, environmental conditions, and stress levels in fish (Witeska et al., 2022). Increases in both parameters suggest enhanced immune responses correlate with improved productivity and survival rates (Purbomartono et al., 2024). This finding aligns with the survival data in the present study, in which treatments with *K. alvarezii* ethanol extracts at 1:3 (P2) and 1:5 (P4) ratios showed higher survival rates than other treatments. Therefore, supplementing fish feed with an ethanol extract of *K. alvarezii* is presumed to enhance the nutritional quality of the feed and contribute to increased red blood cell production. Red blood cells are crucial in metabolic processes and maintaining fish health. Simanjuntak et al. (2022) stated that several factors, including dietary nutrition, can influence erythrocyte and hematocrit levels in fish.

Moreover, the improved metabolic performance and health status observed in treatments supplemented with *K. alvarezii* extract, at varying solvent ratios, are further supported by the water quality parameters measured during the study. Temperature, pH, and dissolved oxygen (DO) levels remained within the optimal range for the survival of Nile Tilapia.

Conclusions

This study investigated the antioxidant potential of ethanol extracts of *K. alvarezii*, prepared at various solvent ratios, to support its potential application as a feed additive in aquaculture. Chemical profile screening revealed that the ethanol extract was dominated by palmitic acid (hexadecanoic acid. The extract prepared at a 1:3 solvent ratio exhibited strong antioxidant activity, with an IC_{50} value of 34.66 ppm. The application of *K. alvarezii* ethanol extract in commercial fish feed over a 40-day rearing period had a positive influence on the growth performance and survival of Nile tilapia. Among the treatments, the 1:3 solvent ratio extract was the most effective, yielding

significantly higher absolute weight gain and specific growth rate, and achieving 100% survival. Additionally, this treatment improved the fish's physiological health, as indicated by elevated erythrocyte counts and hematocrit.

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References

Ambarwati N., Hidayati S., Mujtahidah T. (2022). Hematologic analysis of tilapia (*Oreochromis niloticus*) on ectoparasite infection of the floating net cages in Rawa Pening. Asian Journal of Aquatic Sciences, 5(1): 55-61.

Anova I.T., Yeni G. (2020). The solvent ratio of ethanol and ethyl acetate in the extraction process of the characteristics of the gambier. Jurnal Litbang Industri, 10(2): 121-127.

Araújo M.L., Rema P., Sousa-Pinto I., Cunha L.M., Peixoto M.J., Pires M.A., Seixas F., Brotas V., Beltran C., Valente L.M.P. (2015). Dietary inclusion of IMTA-cultivated *Gracilaria vermiculophylla* in rainbow trout (*Oncorhynchus mykiss*) diets: effects on growth, intestinal morphology, tissue pigmentation, and immunological response. Journal of Applied Phycology, 28(1): 679-689.

Ariano A., Musco N., Severino L., De Maio A., Tramice A., Tommonaro G., Damiano S., Genovese A., Olanrewaju O.S., Bovera F., Guerriero G. (2021). Chemistry of tropical *Eucheumatoids*: Potential for food and feed applications. Biomolecule*,* 11(6): 2-15.

Astika, Ilhamdy F.A., Putri S.M.R. (2022). Characterization of some seaweeds from Natuna waters as cosmetic preparations. Marinade, 5(2): 77-84.

Azeez A., Jainab B., Fathima A. (2020). Antioxidant activity and HPLC analysis of the marine algae *Halymenia dilata* as a potential fish feed for *Poecilia sphenops*. Plant Archives, 20(2): 6145-6150

Badarinath A.V., Rao K.M., Chetty C.M.S., Ramkanth S., Rajan T.V.S., Gnanaprakash K. (2010). A review on in-vitro antioxidant methods: comparisons, correlations and considerations. International Journal of PharmTech Research, 2(2): 1276-1285.

Bhuyar P., Rahim M.H., Sundararaju S., Maniam G.P., Govindan N. (2019). Antioxidant and antibacterial activity of red seaweed *Kappaphycus alvarezii* against pathogenic bacteria. Global Journal of Environmental Science and Management, 6(1): 1-12.

Biris-Dorhoi S.E., Michiu D., Pop C.R., Rotar A.M., Tofana M., Pop O.L., Socaci S., Farcas A. (2020). Macroalgae—A sustainable source of chemical compounds with biological activities. Nutrients, 12(10): 1-25.

Budiyanto, Kasim F., Abadi S.Y. (2019). Growth and carrageenan content of local and tissue culture seed of *Kappaphycus alvarezii* cultivated in floating cage. AACL Bioflux, 12(1): 167-178.

