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Potential use of phytochemical from ethanolic extract of green seaweed *Ulva reticulata* in aquaculture

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Abstract. *Tarigan N, Sudrajat AO, Arfah H, Alimuddin A, Wahjuningrum D. 2023. Potential use of phytochemical from ethanolic extract of green seaweed* Ulva reticulata *in aquaculture. Biodiversitas 24: 6868-6879. Ulva reticulata* is green seaweed with potential phytochemical properties in the aquaculture sector. Therefore, this study aims to determine phytochemical components and evaluate the toxicity of *Ulva reticulata* ethanolic extract on aquatic animals. The primary constituents in the sample were extracted using the maceration method with 96% ethanol. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, while toxicity level was determined using the Brine Shrimp Lethal Test (BSLT) method at concentrations of 0, 10, 100, 300, 500, 1000, and 2000 mg L^{-1}. The results showed that *Ulva reticulata* ethanolic extract was qualitatively composed of alkaloids, flavonoids, saponins, tannins, phenols, and steroids with mid (++), low (+), low (+), low (+), low (+), and mid (++) activity levels, respectively. Based on quantitative analysis, the sample was composed of sitosterol (19.01 mg g^{-1}), stigmasterol (35.00 mg g^{-1}), saponins (17.04 mg g^{-1}), flavonoids (82.16 mg QE 100 g^{-1}), total phenols (144.00 g QE 100 g^{-1}), and tannins (49.03 mg g^{-1}). Furthermore, *Ulva reticulata* ethanolic extract had a strong antioxidant level with a 50% inhibitory concentration (IC_{50}) of 53.00 ppm. The toxicity test showed that the 50% lethal concentration (LC_{50}) of the sample was 481.86 mg L^{-1}. These results showed that *Ulva reticulata* ethanolic extract was a non-toxic material with a high potential for phytochemical properties that could be beneficial for fish growth, reproduction, and health.

Keywords: Antioxidant, ethanolic extract, phytochemicals, toxicity, *Ulva reticulata*

INTRODUCTION

In recent years, there has been an increase in the application of plant extract within the aquaculture sector for various purposes (Awad and Awaad 2017; Gabriel 2019; Lutfi et al. 2023). Furthermore, plant extract is often used as an alternative material, offering enhanced safety, economical feasibility, and environmentally friendliness compared to synthetic variants. Several studies have also shown that plant extract is capable of degrading naturally, thereby avoiding environmental pollution and detrimental effects on other organisms. This shows that their use can reduce the use of chemical materials and synthetic drugs in aquaculture (Abaho et al. 2022; Hoga et al. 2018; Reverter et al. 2014). The exploration of natural plants has been reported to gain widespread popularity (Moreira et al. 2022; Gao and Beardall 2022), particularly seaweed, which contains primary and secondary metabolites (Nielsen et al. 2021; Kamal et al. 2023; Hempel et al. 2023). According to previous studies, the primary metabolites in seaweed include proteins, lipids, carbohydrates, vitamins, and minerals, serving as alternative feed ingredients (Wan et al. 2019; Thiviya et al. 2022). Furthermore, this plant also contains secondary metabolites, including alkaloids, flavonoids, saponins, tannins, phenols, steroids/terpenoids, and antioxidants (Van Doan et al. 2019; Michalak et al. 2022; Abaho et al. 2022; Susanto et al. 2023; Lomartire and Gonçalves 2023). The value of these primary and secondary metabolites has been reported to exhibit variability among various species, geographical locations, and seasons (Park et al. 2022). A previous study reported that *Ulva lactuca* from Kukup Beach, Central Java, had primary metabolites, such as proteins (10.43%), lipids (5.17%), and carbohydrates (60.93%) (Da Costa et al. 2018). Another report stated that *Ulva reticulata* from Pattani waters in Thailand contained 21.06% protein, 0.75% lipids, and 55.77% carbohydrates (Ratana-arporn and Chirapar 2006). Moreover, the secondary metabolites in *Ulva intestinais* from Iran waters comprised 2.16 mgGA/g phenols, 11.7 mgQE/g flavonoid, 18.02 mg g^{-1} saponins, 30.23 mg g^{-1} tannins, and 1,63 mg g^{-1} steroid (Pirian et al. 2017).

Seaweed extract containing primary and secondary metabolites often has physiological effects on the growth, reproduction, and health of animals (Tarkowska 2019; Abo-Raya et al. 2021; Harikrishnan et al. 2021). This result is consistent with Akbary and Aminikhoei (2018), that alkaloids, flavonoids, and saponins from *Ulva rigida* extract have antimicrobial and antioxidant roles, which have positive effects on fish health and growth. In addition, steroids in seaweed, such as sitosterol and stigmasterols, positively impact animal reproduction. These compounds are typically converted to cholesterol, a key ingredient for the production of steroid hormone, which stimulates

reproduction (Nieminen et al. 2010; Tarkowská 2019; Arini 2021; Janeczko 2021). Moreover, antioxidative compounds in seaweed play an essential role in free radical inhibition, thereby improving fish growth and health performance (Harikrishnan et al. 2021). Based on these results, various species of the plant in Indonesian waters exhibit a high potential for aquaculture applications. *Ulva reticulata* is seaweed that can be used in the aquaculture sector. *Ulva reticulata* is abundantly found in several Indonesian waters, including Bali, East Nusa Tenggara, and Sulawesi, but it has not been used for aquaculture activities (Meiyasa et al. 2020). Several studies have reported that *Ulva rigida*, *Ulva chlorate*, *Ulva lactuca*, and *Ulva intestinalis* can serve as alternative nutritional feed ingredients, promoting improvement in fish growth, reproduction, and health (Corral-Rosales et al. 2019; Van doan et al. 2019; Klongklaew et al. 2021; Rahim et al. 2021; Ridwanudin et al. 2022). The use of this species has been confirmed to be non-toxic for fish physiology (Madibana et al. 2017). Despite its importance, there is limited information on the use of *Ulva reticulata* ethanolic extract and its toxicity level. This shows that further investigations regarding the toxicity level of *Ulva reticulata* ethanolic extract are necessary. Toxicity level in ethanolic extract can be measured using the Brine Shrimp Lethality Test (BSLT) (Meyer et al. 1982; Fauziah et al. 2022).

The BSLT method serves as an initial stage in determining the toxicity level of plant extract, facilitating the identification of toxins levels. The results are often presented as LC_{50} (*Lethal Concentration* 50) (Aulia et al. 2023), which is determined by observing brine shrimp (*Artemia salina*). In previous studies, shrimp is an alternative animal for assessing the toxicity level of natural materials using simple and cost-effective methods (Parra et al. 2001). Brine shrimps are also highly sensitive and typically die after being exposed to toxic natural ingredients (Jelita et al. 2020). The BSLT method for plant extract is considered to be more responsive in detecting toxicity (Mirzae and Mirzae 2013). Therefore, this study aims to identify phytochemical components and their toxicity levels in *Ulva reticulata* ethanolic extract as basic information for further use in the aquaculture sector.

MATERIALS AND METHODS

Sample source

Seaweed samples were obtained from the Moudolung waters of the East Sumba District, East Nusa Tenggara, Indonesia (Figure 1). Furthermore, the samples were collected from nature in January 2023 at a weight of 20 kg. Sampling was carried out by exploring the coastal waters of Moudolung from the edge to the edge of the beach at low tide. The samples obtained were cleaned using clean water and stored in plastic, followed by morphological identification at the Integrated Laboratory for Oceanography Research, National Bureau of Research and Innovation (BRIN), Ancol, Jakarta. Seaweed used in this study was *Ulva reticulata* Forsskal, 1775 as shown in Figure 2.

Procedures

Proximate analysis Ulva reticulata

The dried *Ulva reticulata* samples (20 g) were analyzed for their nutrient contents using proximate analysis, containing proteins, lipids, carbohydrates, moisture, and ash. The protein content was measured using the Micro-Kjeldahl method; lipid content was measured using the Soxhlet method; carbohydrate content was determined using the by-difference method; and moisture and ash contents were determined following the AOAC method (Da Costa et al. 2018).

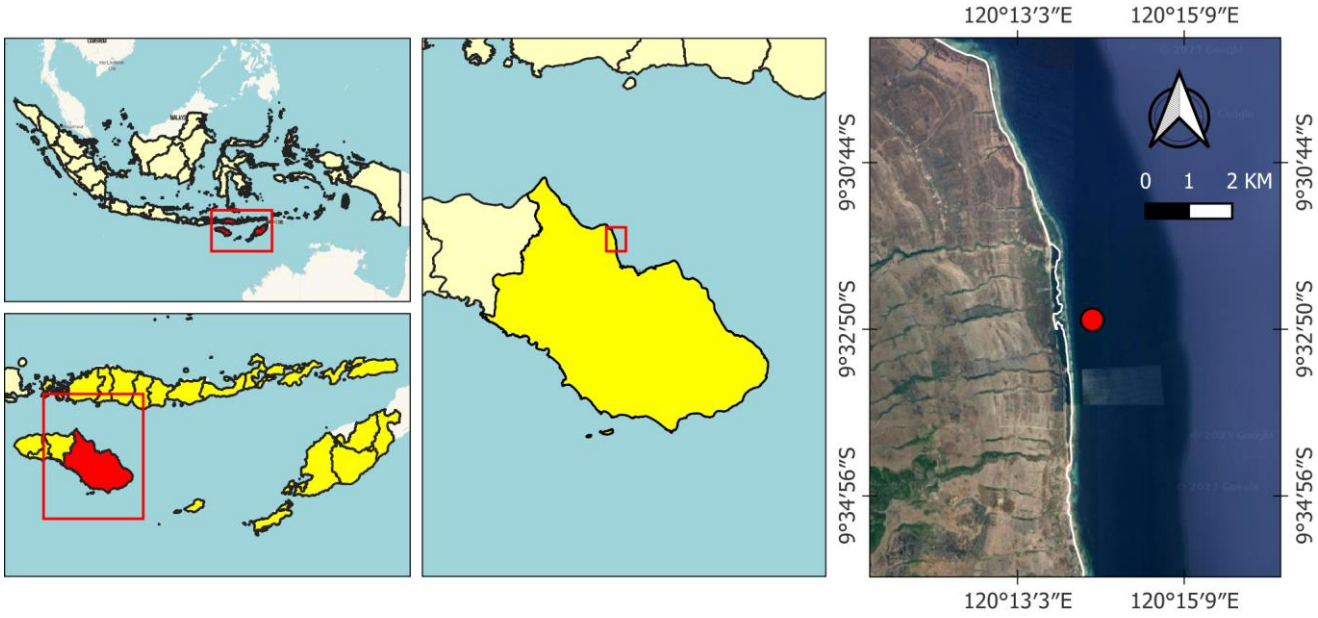

Figure 1. Sampling location for *Ulva reticulata* in Moudolung waters, East Sumba, East Nusa Tenggara, Indonesia

Figure 2. Morphology of the green seaweed *Ulva reticulata* Forsskal, 1775

Ulva reticulata ethanolic extract

The *Ulva reticulata* was extracted using the maceration method, following Zaghbib et al. (2022), with ethanol 96% solvent at a 1:15 ratio. The simplicial *Ulva reticulata* was dried at 10 g, crushed, and transferred to a dark glass container filled with 150 mL of 96% ethanol. Maceration was performed for five days (5×24 h) with ethanol solvent at room temperature and 21°C under darker conditions. The extraction product was filtered through 0.2 Whatman paper and concentrated using a rotary evaporator at 40°C (Yao et al. 2022). After the extraction process, the yield was determined based on the ratio of the extract weight to the simplicial *U. reticulata* weight as the initial sample weight (Vega et al. 2020).

Yield (%) = [Extract weight (g) / Initial sample weight (g)] × 100

Qualitative screening phytochemical analysis

Phytochemical screening of *Ulva reticulata* ethanolic extract was performed qualitatively to identify the secondary metabolites, namely alkaloids, flavonoids, phenols, saponins, tannins, and steroids/terpenoids, using the Harborne (1998) method. Phytochemical screening results are presented as non-detected (-), weak (+), moderate (++), and strong (+++) symbols. Alkaloid analysis was performed by adding 1 mL of the sample dissolved in a few drops of 2N sulfuric acid. Subsequently, the mixed solution was tested with Dragendorff's reagent, Mayers's reagent, and Wagner's reagent to determine the presence of alkaloids in the sample. A positive test result for Dragendorff's reagent was a red-orange precipitate. A yellowish-white precipitate was obtained using Mayer's reagent as a positive test result. A brown precipitate indicated a positive result when Wagner's reagent was used. Flavonoid testing was performed by adding a few drops of concentrated HCl and Mg powder to the sample. If the color changes to orange and foam is formed, the sample is positive for flavonoid compounds. Then, the saponin phytochemical compounds were tested by dissolving the sample in 2 ml of hot water. If a stable foam was formed after 30 min, the sample was positive for saponin compounds. The phenolic/tannin content test was performed by mixing the sample with $FeCl_3$. If a blackish-purple color is formed, the sample contains tannin compounds. Finally, qualitative steroids/terpenoids compounds were analyzed by dripping the Lieberman-Burchard reagent into the sample. A purple-red color reaction indicated a positive result for terpenoids, whereas a blue-green color reaction indicated a positive result for steroids.

Quantitative phytochemical analysis

Quantitative phytochemical analysis was performed at the Center for Spices and Medicinal Plants, Agency for Agricultural Research and Development Laboratory, and Laboratory for Analysis, Center for Agricultural Postharvest Research and Development, Indonesian Ministry of Agriculture. The analysis was performed using a standard thin-layer chromatography (TLC) Scanner. TLC scanner is a chromatographic method that uses a thin stationary phase supported by an inert layer to separate the mixture. Silica gel G_{60} thin TLC plates were activated in a 50°C oven for 30 minutes. A capillary tube was used to apply the 25 µL sample to the TLC plate. Each 25 µL extract was dissolved in ethanol and transferred onto the TLC plate using a capillary tube. After developing the TLC in the mobile phase, the TLC plate was sprayed with vanillin sulfate reagent (Indriaty et al. 2023).

Phytochemical characterization with GC-MS (Gas Chromatography and Mass Spectrometry)

The compound components of *Ulva reticulata* extract were identified using the GC-MS. GC-MS analysis was performed in the Laboratory of Forensics, Police Headquarters, Sentul, Bogor, Indonesia. The GC-MS analysis procedure followed that of Masyudi et al. (2022). The GC-MS analysis of *Ulva reticulata* extract was performed using the GC-MS with Auto Sampler 5975A (Agilent Technologies 7890A), Mass Selective Detector, and data system in Chemstation. The sample was dissolved in methanol p.a., followed by the injection of 5 µL into the GC-MS using helium (He) gas through a capillary column with a 1.2 mL/minute rate and a split ratio of 8:1 psi. The injector and detector were programmed at 250°C and 230°C, with operating temperatures of 280°C and 140°C, respectively. The National Institute of Standards and Mass Spectral Technology (NIST-MS) spectrometer database interpreted the mass spectrum fragmentation pattern.

Antioxidant analysis with DPPH (2,2- diphenyl-1-picrylhydrazyl) method

The antioxidant analysis procedure was performed using a UV-Vis spectrophotometer at a wavelength of 517 nm. This analysis was carried out based on a study by Hidayat et al. (2020). Furthermore, *Ulva reticulata* extract solution was prepared at various concentrations (20, 40, 60, 80, and 100 mg/L). Trolox, as a positive control, was prepared at concentrations of 20, 40, 60, 80, and 100 mg/L. The samples were pipetted and added to the DPPH solution at a 1:4 ratio in 96-wells of a clear polystyrene microplate

until homogenous. The mixture obtained was incubated at 37°C for 30 minutes, and the absorbance was measured using a microplate reader at a wavelength of 517 nm.

Toxicity test of Ulva reticulata ethanolic extract

Toxicity analysis of *Ulva reticulata* ethanolic extract was performed using the BSLT method (Fauziah et al. 2022). The BSLT analysis was used to determine toxicity of bioactive compounds from *Ulva reticulata* extract at various concentrations, including 0, 10, 100, 300, 500, 1000, and 2000 mg/L, with 4 replications. Extract concentration test was carried out using brine shrimp (*Artemia salina*) with 10 mL of seawater as the medium. Brine shrimp larvae (0.1 g) were incubated for 48 h in a conical container at 28-30°C. Furthermore, this container was equipped with aeration and a 40-watt lamp. A total of 20 brine shrimp larvae were harvested by separating them from shells, followed by transfer into a vial filled with 4 mL of seawater using a dropper based on *Ulva reticulata* extract concentration. The numbers of dead and live brine shrimp larvae were counted after 24 h. The LC_{50} concentration was determined by calculating brine shrimp larvae mortality using the formula proposed by Awaludin et al. (2020).

$$Y = a + Bx$$

Where: Y = 50, and the X = LC_{50}

Data analysis

Qualitative and quantitative phytochemical tests and GC-MS characterization were analyzed descriptively and are presented in figures and tables. The DPPH-antioxidant activity was analyzed descriptively, while the BSLT analysis used probit analysis with SPSS 22.0.

RESULTS AND DISCUSSION

Proximate content

The proximate analysis results for simplicial *Ulva reticulata* from Moudolung-Sumba waters, East Nusa Tenggara, showed 15.24% moisture, 23.10% ash, 0.33% lipid, 10.08% protein, 33.82% carbohydrates (Table 1). This result was inconsistent with Anh et al. (2020), who found that *Ulva reticulata* from Vietnam waters had 1.8% protein, 10.6% lipid, 65.5% carbohydrates, 19.20% moisture, and 10.6% ash. Furthermore, samples from Thai waters contained 21.06% protein, 0.75% lipid, 55.7% carbohydrates, 22.51% moisture, and 17.58% ash (Ratana-Arporn and Chirapart 2006). This showed that *Ulva reticulata* from different waters had varying proximate contents. Liminana et al. (2023) reported that variations in content were influenced by location, geographical location, season, and environment. Based on the proximate analysis results, the sample had a moderate amount of protein, low lipid, carbohydrate, and high ash content. Saleh (2020) also stated that the amount of seaweed nutrients contained moderate and high protein by 10-47%, low lipid at 0-3%, high carbohydrates at 30-60%, and ash content at 7-38%. The nutrient components had potential to be used as ingredients for fish feed to meet nutritional needs. This condition was similar to that of Øverland et al. (2019), who reported the use of seaweed with high protein and carbohydrate content as nutritional ingredients. The protein content of *Ulva* sp. consisted of several non-essential amino acids, such as aspartic acid, glutamic acid, alanine, histidine, arginine, leucine, and threonine, which had potential to be used as fish feed (Brien et al. 2022; Thiviya et al. 2022). Several amino acids, including alanine, histidine, arginine, and lysine, were the most important nutrients in feed development that supported the nutrient requirements (Santiago and Lovell 1988; Miranda et al. 2017; Li et al. 2021). The high carbohydrate content in *Ulva reticulata* could be used in the manufacture of feed to support fish growth. Suryaningrum and Samsudin (2020) reported that it had potential to be developed as an alternative fish feed supplement. Several types of *Ulva* spp. had been used to support the growth of aquatic organisms. In a previous study, *Ulva lactuca* had been developed as a supplement for catfish and tilapia (Abdel-Warith et al. 2016; Mahasu et al. 2016), while *Ulva intestinalis* was explored used as an ingredient for tilapia (Siddik et al. 2015). *Ulva reticulata* had also been reported to be a supplement that supported the growth and health of goldfish (Rama et al. 2014). Based on these results, the nutrient components of *Ulva reticulata* had potential to be used as fish feed supplements.