Cahyani H., Hardianti, Kurnia D.P., Aslan L.O.M. (2020). Cultivation of seaweed *Kappaphycus alvarezii* (Doty) Doty ex silva using tissue-cultured seedlings in encircling tank culture system. IOP Conference Series Earth and Environmental Science, 473: 012009: 1-8.

Carev I., Gelemanovic A., Glumac M., Tutek K., Dželalija M., Paiardini A., Prosseda G. (2023). *Centaurea triumfetii* essenial oil chemical composition, comparative analysis, and antimicrobial activity of selected compounds. Scientific Reports, 13(1): 1-13.

Chandimali N., Park E.H., Bak S.G., Lim H.J., Won Y.S., Lee S.J. (2023). Seaweed callus culture: A comprehensive review of current circumstances and future perspectives. Algal Research, 77(10): 1-9.

Djaeni M., Ariani N., Hidayat R., Utari F.D. (2017). Ultrasonic aided anthocyanin extraction of *Hibiscus sabdariffa* L. flower petal: Antioxidant activity. Jurnal Aplikasi Teknologi Pangan, 6(3): 148-151.

El-Beltagi H.S., Mohamed A.A., Mohamed H.I., Ramadan K.M.A., Barqawi A.A., Mansour A.T. (2022). Phytochemical and potential properties of seaweeds and their recent applications: A review. Marine Drugs, 20(6): 1-49.

Fayaz M., Namitha K.K., Murthy K.N.C., Swamy M.M., Sarada R., Khanam S., Subbarao P.V., Ravishankar G.A. (2005). Chemical composition, iron bioavailability, and antioxidant activity of *Kappaphycus alvarezii* (Doty). Journal of Agricultural and Food Chemistry, 53(3): 792-797.

Fratianni F., D'Acierno A., Ombra M.N., Amato G., De

Feo V., Ayala-Zavala J.F., Coppola R., Nazzaro F. (2021). Fatty acid composition, antioxidant, and in vitro anti-inflammatory activity of five cold-pressed *Prunus* seed oils, and their anti-biofilm effect against pathogenic bacteria. Frontiers in Nutrition, 8(775751): 1-13.

Fujaya Y., Azis H.Y., Hidayani A.A., Badraeni, Arifin N.H., Khor W., Fazhan H., Nurhidayat, Hardi E.H. (2022). Tilapia aquaculture water quality in response to the addition of fermented herbal extract. IOP Conference Series: Earth and Environmental Science, 1119(1): 012080:

Ganesan A.R., Tiwari U., Rajauria G. (2019). Seaweed nutraceuticals and their therapeutic role in disease prevention. Food Science and Human Wellness, 8(3): 252-263.

Hartika R., Mustahal, Putra A.N. (2014). Tilapia blood parameters with the addition of different dose of prebiotics in feed. Jurnal Perikanan dan Kelautan, 4(4): 259-267.

Ika F.S., Haeruddin, Mulyana W.O. (2023). Test antioxidant activity of ethyl acetate extract of startfruit leaves *(Averrhoa bilimbi L.)* with the DPPH method. Sains: Jurnal Kimia dan Pendidikan Kimia, 12(1): 41-49.

Jayasinghe G.D.T.M., Jinadasa B.K.K.K., Chinthaka S.D.M. (2018). Study on lipid content and fatty acid profile of four marine macro algae (seaweeds) collected from south east coast of Sri Lanka. Asian Journal of Chemistry and Pharmaceutical Sciences, 3(1): 1-6.

Kim D.H., Park M.H., Choi Y.J., Chung K.W., Park C.H., Jang E.I., An H.J., Yu B.P., Chung H.Y. (2013). Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-kB in the aged kidney. PLoS ONE, 8(3): 1-10.

Kumaresan S., Senthilkumar V., Stephen A., Balakumar B.S. (2015). GC-MS analysis and pass-assisted prediction of biological activity spectra of extract of *Phomopsis* sp. isolated from *Andrographis paniculata*. World Journal of Pharmaceutical Research, 4(1): 1035-1053

Lopes D., Rey F., Leal M.C., Lillebø A.I., Calado R., Domingues M.R. (2021). Bioactivities of lipid extracts and complex lipids from seaweeds: Current knowledge and future prospects. Marine Drugs, 19(12): 1-24.

Lugert V., Thaller G., Tetens J., Schulz C., Krieter J. (2016). A review on fish growth calculation: Multiple functions in fish production and their specific application. Reviews in Aquaculture, 8(1): 30-42.

Lumbessy S.Y., Abidin Z., Lestari D.P., Diamahesa W., Rahmadani T.B.C. (2024). Phytochemical screening of seaweed flour *(Eucheuma cottonii*) using various organic solvents and its application in tilapia feed (*Oreochromis niloticus*). Asian Journal of Fisheries and Aquatic Research, 26(11): 100-108.

Morais T., Inácio A., Coutinho T., Ministro M., Cotas J., Pereira L., Bahcevandziev K. (2020). Seaweed potential in the animal feed: A review. Journal of Marine Science and Engineering, 8(8): 1-24.

Muralidhar A.P., Syamala K., Prakash C., Kalidas C., Naik R.P. (2010). Comparative studies on fatty acid composition of three marine macroalgae collection from Mandapam region: South East coast of India. World Applied Sciences Journal, 11(8): 958-965.

Natarajan A., Prabakarakrishnan R., Govindaraju K., Sugumar V., Sathiskumar K., Narenkumar J., Ramanan A., Kumar B.S. (2023). Hepato and renoprotective activity of *Kappaphycus alvarezii* ethanolic extract in cisplatin causes hepatic and kidney harm in *Albino* Wistar rats. Aquaculture International, 31(4): 1925-1940.

Noviyanty A., Salingkat C.A., Syamsiar S. (2019). The effect of solvent type to the quality of red dragon fruit peel (*Hylocereus polyrhizus*) extracts. KOVALEN: Jurnal Riset Kimia, 5(3): 271-279.

Oke J.M., Hamburger M.O. (2002). Screening of some Nigerian medicinal plants for activity using 2,2-diphenyl-picrylhydrazyl (DPPH) radical. African Journal of Biomedical Research, 5(1): 77-79.

Pereira L., Valado A.M. (2021). The seaweed diet in prevention and treatment of the neurodegenerative diseases. Marine Drugs, 19(3): 1-25.

Podungge A., Damongilala L.J., Mewengkang H.W. (2017). Antioxidant activity of seaweed *Eucheuma spinosum* extracted with methanol and ethanol. Media Teknologi Hasil Perikanan, 6(1): 1-5.

Pratama I.S., Siahaan E.A., Anggorowati D.A., Putra Y., Sujangka A., Pangestuti R. (2022). Seaweed phytochemicals utilization in marine aquaculture. AACL Bioflux, 15(4): 1817-1836.

Purbomartono C., Husein A., Mulia D.S., Wuliandari J.R., Suwarsito. (2024). Effectiveness of dietary supplementation with Aloe vera and garlic (*Alium sativum*) on growth, hematology and immunity of North African catfish (*Clarias gariepinus*). AACL Bioflux, 17(6): 3076-3085.

Putra I.W.D.P, Dharmayudha A.A.G.O, Sudimartini L.M. (2016). Identification of chemical compounds ethanol extract leaf moringa (*Moringa oleifera* L) in Bali, Indonesia. Medicusveterinus, 5(5): 464-473.

Putra I.K.W., Putra G.P.G., Wrasiati L.P. (2020). The effect of ratio between material and solvent and maceration time on cocoa beans husk extract (*Theobroma cacao* L.) as a source of antioxidants. Jurnal Rekayasa dan Manajemen Agroindustri, 8(2): 167-176.

Rama R., Aslan L.O.M., Iba W., Nurdin A.R., Armin A., Yusnaeni Y. (2018). Seaweed cultivation of micropropagated seaweed (*Kappaphycus alvarezii*) in Bungin Permai coastal waters, Tinanggea Sub-District, South Konawe Regency. South East Sulawesi. IOP Conference Series Earth and Environmental Science, 175: 012219.

Rashad S., Rawash E.A., El-Chaghaby G. (2025). Investigation of the bioactive compounds, antimicrobial activity, and phyco-pigment contents of some microalgae extracts. Egyptian Journal of Aquatic Biology and Fisheries, 29(1): 613-634.

Reddy C.R.K., Yokoya N.S., Yong W.T.L., Luhan M.R.J., Hurtado A.Q. (2017). Micro-propagation of Kappaphycus and Eucheuma: trends and prospects. In: A.Q. Hurtado, A.T. Critchley, I.C. Neish (Eds.), Tropical seaweed farming trends, problems and opportunities, Springer. pp: 91-110.

Santos S.A.O., Félix R., Pais A.C.S., Rocha S.M., Silvestre A.J.D. (2019). The quest for phenolic compounds from macroalgae: A review of extraction and identification methodologies. Biomolecules, 9(12): 1-56.