Yield percentage

Yield was a ratio of extract weight to the simplicial weight, and extract yield was often used to determine the amount of extract obtained during extraction (Yainahu et al. 2023). A total of 1000 g of *Ulva reticulata* extract processed using the maceration method for 5 days with 96% ethanol obtained a yield value of 24.49% (Table 1). The value of ethanolic extract of *Ulva reticulata* was higher compared to *Ulva lactuca* extract using 96% ethanol after 2 days of maceration (13.54%) (Arbi et al. 2016). Furthermore, the yield of *Ulva rigida* using 95% ethanol with a 3-day maceration time was higher compared to this present study (26.08%) (Wulanjati et al. 2020). The yield of *Ulva lactuca* extract using 99% ethanol with a 4-day maceration time was 27.38% (Nufus and Nurjanah 2017). The value was lower compared to that of *Ulva reticulata* extract with a 4-day maceration time using 96% ethanol at 70.11% (Panjaitan et al. 2022). This showed that the yield of *Ulva extract* varied across various studies. These differences were caused by variations in simplicial types, geographical locations, temperatures, and extraction times. This theory was in line with Aminudin et al. (2020) that the simplicial types and extraction duration affected the yield value.

Qualitative screening and quantitative phytochemical contents

Based on the qualitative phytochemical screening, *Ulva reticulata* ethanolic extract from Moudolung waters, East Sumba, contained bioactive compounds, including alkaloids, flavonoids, saponins, tannins, phenols, and

steroids/terpenoids (Table 2). The results showed that flavonoids, saponins, tannins, and phenols had weak activity (+), while alkaloids and steroids/terpenoids were classified as moderately active (++). This phytochemical screening was different from Widyaningsih et al. (2016), that *Ulva lactuca* ethanolic extract from Drini Waters, Gunung Kidul, Yogyakarta-Indonesia, contained flavonoids and alkaloids with weak activity (+), while saponins, steroids and tannins had no activity (-). In addition, phytochemical content of the sample from Cemara Sewu waters, Yogyakarta, contained alkaloids, flavonoids, saponins, tannins, phenols, and steroids with weak activity (+) (Panjaitan et al. 2022). *Ulva reticulata* acetone extract from Indian waters had phenols and tannins with relatively weak activity (+) as well as steroids/terpenoids with moderate activity (++), while alkaloids and saponins were not found active (Jeeva et al. 2012). This condition showed that phytochemical compounds in seaweed differed between locations. These variations could be attributed to the location and solvent type used in extraction process. The quantitative phytochemical content of *Ulva reticulata* ethanolic extract using TLC also had varying values when compared to various types of seaweed extract, as shown in Table 3.

As shown in Table 3, the quantitative phytochemical content of Ulva *reticulata* ethanolic extract using a TLC scanner showed the presence of sitosterol at 19.01 mg g^{-1}. This sitosterol value was greater compared to *Desmarestia antarctica* hexane extract at 5.29 mg g^{-1} (Pereira et al. 2017). However, it was still lower than that of the chloroform extract at 38.00 mg g^{-1} (Serviere-Zaragoza et al. 2021), *Melastoma malabathricum* ethanolic extract at 160.80 mg g^{-1} (Awaludin et al. 2020), and *Biophytum umbraculum* ethanolic extract at 26.80 mg g^{-1} (Lutfi et al. 2023).

Table 1. Chemical composition and yield value of *Ulva reticulata*

Parameter	Composition (%)
Moisture	15.24
Ash	23.1
Lipid	0.33
Protein	10.68
Carbohydrates	33.82
Yield*	24.49

Note: **Ulva reticulata* ethanolic extract

Table 2. Qualitative screening phytochemical on *Ulva reticulata* ethanolic extract

Bioactive compounds	Results
Alkaloids	++
Flavonoids	+
Saponins	+
Tannins	+
Phenols	+
Steroids/terpenoids	++

Note: (+) weak; (++) moderate

In addition to sterols, *Ulva reticulata* ethanolic extract also contained stigmasterol at 35.00 mg g^{-1}. This stigmasterol content had a greater value than *Desmarestia antarctica* hexane extract at 0.02 mg g^{-1} (Pereira et al. 2017), *Ulva lactuca* chloroform extract at 20.00 mg g^{-1} (Serviere-Zaragoza et al. 2021), and *Biophytum umbraculum* ethanolic extract at 24.60 mg g^{-1} (Lutfi et al. 2023). The results showed that the sitosterol and stigmasterol compounds had varying content levels in different plants. The value of sitosterol and stigmasterol compounds in plants was influenced by several factors, including plant type, solvent type, location, and seasonal changes. According to Serviere-Zaragoza et al. (2021), seasonal changes greatly affected sterol and stigmasterol contents in seaweed.

Ulva reticulata ethanolic extract also contained saponins at 17.04 mg g^{-1}, which was lower compared to *Melastoma malabathricum* ethanolic extract at 114.60 mg g^{-1} (Noviyanty et al. 2020), *Sargassum vulgare* chloroform extract at 19.31 mg g^{-1} (Abu-ahmed et al. 2021), *Ulva lactuca* methanolic extract at 17.70 mg g^{-1} (Pappou et al. 2022), and *Biophytum umbraculum* ethanolic extract at 17.10 mg g^{-1} (Lutfi et al. 2023). However, this value was different from that of the flavonoids and phenolic compounds in *Ulva reticulata*. The flavonoid content of *Ulva reticulata* ethanolic extract was 82.16 mg QE g^{-1}, which was higher compared to *Sargassum vulgare* at 67.09 mg QE g^{-1} (Abu-ahmed et al. 2021), *Ulva lactuca* methanolic extract at 59.49 mg QE g^{-1} (Pappou et al. 2022), and *Biophytum umbraculum* ethanolic extract at 2.50 mg QE g^{-1} (Lutfi et al. 2023). A similar condition was also observed for total phenols in *Ulva reticulata* ethanolic extract, which was obtained at 144.00 mg GAE 100 g^{-1}. This value was lower than in *Sargassum vulgare* chloroform extract at 918 mg GAE 100 g^{-1} (Abu-ahmed et al. 2021) and *Biophytum umbraculum* ethanolic extract at 531.87 mg GAE 100 g^{-1}. However, the total phenol value produced in *Ulva reticulata* ethanolic extract was greater than in *Ulva lactuca* at 45.32 mg GAE 100 g^{-1} (Pappou et al. 2022) and *Melastoma malabathricum* at 2.47 mg GAE 100 g^{-1} (Awaludin et al. 2020). The total tannin value in the sample was 49.03 mg g^{-1}, which was greater than that of *Sargassum vulgare* chloroform extract at 34.00 mg g^{-1} (Abu-ahmed et al. 2021), *Ulva lactuca* at 22.52 mg g^{-1} (Pappou et al. 2022), and *Biophytum umbraculum* at 15.37 mg g^{-1} (Lutfi et al. 2023). The total value of phytochemical contents in saponins, flavonoids, phenols, and tannins varied among different plant species. This condition was caused by variations in the plant type, sampling location, and solvent type used during extraction. This condition was similar to that of Tarakanita et al. (2020), who found that variations in plant phytochemical content were influenced by sampling location and plant type.

Phytochemical compounds in *Ulva reticulata* ethanolic extract had potential to be used in aquaculture sector, as supported by Lutfi et al. (2023), who focused on phytochemical content derived from plant extract. The results showed that *Ulva reticulata* ethanolic extract contained sterols, including sitosterol and stigmasterol. These sterols had chemical structures similar to cholesterol,

which was a precursor of steroid hormones in animals. Tarkowska (2019) found that the stigmasterol found in plants had a structure similar to that of cholesterol, which was the main ingredient in the biosynthesis of steroid hormones. Furthermore, sterols derived from plants were converted into cholesterol and later became pregnenolone through cytochrome P450 enzymes, which led to the biosynthesis of steroid hormones, including testosterone and estrogen (Lafont et al. 2021). The sitosterol and stigmasterol contained in *Ulva reticulata* ethanolic extract were thought to play a role in cultured fish reproduction. Previous studies had reported the use of several *Ulva* species in animals. Corral-Rosales et al. (2019) stated that the administration of *Ulva clathrata* could increase the steroid components in shrimp (*Litopenaeus vannamei*), while *Ulva lactuca* extract in rabbits could increase the concentration of testosterone and sperm motility (Okab et al. 2013).