Simanjuntak R., Husni S., Cahyadi J. (2022). Bioenrichment of papaya leaf meal (*Carica papaya*) with different feed formulations to increase Nile tilapia (*Oreochromis niloticus*) growth. IOP Conference Series: Earth and Environmental Science, 1083(1): 012089.

Siswadi, Saragih G.S. (2021). Phytochemical analysis of bioactive compounds in ethanolic extract of *Sterculia quadrifida* R.Br. AIP Conference Proceedings, 2353(1): 030098.

Subri M., Zin M. (2020). Antioxidative properties and proximate analysis of spent coffee ground (SCG) extracted using ultrasonic-methanol assisted technique as a potential functional food ingredient. Food Research, 4(3): 636-644.

Sudirman I., Syawal H., Lukistyowati I. (2021). Erythrocyte profile of *Cyprinus carpio* feed contains *Aeromonas hydrophila* vaccine. Ilmu Perairan (Aquatic Science), 9(2): 144-151.

Sunita A., Meena S. (2018). Pharmacological studies on flowers of *Ceropegia bulbosa* Roxb. var. *bulbosa* and *lushi*i (Grah.) Hook. F from Thar Desert of Rajasthan, India. Research Journal of Pharmacognosy and Phytochemistry, 10(3): 226-232.

Tamat S., Wikanta T., Maulina L. (2007). Antioxidant activity and toxicity of bioactive compounds from green seaweed extract *Ulva reticulata* Forsskal. Jurnal Ilmu Kefarmasian Indonesia, 5(1): 31-36.

Tanbiyaskur, Wijayanti M., Rarassari M., Mukti R. (2022). Total erythrocytes, hematocrit and survival rate of javan combtail (*Belontia hasselti*) feed with probiotic of swamp. Jurnal Ruaya: Jurnal Penelitian dan Kajian Ilmu Perikanan dan Kelautan, 10(2): 99-104.

Tiwari P., Kumar B., Kaur M., Kaur G., Kaur H. (2011). Phytochemical screening and extraction: a review. Internationale Pharmaceutica Sciencia, 1(1): 98-106.

Tripathy A. (2016). Oxidative stress, reactive oxygen species (ROS) and antioxidative defense system, with special reference to fish. International Journal of Current Research in Biosciences and Plant Biology, 3(10): 79-89.

Uma M., Jothinayaki S., Kumaravel S., Kalaiselvi P. (2011). Determination of bioactive components of *Plectranthus amboinicus* Lour by GC–MS analysis. New York Science Journal, 4(8): 66-69.

Valente L.M.P., Rema P., Ferraro V., Pintado M., Sousa-Pinto I., Cunha L.M., Oliveira M.B., Araújo M.L. (2015). Iodine enrichment of rainbow trout flesh by dietary supplementation with the red seaweed *Gracilaria vermiculophylla*. Aquaculture, 446: 132-139.

Vazirzadeh A., Marhamati A., Rabiee R., Faggio C. (2020). Immunomodulation, antioxidant enhancement and immune genes up-regulation in rainbow trout (*Oncorhynchus mykiss*) fed on seaweeds included diets. Fish and Shellfish Immunology, 106(6): 852-858.

Vijayalingam T.A., Rajesh N.V. (2019). Seagrasses as potential source of fodder for livestock: Complete proximate and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Annals of Phytomedicine An International Journal, 8(2): 93-98.

Wei C.C., Yen P.L., Chang S.T., Cheng P.L., Lo Y.C., Liao V.H.C. (2016). Antioxidative activities of both oleic acid and *Camellia tenuifolia* seed oil are regulated by

the transcription factor DAF-16/FOXO in *Caenorhabditis elegans*. PLoS ONE, 11(6): 1-15.

Witeska M., Kondera E., Lugowska K., Bojarski B. (2022). Hematological methods in fish – Not only for beginners. Aquaculture, 547: 1-17.

Wu H., Lu P., Liu Z., Sharifi-Rad J., Suleria H.A.R. (2022). Impact of roasting on the phenolic and volatile compounds in coffee beans. Food Science and Nutrition, 10(7): 2408-2425.

Yong S.Y., Yong W.T.L., Ng S.E., Anton A., Yassir S. (2014). Chemical composition of farmed and micropropagated *Kappaphycus alvarezii* (Rhodophyta, Gigartinales), a commercially important seaweed in Malaysia. Journal of Applied Phycology, 27(3): 1271-1275.