Ethanolic extract of *Ulva reticulata* contained saponins, which were phytochemical components that met the nutrient requirements of animals. These compounds exhibited antioxidant, immunostimulatory, and anti-inflammatory activities, which had beneficial effects on animal growth and health. A similar condition was reported by Chakraborty et al. (2014), who reported that saponins had antioxidant, immunostimulatory, and anti-inflammatory roles in animal physiological processes. Furthermore, Zhang et al. (2022) reported that plants containing these compounds could be used as immunostimulants, antivirals, and antibacterials to improve the health status and growth of goldfish. Saponins also played a role in fish reproduction and could induce luteinizing hormone (LH) release. Several studies had shown that the compounds were reported to affect the release of reproductive hormones, namely LH from the pituitary during the reproductive phase for oocyte maturation and ovulation (Makkar et al. 2007).

In addition to saponins, polyphenolic compounds had also been found in ethanolic extract of *Ulva reticulata*, including flavonoids and phenols. These compounds had potential to be used in aquaculture, as well as antioxidative and anti-inflammatory properties that could be used for dietary supplementation of fish feed. Polyphenols also exhibited protective effects against lipid oxidation and free radicals, which affected the growth, reproduction, and health of fish. This condition was supported by Ahmadifar et al. (2021), who reported that plants containing polyphenols could be used as functional feed additives. These plants often had antioxidant and anti-inflammatory roles and could affect growth performance, reproductive performance, and health status. Polyphenols played a role in preventing lipid oxidation to protect the cell membranes from free radicals (Purbosari et al. 2021)

Ethanolic extract of *Ulva reticulata* also contained tannins, which were water-soluble polyphenols found in plant extract. These compounds derived from plants served as antioxidants that supported protein digestion and animal growth (Delimont et al. 2017). Furthermore, tannins had a synergistic relationship with other phytochemical compounds, which served as antioxidants. In previous studies, these compounds also had potential to support the quality of spermatozoa (Aminudin et al. 2020). This showed that tannins derived from *Ulva reticulata* ethanolic extract had potential to be used in aquaculture. Abaho et al. (2022) reported that bioactive components, such as flavonoids, tannins, terpenoids, alkaloids, and steroids in plants worked synergistically to support androgenic and anabolic processes, along with stimulating digestion, appetite, and immunity in fish. Use of plant extract compounds had a positive effect on fish growth, reproduction, and health (Chakraborty et al. 2014; Corral-Rosales et al. 2019; Abo-Raya et al. 2021). The presence of phytochemical compounds in plants could stimulate the immune system and improve growth performance (Beltran and Esteban 2022). As shown in Table 3, *Ulva reticulata* ethanolic extract had a relatively higher phytochemical content, including stigmasterol, flavonoids, and tannins, when compared to extract that had been studied. Based on these results, *Ulva reticulata* ethanolic extract could potentially be used in aquaculture to support fish growth, reproduction, and health.

Determination of phytochemical compound in extract *Ulva reticulata* using GC-MS

The identification of phytochemical compounds using GC-MS was a method for quantitatively determining unidentified components (Olivia et al. 2021). The results of the process for ethanolic extract of *Ulva reticulata* showed 9 types of compounds with different retention times (RT) and area percentages, as shown in Table 4. These components included palmitic acid (22.11%), 1,2-benzenedicarboxylic acid (5.16%), 9,12,15-octadecatrienoic acid (3.05%), hexadecanoic acid (2.28%) belonging to the fatty acid group. Other compounds included 3,7,11,15-tetramethyl-2-hexadecen-1-ol- (8.63%), neophytadiene (5.91%), and phytol (2.72%) belonging to the terpenoid component, 1H- Benzimidazole (4.17%) categorized as a phenol, and 9,19-Cyclolanost-24-en-3-ol acetate (4.00%) belonging to the sterol component. Several compounds identified from *Ulva reticulata* ethanolic extract had potential for use in aquaculture sector, particularly for fish production (Alagawany et al. 2021). Yu et al. (2020) suggested that palmitic acid was a saturated fatty acid that played a role in intracellular signaling and improved fish health status. Kumar et al. (2020) also reported that it could serve as a precursor for binding ligands to various cellular processes and modulating fish growth and health. Furthermore, fatty acid components also played a role in reproduction. Zeng et al. (2023) added that these components played a role in increasing FSH and LH hormone precursors through molecular mechanisms, which could trigger follicular development, as well as facilitate oocyte maturation, and embryo development in animals. Terpenoid compounds were one of the bioactive components of seaweed, which had a role in animal health and reproduction.

Table 3. Quantitative phytochemical compounds of *Ulva reticulata* ethanolic extract using a TLC scanner compared to those of several phytochemical plants

Compounds	Phytochemical plants					Functions	References
	***Ulva reticulata* (*This research*)**	***Biophytum umbraculum* (Lutfi et al. 2023)**	***Melastoma malabathricum* (Awaludin et al. 2020; Noviyanty et al. 2020)**	***Sargassum vulgare* (Pereira et al. 2017; Abu Ahmed et al. 2021)**	***Ulva lactuca* (Pappou et al. 2022; Serviere-Zaragoza et al. 2021)**		
Sitosterol	19.01	26.80	160.80	5.29	38.00	Animal reproduction	Nieminen et al. 2010; Chakraborty et al. 2011
Stigmasterol (mg g^{-1})	35.00	24.60	-	2.69	20.00	Fish reproduction	Lafont et al. 2021; Tarkowská 2019
Saponins (mg g^{-1})	17.04	17.10	114.60	19.31	17.70	Fish reproduction, growth, and health	Chakraborty et al. 2014; Makkar et al. 2007
Flavonoids (mg QE 100 g^{-1})	82.16	2.50	1.63	67.09	59.49	Fish health	Purbosari et al. 2021
Total phenols (mg GAE 100 g^{-1})	144.00	531.87	2.47	918.00	45.32	Fish growth and health	Purbosari et al. 2021; Pappou et al. 2022
Tannins (mg g^{-1})	49.03	15.37	0.01	34.00	22.52	Fish growth	Beltran and Esteban 2022

Table 4. Phytochemical compounds of *Ulva reticulata* ethanolic extract using GC-MS analysis

Compound	Component group	RT	Relative area (%)	Potential compound function utilization on cultivation (References)
9,12,15-Octadecatrienoic acid	Fatty acid (omega 6)	15.71	3.05	Fish Reproduction (Lutfi et al. 2023) and nutrition (Awaludin et al. 2020)
Palmitic acid	Fatty acid	14.77	22.11	Fish growth and health (Librán-Pérez et al. 2019) and fish reproduction (Hilbig et al. 2019)
1,2-Benzenedicarboxylic acid	Fatty acid	19.47	5.16	Fish reproduction (Lutfi et al. 2023) and antioxidant (Beulah et al. 2018)
Hexadecanoic acid,	Fatty acid	14.00	2.28	Fish nutrition (Glencross 2009) and health (Librán-Pérez et al. 2019)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	terpenoids	13.57	8.63	Fish health (Sutili et al. 2018)
Neophytadiene	terpenoids	13.13	5.91	Fish health (Chakraborty et al. 2011)
Phytol	Terpenoids	15.81	2.72	Fish health (Hoseini et al. 2021)
9,19-Cyclolanost-24-en-3-ol acetate	Sterol	5.36	4.00	Fish reproduction (Awaludin et al. 2020)
1H-Benzimidazole	Phenol	27.73	4.17	Fish health (Hoseini et al. 2021; Alagawany et al. 2020)

Hoseini et al. (2021) reported that terpenoids could stimulate the activity of antioxidant enzymes to suppress oxidative stress, thereby affecting fish growth and health. Furthermore, these compounds, such as phytol, also served as precursors in the synthesis of vitamins E and K (Awaludin et al. 2020). In addition to terpenoids, sterol and phenol groups were found in ethanolic extract of *Ulva reticulata*. Sterols were a group of steroid alcohols with chemical structures similar to those of cholesterols (Chakraborty et al. 2014). The presence of these components in plant extract influenced the production of reproductive hormones in animals. Abaho et al. (2022) and Ghosal et al. (2015) supported that sterols derived from plants affected the secretion of estrogen and testosterone hormones through the aromatase enzyme action.

Phenol compounds were a group of small molecules in plants that contained several simple compounds. Phenolics also served as immunostimulants and antioxidants in fish production (Naiel et al. 2021). Information on the use of plants containing phenolic compounds in fish production had been widely reported. These compounds had been shown to function as antioxidants through lysozyme and complement pathway activities, thereby affecting production performance and health (Alagawany et al. 2020; Hosseini et al. 2021). Based on these results, phytochemical compounds in *Ulva reticulata* ethanolic extract could be used as alternative ingredients that affected fish growth, reproduction, and health.

Antioxidant activity

Based on the results of the DPPH method on *Ulva reticulata* ethanolic extract, an IC_{50} value of 53.00 ppm was obtained, showing strong antioxidant activity. Hamit et al. (2022) reported that the antioxidant activity of *Ulva* sp. species was strong with IC_{50} of 50-100 ppm, while intermediate and weak activities were showed by values of 100-150 ppm and 151-200 ppm, respectively. Furthermore, a smaller IC_{50} value showed that the activity was higher (Nufus and Nurjanah 2017). The value obtained in this study was higher than *Ulva lactuca* extract with an IC_{50} value of 46.68 ppm (Rompas and Gasah 2022). The antioxidant activity of seaweed was more stable, showing its potential as a natural antioxidant (Kumar et al. 2021). Based on these results, natural antioxidant activity derived from seaweed could prevent the production of free radicals in animals. Kumar et al. (2022) reported that antioxidant compounds derived from plants could be used to prevent free radicals in fish. Furthermore, Abo-Raya et al. (2021) reported that 100 mg kg^{-1} of *Ulva fasciata* extract could improve health status, antioxidant activity and growth in tilapia fish. A strong activity was often quickly absorbed in the body to inhibit the occurrence of these compounds in cellular structures, such as DNA, proteins, and lipids, during cell metabolism processes (Michalak et al. 2022). Free radicals or reactive oxygen species (ROS) were highly reactive compounds that attacked biological molecules, such as lipids, proteins, enzymes, DNA, and RNA. These compounds typically caused cell and tissue damage, thereby affecting physiological and pathological activities in animals (Tziveleka et al. 2021). Antioxidants inhibited ROS by increasing antioxidant enzyme activity, inhibiting lipid peroxidation, and reducing oxidation through metal ion chelation (Yan et al. 2020). Furthermore, antioxidant compounds in the body regulated the activity of enzymes, such as superoxide dismutase (SOD), glutathione transferase (GST), and glutathione peroxidase (GSH-Px), to reduce structural and functional damage caused by oxidative damage to the mitochondria (Surai 2020). Previous studies had also reported that natural compounds from seaweed could be applied to fish. The antioxidant activity of green seaweed could reduce malondialdehyde (MDA) levels and increase growth, hematological, antioxidant enzymes, and the immune system in striped catfish (*Pangasianodon hypothalamus*) and rainbow trout (*Oncorhynchus mykiss*) (Abdelhamid et al. 2021; Kiadaliri et al. 2020). The components had also been found to act on various reproductive organs, including the hypothalamus and pituitary. Antioxidant activity affected reproductive hormones by binding to and activating the estrogen receptors (Cipolletti et al. 2018). Furthermore, it regulated animal reproduction through the modulation of neurohormones (GnRH) and gonadotropins, including follicle-stimulating hormone (FSH), LH, and steroids (estrogen, testosterone, and prostaglandins) (Hashem et al. 2020). Based on the results, natural antioxidants in *Ulva reticulata* extract had potential as a dietary supplement to improve fish growth, reproduction, and health (Gunathilake et al. 2022).

Toxicity activity analysis

Toxicity test of the plant extracts was carried out as a preliminary test in the use of natural materials to determine the appropriate concentrations of natural ingredients. This tests could be used to obtain basic information for selecting long-term doses in animal (Maheshwari and Shaikh 2016). Futhermore, Figure 3 showed that variations in *Ulva reticulata* ethanolic extract concentration affected the mortality rate of brine shrimp larvae. This condition led to an increased mortality rate andalong with an elevated ethanolic extract. These results were similar to those of Awaludin et al. (2020), who reported that the higher the plant extract concentration, the higher the mortality rate of shrimp larvae.

The probit analysis results for *Ulva reticulata* ethanolic extract showed an LC_{50} value of 481,865 mg/L, which could be categorized as a low toxicity value. This condition was supported by Meyer et al. (1982), who found that compounds in plant extracts had a toxic effect if the LC_{50} value was below 1000 mg/L, while no toxic effect was present if the LC_{50} value was above 1000 mg/L. These results showed that *Ulva reticulata* ethanolic extract had no toxic effects. Furthermore, the highest dose of extract led to a low mortality rate in brine shrimp larvae. Therefore, the use *Ulva reticulata* ethanolic extract was safe for aquaculture applications.

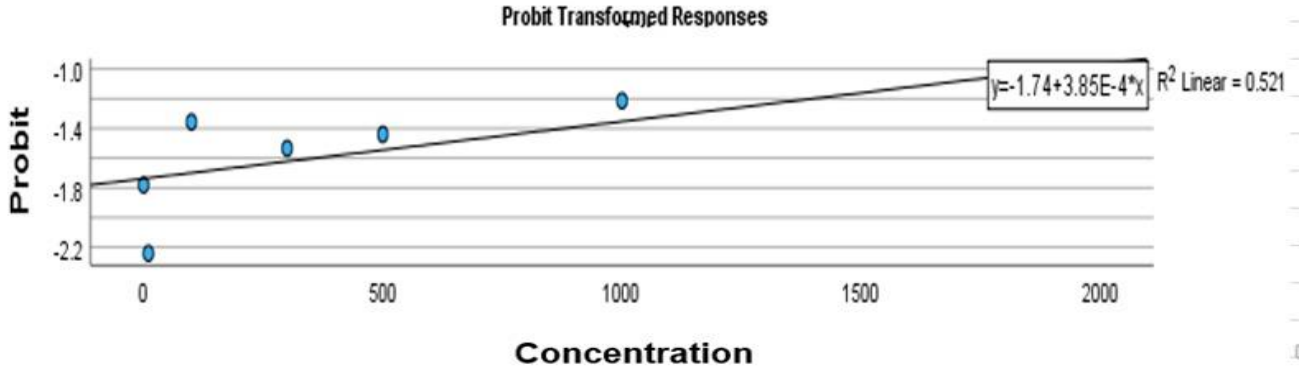

Figure 3. Probit scale and concentration log of *Ulva reticulata* ethanolic extract

This study concluded that *Ulva reticulata* ethanolic extract contained phytochemical compounds, including alkaloids, flavonoids, saponins, tannins, phenols, and steroids/terpenoids. Based on the results, a total of 9 compounds were identified using GC-MS, including 9, 12,15-octadecatrienoic acid, palmitic acid, 1,2-benzenedicarboxylic acid, hexadecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, phytol, neophytadiene, 9,19-Cyclolanost-24-en-3-ol acetate, and 1H-benzimidazole. Ulva *reticulata* ethanolic extract also had strong antioxidant activity based on the IC_{50} of 53.00 ppm and low toxicity value with an LC_{50} of 481.865 m/L. These results showed that phytochemical components of *Ulva reticulata* extract had potential as safe dietary supplements for fish growth, reproduction, and health.

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REFERENCES

Abaho I, Masembe C, Akoll P, Jones CLW. 2022. The use of plant extracts to control tilapia reproduction: Current status and future perspectives. J World Aquac Soc 53 (3): 593-619. DOI: 10.1111/jwas.12863.

Abdelhamid AF, Ayoub HF, Abd El-Gawad EA, Abdelghany MF, Abdel-Tawwab M. 2021. Potential effects of dietary seaweeds mixture on the growth performance, antioxidant status, immunity response, and resistance of striped catfish (*Pangasianodon hypophthalmus*) against *Aeromonas hydrophila* infection. Fish Shellfish Immunol 119: 76-83. DOI: 10.1016/j.fsi.2021.09.043.

Abdel-Warith A-WA, Younis E-SMI, Al-Asgah NA. 2016. Potential use of green macroalgae *Ulva lactuca* as a feed supplement in diets on growth performance, feed utilization and body composition of the African catfish, *Clarias gariepinus*. Saudi J Biol Sci 23 (3): 404-409. DOI: 10.1016/j.sjbs.2015.11.010.

Abo-Raya MH, Alshehri KM, Abdelhameed RFA, Elbialy ZI, Elhady SS, Mohamed RA. 2021. Assessment of growth-related parameters and immune-biochemical profile of nile tilapia (*Oreochromis niloticus*) fed dietary *Ulva fasciata* extract. Aquac Res 52 (7): 3233-3246. DOI: 10.1111/are.15169.

Abu Ahmed SE-S, Deyab MA, El-Ashry FS, El-Adl MF. 2021. Qualitative and quantitative phytochemical composition of *Sargassum vulgare* at Hurghada Red Sea Coast-Egypt. Sci J Damietta Fac Sci 11 (1): 10-19. DOI: 10.21608/sjdfs.2021.195585.

Ahmadifar E, Pourmohammadi Fallah H, Yousefi M, Dawood MAO, Hoseinifar SH, Adineh H, Yilmaz S, Paolucci M, Doan HV. 2021. The gene regulatory roles of herbal extracts on the growth, immune system, and reproduction of fish. Animals 11 (8): 2167. DOI: 10.3390/ani11082167.

Akbary P, Aminikhoei Z. 2018. Effect of water-soluble polysaccharide extract from the green alga *Ulva rigida* on growth performance, antioxidant enzyme activity, and immune stimulation of grey mullet *Mugil cephalus*. J Appl Phycol 30 (2): 1345-1353. DOI: 10.1007/s10811-017-1299-8.

Alagawany M, Farag MR, Salah AS, Mahmoud MA. 2020. The role of oregano herb and its derivatives as immunomodulators in fish. Rev Aquac 12 (4): 2481-2492. DOI: 10.1111/raq.12453.

Alagawany M, Taha AE, Noreldin A, El-Tarabily KA, Abd El-Hack ME. 2021. Nutritional applications of species of *Spirulina* and *Chlorella* in farmed fish: A review. Aquaculture 542: 736841. DOI: 10.1016/j.aquaculture.2021.736841.

Aminudin A, Andarwulan N, Palupi NS, Arifiantini I. 2020. Characteristics and antioxidant activity of kebar grass (*Biophytum petersianum*) extract. Biosaintifika: J Biol Biol Educ 12 (2): 178-185. DOI: 10.15294/biosaintifika.v12i2.23820.

Anh HTL, Kawata Y, Tam LT, Thom LT, Ha NC, Hien HTM, Thu NTH, Huy PQ, Hong DD. 2020. Production of pyruvate from *Ulva reticulata* using the alkaliphilic, halophilic bacterium *Halomonas* sp. BL6. J Appl Phycol 32: 2283-2293. DOI: 10.1007/s10811-020-02035-1.

Arbi B, Ma'ruf WF, Romadhon R. 2016. The activity of bioactive compounds from sea lettuce (*Ulva lactuca*) as antioxidant in fish oil. Saintek Perikanan: Indones J Fish Sci Technol 12 (1): 12-18. DOI: 10.14710/ijfst.12.1.12-18. [Indonesian]

Arini LA. 2021. The study of sex steroid hormone compound in green algae (*Chlorophyta*) for female fertility: A literature review. IOP Conf Ser: Earth Environ Sci 913 (1): 012085. DOI: 10.1088/1755-1315/913/1/012085.

Aulia NE, Yudiati E, Hartati R. 2023. Increased growth of *Artemia* sp. through *Ulva* sp. extract application. J Mar Res 12 (2): 196-202.

Awad E, Awaad A. 2017. Role of medicinal plants on growth performance and immune status in fish. Fish Shellfish Immunol 67: 40-54. DOI: 10.1016/j.fsi.2017.05.034.

Awaludin A, Kartina K, Maulianawati D, Manalu W, Andriyanto A, Septiana R, Arfandi A, Lalang Y. 2020. Short Communication: Phytochemical screening and toxicity of ethanol extract of *Sauropus*

androgynus. Biodiversitas 21 (7): 2967-2970. DOI: 10.13057/biodiv/d210712.
Beltran JMG, Esteban MÁ. 2022. Nature-identical compounds as feed additives in aquaculture. Fish Shellfish Immunol 123 (2): 409-416. DOI: 10.1016/j.fsi.2022.03.010.
Beulah GG, Soris PT, Mohan VR. 2018. GC-MS determination of bioactive compounds of *Dendrophthoe falcata* (LF) Ettingsh: An epiphytic plant. Intl J Health Sci Res 8: 261-269.
Brien RO, Hayes M, Sheldrake G, Tiwari B, Walsh P. 2022. Macroalgal proteins: A review. Foods 11 (4): 571. DOI: 10.3390/foods11040571.
Chakraborty SB, Hancz C. 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. Rev Aquac 3 (3): 103-119. DOI: 10.1111/j.1753-5131.2011.01048.x.
Chakraborty SB, Horn P, Hancz C. 2014. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Rev Aquac 6 (1): 1-19. DOI: 10.1111/raq.12021.
Cipolletti M, Solar Fernandez V, Montalesi E, Marino M, Fiocchetti M. 2018. Beyond the antioxidant activity of dietary polyphenols in cancer: The modulation of estrogen receptors (ERs) signaling. Intl J Mol Sci 19 (9): 2624. DOI: 10.3390/ijms19092624.
Corral-Rosales C, Ricque-Marie D, Cruz-Suárez LE, Arjona O, Palacios E. 2019. Fatty acids, sterols, phenolic compounds, and carotenoid changes in response to dietary inclusion of *Ulva clathrata* in shrimp *Litopenaeus vannamei* broodstock. J Appl Phycol 31: 4009-4020. DOI: 10.1007/s10811-019-01829-2.
Corral-Rosales DC, Cruz-Suárez LE, Ricque-Marie D, Rodríguez-Jaramillo C, Palacios E. 2018. Modulation of reproductive exhaustion using *Ulva clathrata* in pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) broodstock during commercial maturation. Aquac Res 49 (12): 3711-3722. DOI: 10.1111/are.13839.
Da Costa JF, Merdekawati W, Otu FR. 2018. Proximate analysis, antioxidant activity, and pigment composition of *Ulva lactuca* from Kukup Beach. J Food Technol Nutr 17 (1): 1-17.
Delimont NM, Haub MD, Lindshield BL. 2017 The impact of tannin consumption on iron bioavailability and status: A narrative review. Curr Dev Nutr 1 (2): 1-12. DOI: 10.3945/cdn.116.000042.
Fauziah F, Maulinasari, Harnelly E, Ismail YS, Fitri L. 2022. Toxicity test of rose periwinkle (*Catharanthus roseus*) leaves endophytic bacteria using Brine Shrimp Lethality Test (BSLT) method. Biodiversitas 23 (1): 171-177. DOI: 10.13057/biodiv/d230122.
Gabriel NN. 2019. Review on the progress in the role of herbal extracts in tilapia culture. Cogent Food Agric 5 (1): 1619651. DOI: 10.1080/23311932.2019.1619651.
Gao K, Beardall J. 2022. Using macroalgae to address UN sustainable development goals through CO_2 remediation and improvement of the aquaculture environment. Appl Phycol 3 (1): 360-367. DOI: 10.1080/26388081.2022.2025617.
Ghosal I, Mukherjee D, Hancz C, Chakraborty SB. 2015. Efficacy of *Basella alba* and *Tribulus terrestris* extracts for production of monosex nile tilapia, *Oreochromis niloticus*. J Appl Pharm Sci 5 (8): 152-158. DOI: 10.7324/JAPS.2015.50824.
Glencross BD. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev Aquac 1 (2): 71-124. DOI: 10.1111/j.1753-5131.2009.01006.x.
Gunathilake T, Akanbi TO, Suleria HAR, Nalder TD, Francis DS, Barrow CJ. 2022. Seaweed phenolics as natural antioxidants, aquafeed additives, veterinary treatments and cross-linkers for microencapsulation. Mar Drugs 20 (7): 445. DOI: 10.3390/md20070445.
Hamit CS, Nam KC, Abeyrathne EDNS. 2022. Bioactive peptide production and determination of functional properties using crude water extracts of *Ulva lactuca* (Chlorophyta) and *Sargassum crassifolium* (Phaeophyceae) collected from Sri Lanka. Appl Phycol 3 (1): 72-81. DOI: 10.1080/26388081.2022.2063758.
Harborne AJ. 1998. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science and Business Media.
Harikrishnan R, Devi G, Van Doan H, Balasundaram C, Arockiaraj J, Jagruthi C. 2021. Efficacy of ulvan on immune response and immuno-antioxidant gene modulation in *Labeo rohita* against columnaris disease. Fish Shellfish Immunol 117 (5): 262-273. DOI: 10.1016/j.fsi.2021.08.004.
Hashem NM, Gonzalez-Bulnes A, Simal-Gandara J. 2020. Polyphenols in farm animals: Source of reproductive gain or waste? Antioxidants 9 (10): 1023. DOI: 10.3390/antiox9101023.
Hempel MDSS, Colepicolo P, Zambotti-Villela L. 2023. Macroalgae biorefinery for the cosmetic industry: Basic concept, green technology, and safety guidelines. Phycology 3 (1): 211-241. DOI: 10.3390/phycology3010014.
Hidayat T, Jacoeb AM, Putera BA. 2020. Antioxidant activity of *Caulerpa* sp. fresh and boiled. J Indones Fish Prod Process 23 (3): 566-575. DOI: 10.17844/jphpi.v23i3.33869. [Indonesian]
Hilbig CC, Nascimento NFD, Heinen AL, Neto AT, Funghetto JP, Bombardelli RA, Meurer F, Nakaghi LSO. 2019. Effects of dietary fatty acids on the reproduction of South American female catfish *Rhamdia quelen* (Quoy & Gaimard, 1824). Lat Am J Aquat Res 47 (3): 456-466. DOI: 10.3856/vol47-issue3-fulltext-8.
Hoga CA, Almeida FL, Reyes FGR. 2018. A review on the use of hormones in fish farming: Analytical methods to determine their residues. CyTA - J Food 16 (1): 679-691. DOI: 10.1080/19476337.2018.1475423.
Hoseini SM, Gharavi B, Mirghaed AT, Hoseinifar SH, Van Doan H. 2021. Effects of dietary phytol supplementation on growth performance, immunological parameters, antioxidant and stress responses to ammonia exposure in common carp, *Cyprinus carpio* (Linnaeus, 1758). Aquaculture 545: 737151. DOI: 10.1016/j.aquaculture.2021.737151.
Indriaty, Djufri, Ginting B, Hasballah K. 2023. Phytochemical screening, phenolic and flavonoid content, and antioxidant activity of Rhizophoraceae methanol extracts from Langsa, Aceh, Indonesia. Biodiversitas 24 (5): 2865-2876. DOI: 10.13057/biodiv/d240541.
Janeczko A. 2021. Estrogens and androgens in plants: The last 20 years of studies. Plants 10 (12): 2783. DOI: 10.3390/plants10122783.
Jeeva S, Marimuthu J, Domettila C, Anantham B, Mahesh M. 2012. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India. Asian Pac J Trop Biomed 2 (1): S30-S33. DOI: 10.1016/S2221-1691(12)60125-7.
Jelita SF, Setyowati, Ferdinand M. 2020. Toxicological test of acalypha siamensis infusion using the Brine Shrimp Lethality Test (BSLT). Method Farmaka 18 (1): 14-22.
Kamal M, Abdel-Raouf N, Alwutayd K, Abdelgawad H, Abdelhameed MS, Hammouda O, Elsayed KNM. 2023. Seasonal changes in the biochemical composition of dominant macroalgal species along the Egyptian Red Sea Shore. Biology 12 (3): 411. DOI: 10.3390/biology12030411.
Kiadaliri M, Firouzbakhsh F, Deldar H. 2020. Effects of feeding with red algae (*Laurencia caspica*) hydroalcoholic extract on antioxidant defense, immune responses, and immune gene expression of kidney in rainbow trout (*Oncorhynchus mykiss*) infected with *Aeromonas hydrophila*. Aquaculture 526: 735361. DOI: 10.1016/j.aquaculture.2020.735361.
Klongklaew N, Praiboon J, Tamtin M, Srisapoome P. 2021. Chemical composition of a Hot Water Crude Extract (HWCE) from *Ulva intestinalis* and its potential effects on growth performance, immune responses, and resistance to white spot syndrome virus and yellowhead virus in Pacific white shrimp (*Litopenaeus vannamei*). Fish Shellfish Immunol 112 (2): 8-22. DOI: 10.1016/j.fsi.2021.02.004.
Kumar N, Chandan NK, Gupta SK, Bhushan S, Patole PB. 2022. Omega-3 fatty acids effectively modulate growth performance, immune response, and disease resistance in fish against multiple stresses. Aquaculture 547: 737506. DOI: 10.1016/j.aquaculture.2021.737506.
Kumar Y, Tarafdar A, Badgujar PC. 2021. Seaweed as a source of natural antioxidants: Therapeutic activity and food applications. J Food Qual 2021: 1-17. DOI: 10.1155/2021/5753391.
Kumar Y, Tarafdar A, Kumar D, Saravanan C, Badgujar PC, Pharande A, Pareek S, Fawole OA. 2022. Polyphenols of edible macroalgae: Estimation of in vitro bio-accessibility and cytotoxicity, quantification by LC-MS/MS and potential utilization as an antimicrobial and functional food ingredient. Antioxidants 11 (5): 993. DOI: 10.3390/antiox11050993.
Lafont R, Balducci C, Dinan L. 2021. Ecdysteroids. Encyclopedia 1 (4): 1267-1302. DOI: 10.3390/encyclopedia1040096.
Librán-Pérez M, Pereiro P, Figueras A, Novoa B. 2019. Antiviral activity of palmitic acid via autophagic flux inhibition in zebrafish (*Danio rerio*). Fish Shellfish Immunol 95 (2): 595-605. DOI: 10.1016/j.fsi.2019.10.055.
Li X, Zheng S, Wu G. 2021. Nutrition and functions of amino acids in fish. Amino Acids in Nutrition and Health: Amino Acids in the Nutrition of Companion, Zoo and Farm Animals: 133-168. DOI: 10.1007/978-3-030-54462-1_8.

Limiñana VA, Benoist T, Sempere SA, Pérez SEM, Moya MSP. 2023. Chemical composition of sustainable Mediterranean macroalgae obtained from land-based and sea-based aquaculture systems. Food Biosci 54: 102902. DOI: 10.1016/j.fbio.2023.102902.

Lomartire S, Gonçalves AMM. 2023. An overview on antimicrobial potential of edible terrestrial plants and marine macroalgae Rhodophyta and Chlorophyta extracts. Mar Drugs 21 (3): 163. DOI: 10.3390/md21030163.

Lutfi L, Sudrajat AO, Carman O, Wahjuningrum D, Widanarni W, Boediono A. 2023. Phytochemical screening and toxicity analysis of South Pacific palm (*Biophytum umbraculum*) ethanolic extract for potential utilization in aquaculture. Aquacult Aquarium Conserv Legislation 16 (2): 837-852.

Madibana MJ, Mlambo V, Lewis B, Fouché C. 2017. Effect of graded levels of dietary seaweed (*Ulva* sp.) on growth, hematological and serum biochemical parameters in dusky kob, *Argyrosomus japonicus*, sciaenidae. Egypt J Aquat Res 43 (3): 249-254. DOI: 10.1016/j.ejar.2017.09.003.

Mahasu NH, Jusadi D, Setiawati M, Giri INAA. 2016. Potential use of *Ulva lactuca* as feed ingredient for tilapia. Jurnal Ilmu dan Teknologi Kelautan Tropis 8 (1): 259-267. [Indonesian]

Maheshwari DG, Shaikh NK. 2016. An overview of toxicity testing method. Intl J Pharm Technol 8 (2): 3834-3849.

Makkar HPS, Francis G, Becker K. 2007. Bioactivity of phytochemicals in some lesser known plants and their effects and potential applications in livestock and aquaculture production systems. Animal 1 (9): 1371-1391. DOI: 10.1017/S1751731107000298.

Masyudi, Hanafiah M, Rinidar, Usman S, Marlina. 2022. Phytochemical screening and GC-MS analysis of bioactive compounds of *Blumea balsamiferaleaf* extracts from South Aceh, Indonesia. Biodiversitas 23 (3): 1344-1352. DOI: 10.13057/biodiv/d230319.

Meiyasa F, Tega YR, Henggu KU, Tarigan N, Ndahawali S. 2020. Identification of macroalgae in Moudolung Waters, East Sumba Regency. Quagga: Jurnal Pendidikan dan Biologi 12 (2): 202-210. DOI: 10.25134/quagga.v12i2.2751. [Indonesian]

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DEJ, McLaughlin JL. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica 45 (5): 31-34. DOI: 10.1055/s-2007-971236.

Michalak I, Tiwari R, Dhawan M, Alagawany M, Farag MR, Sharun K, Emran TB, Dhama K. 2022. Antioxidant effects of seaweeds and their active compounds on animal health and production-A review. Vet Q 42 (1): 48-67. DOI: 10.1080/01652176.2022.2061744.

Miranda M, López-Alonso M, García-Vaquero M. 2017. Macroalgae for Functional Feed Development: Applications in Aquaculture, Ruminant. In: Newton P (eds.). Seaweeds: Biodiversity, Environmental Chemistry and Ecological Impacts.

Mirzaei M, Mirzaei A. 2013. Comparison of the *Artemia salina* and *Artemia uramiana* bioassays for toxicity of Iranian medicinal plants. Intl Res J Biol Sci 49 (2): 49-54.

Moreira A, Cruz S, Marques R, Cartaxana P. 2022. The underexplored potential of green macroalgae in aquaculture. Rev Aquac 14 (1): 5-26. DOI: 10.1111/raq.12580.

Naiel MAE, Alagawany M, Patra AK, El-Kholy AI, Amer MS, Abd El-Hack ME. 2021. Beneficial impacts and health benefits of macroalgae phenolic molecules on fish production. Aquaculture 534: 736186. DOI: 10.1016/j.aquaculture.2020.736186.

Nielsen CW, Rustad T, Holdt SL. 2021. Vitamin C from seaweed: A review assessing seaweed as contributor to daily intake. Foods 10 (1): 198. DOI: 10.3390/foods10010198.

Nieminen P, Pölönen I, Mustonen AM. 2010. Increased reproductive success in the white American mink (*Neovison vison*) with chronic dietary β-sitosterol supplement. Anim Reprod Sci 119 (3-4): 287-292. DOI: 10.1016/j.anireprosci.2010.01.008.

Noviyanty Y, Hepiyansori H, Dewi BR. 2020. Identification and determination of saponin content of the ethanol extract of senggani flower *Melastoma malabathricum* L. gravimetric method. Oceana Biomed J 3 (1): 45-53. DOI: 10.30649/obj.v3i1.46. [Indonesian]

Nufus C, Nurjanah N, Abdullah A. 2017. Characteristics of green seaweeds from Seribu Islands and Sekotong West Nusa Tenggara Antioxidant. Jurnal Pengolahan Hasil Perikanan Indonesia 20 (3): 620-632. DOI: 10.17844/jphpi.v20i3.19819. [Indonesian]

Okab AB, Samara EM, Abdoun KA, Rafay J, Ondruska L, Parkanyi V, Pivko J, Ayoub MA, Al-Haidary AA, Aljumaah RS, Peter M. 2013. Effects of dietary seaweed (*Ulva lactuca*) supplementation on the reproductive performance of buck and doe rabbits. J Appl Anim Res 41 (3): 347-355. DOI: 10.1080/09712119.2013.783479.

Olivia NU, Goodness UC, Obinna OM. 2021. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. Futur J Pharm Sci 7: 1-5. DOI: 10.1186/s43094-021-00208-4.

Øverland M, Mydland LT, Skrede A. 2019. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. J Sci Food Agric 99 (1): 13-24. DOI: 10.1002/jsfa.9143.

Panjaitan RS, Thalib N, Sumantri S. 2022. Antibacterial activity of 96% ethanolic extract of *Ulva reticulata* against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Indones J Pharm Res 2 (1): 13-19.

Pappou S, Dardavila MM, Savvidou MG, Louli V, Magoulas K, Voutsas E. 2022. Extraction of bioactive compounds from *Ulva lactuca*. Appl Sci 12 (4): 2117. DOI: 10.3390/app12042117.

Park E, Yu H, Lim J-H, Choi JH, Park K-J, Lee J. 2022. Seaweed metabolomics: A review on its nutrients, bioactive compounds and changes in climate change. Food Res Intl 163: 112221. DOI: 10.1016/j.foodres.2022.112221.

Parra AL, Yhebra RS, Sardiñas IG, Buela LI. 2001. Comparative study of the assay of *Artemia salina L.* and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine 8 (5): 395-400. DOI: 10.1078/0944-7113-00044.

Pereira CMP, Nunes CFP, Zambotti-Villela L, Streit NM, Dias D, Pinto E, Gomes CB, Colepicolo P. 2017. Extraction of sterols in brown macroalgae from Antarctica and their identification by liquid chromatography coupled with tandem mass spectrometry. J Appl Phycol 29: 751-757. DOI: 10.1007/s10811-016-0905-5.

Pirian K, Piri K, Sohrabipour J, Tamadoni Jahromi S, Rabiei R. 2017. Evaluation of chemical components and physicochemical properties of two green macroalgae species *Ulva intestinalis* and *Ulva linza* from Persian Gulf. Iran J Med Aromat Plants Res 33 (1): 62-72. DOI: 10.22092/ijmapr.2017.109708.

Purbosari N, Warsiki E, Syamsu K, Santoso J, Effendi I. 2021. Evaluation of the application of seaweed (*Eucheuma cottonii*) extract as fish anesthetic agent. Aquac Intl 29: 1545-1560. DOI: 10.1007/s10499-021-00693-7.

Rahim N, Wulan S, Zainuddin EN. 2021. Potential of *Ulva reticulata* extract in increasing lysozyme activity and hemocyte differentiation in tiger prawns (*Penaeus monodon*). Aquafish Sci J 1 (1): 1-9.

Rama NP, Elezabeth MA, Uthayasiva M, Arularasan S. 2014. Seaweed *Ulva reticulata* a potential feed supplement for growth, colouration and disease resistance in fresh water ornamental gold fish, *Carassius auratus*. J Aquat Reseour Dev 5 (254): 2-12.

Ratana-Arporn P, Chirapart A. 2006. Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulata*. Agric Nat Resour 40 (6): 75-83.

Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433: 50-61. DOI: 10.1016/j.aquaculture.2014.05.048.

Ridwanudin A, Anggorowati DA, Sujangka A, Badi BF, Tarmin N, Wahab A. 2022. The effect of using artificial feed made from green macroalgae *Ulva* sp. on the growth of juvenile Abalone Haliotis squamata. Oceanol Limnol Indones 7 (2): 53-64.

Rompas IFX, Gasah O. 2022. Effectiveness of green seaweed (*Ulva lactuca*) extract on antioxidant activity as a sustainable food source. J Biol Educ 7 (3): 172-189. DOI: 10.32938/jbe.v7i3.1917. [Indonesian]

Saleh HHE. 2020. Review on using of macro algae (seaweeds) in fish nutrition. J Zool Res 2 (2): 6-11. DOI: 10.30564/jzr.v2i2.2054.

Santiago CB, Lovell RT. 1988. Amino acid requirements for growth of nile tilapia. J Nutr 118 (12): 1540-1546. DOI: 10.1093/jn/118.12.1540.

Serviere-Zaragoza E, Hurtado-Oliva MÁ, Mazariegos-Villarreal A, Arjona O, Palacios E. 2021. Seasonal and interannual variation of sterols in macrophytes from the Pacific coast of Baja California Peninsula (Mexico). Phycol Res 69 (1): 41-47. DOI: 10.1111/pre.12440.

Siddik MAB, Rahman MM, Anh NTN, Nevejan N, Bossier P. 2015. Seaweed, *Enteromorpha intestinalis*, as a diet for nile tilapia *Oreochromis niloticus* fry. J Appl Aquac 27 (2): 113-123. DOI: 10.1080/10454438.2015.1008286.

Surai PF. 2020. Antioxidants in poultry nutrition and reproduction: An update. Antioxidants 9 (2): 105. DOI: 10.3390/antiox9020105.

Suryaningrum LH, Samsudin R. 2020. Nutrient digestibility of green seaweed Ulva meal and the influence on growth performance of nile tilapia (*Oreochromis niloticus*). Emir J Food Agric 32 (7): 488-494. DOI: 10.9755/ejfa.2020.v32.i7.2131.

Sutili FJ, Gatlin DM, Heinzmann BM, Baldisserotto B. 2018. Plant essential oils as fish diet additives: Benefits on fish health and stability in feed. Rev Aquac 10 (3): 716-726. DOI: 10.1111/raq.12197.

Susanto AB, Santosa GW, Djunaedi A, Pringgenies D, Ariyanto D. 2023. The impact of mixed feed of seaweed *Gracilaria sp*. and transfer factor formula on the immune response of nile tilapia (*Oreochromis niloticus*). Aquacult Aquarium Conserv Legislation 16 (1): 242-251.

Tarakanita DNS, Satriadi T, Jauhari A. 2020. Potensi keberadaan fitokimia kamalaka (*Phyllanthus emblica*) berdasarkan perbedaan ketinggian tempat tumbuh. J Sylva Scienteae 2 (4): 645-654. [Indonesian]

Tarkowska D. 2019. Plants are capable of synthesizing animal steroid hormones. Molecules 24 (14): 2585. DOI: 10.3390/molecules24142585.

Thiviya P, Gamage A, Gama-Arachchige NS, Merah O, Madhujith T. 2022. Seaweeds as a source of functional proteins. Phycology 2 (2): 216-243. DOI: 10.3390/phycology2020012.

Tziveleka L-A, Tammam MA, Tzakou O, Roussis V, Ioannou E. 2021. Metabolites with antioxidant activity from marine macroalgae. Antioxidants 10 (9): 1431. DOI: 10.3390/antiox10091431.

Van Doan H, Hoseinifar SH, Esteban MÁ, Dadar M, Thu TTN. 2019. Mushrooms, seaweed, and their derivatives as functional feed additives for aquaculture: An updated view. Stud Nat Prod Chem 62: 41-90. DOI: 10.1016/B978-0-444-64185-4.00002-2.

Vega J, Álvarez-Gómez F, Güenaga L, Figueroa FL, Gómez-Pinchetti JL. 2020. Antioxidant activity of extracts from marine macroalgae, wild-collected and cultivated, in an integrated multi-trophic aquaculture system. Aquaculture 522: 735088. DOI: 10.1016/j.aquaculture.2020.735088.

Wan AHL, Davies SJ, Soler-Vila A, Fitzgerald R, Johnson MP. 2019. Macroalgae as a sustainable aquafeed ingredient. Rev Aquac 11 (3): 458-492. DOI: 10.1111/raq.12241.

Widyaningsih W, Pramono S, Widyarini S, Sugiyanto S. 2016. Skrining fitokimia ekstrak etanol *Ulva lactuca* L. dengan metode kromatografi lapis tipis. Media Farmasi: Jurnal Ilmu Farmasi 13 (2): 199-206. DOI: 10.12928/mf.v13i2.7772. [Indonesian]

Wulanjati MP, Indrianingsih AW, Darsih C, Apriyana W, Batrisya. 2020. Antioxidant and antibacterial activity of ethanolic extract from *Ulva* sp. IOP Conf Ser: Earth Environ Sci 462 (1): 012028. DOI: 10.1088/1755-1315/462/1/012028.

Yainahu J, Mile L, Suherman SP. 2023. Yield analysis and phytochemical screening of fresh and dried red seaweed (*Eucheuma spinosium*) extracts. Jambura Fish Process J 5 (2): 1-8.

Yan Z, Zhong Y, Duan Y, Chen Q, Li F. 2020. Antioxidant mechanism of tea polyphenols and its impact on health benefits. Anim Nutr 6 (2): 115-123. DOI: 10.1016/j.aninu.2020.01.001.

Yao L, Liang Y, Sun M, Song S, Wang H, Dong Z, Feng T, Yue H. 2022. Characteristic volatile fingerprints of three edible marine green algae (*Ulva* spp.) in China by HS-GC-IMS and evaluation of the antioxidant bioactivities. Food Res Intl 162: 112109. DOI: 10.1016/j.foodres.2022.112109.

Yu Y, Li C, Liu J, Zhu F, Wei S, Huang Y, Huang X, Qin Q. 2020. Palmitic acid promotes virus replication in fish cell by modulating autophagy flux and TBK1-IRF3/7 pathway. Front Immunol 11: 1764. DOI: 10.3389/fimmu.2020.01764.

Zaghbib I, Hassouna M. 2022. Functional properties and biological potentials of the tunisian green seaweed *Ulva lactuca*. Am Acad Sci Res J Eng Technol Sci 85 (1): 89-99.

Zeng X, Li S, Liu L, Cai S, Ye Q, Xue B, Zeng X. 2023. Role of functional fatty acids in modulation of reproductive potential in livestock. J Anim Sci Biotechnol 14 (1): 24-30. DOI: 10.1186/s40104-022-00818-9.

Zhang W, Zhao J, Ma Y, Li J, Chen, X. 2022. The effective components of herbal medicines used for prevention and control of fish diseases. Fish Shellfish Immunol 126: 73-83. DOI: 10.1016/j.fsi.2022.05.036.