

Benefits of *Sargassum* sp. Extract for Feed Supplementation on the Growth and pH Shock Resistance of Zebrafish (*Danio rerio*)

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Abstract

Sargassum sp. is an abundant marine biological resource in Indonesia rich in bioactive compounds, yet its utilization as a feed supplement in fish culture remains limited. Feed represents the highest cost component in aquaculture, necessitating economical natural additives to improve fish performance and their resistance to environmental stressors such as pH fluctuations. This research aimed to evaluate the effect of *Sargassum* sp. water extract, obtained using acidic (pH 3) and alkaline (pH 12) methods, on the survival rate, growth, and resistance of zebrafish (*Danio rerio*) to extremely low pH shock. The study employed a Completely Randomized Design (CRD) with nine treatments (control, four alkaline extract doses, and four acidic doses) over a 30-day rearing period. Doses were determined based on BSLT toxicity tests, which indicated that both extracts were non-toxic ($LC_{50} > 1000$ ppm). The parameters observed included survival rate, absolute weight gain, and mortality during a pH 3 shock test. The results showed that supplementation had no significant effect on survival rate ($P > 0.05$). However, all extract treatments (acidic and alkaline) significantly increased absolute weight gain ($P < 0.05$) compared to the control, with the highest average weight found at the lowest alkaline *Sargassum* sp. extract 1.375 ppm. Fish supplemented with the extract showed significantly lower mortality in the low pH shock test ($P < 0.05$), with the best resistance value observed at the lowest acidic treatment (1,875 ppm). These findings indicate that *Sargassum* sp. extract has the potential as an effective natural feed supplement to promote growth and enhance the physiological resistance of fish to environmental stress, even at low concentrations.

Keywords: Brown Seaweed; Acidic-Alkaline Extraction; pH; *Danio rerio*

INTRODUCTION

Indonesia is a maritime country with vast potential for marine biological resources, including various species of seaweed that grow abundantly in coastal waters. One of the most commonly found brown seaweeds is *Sargassum* sp. This alga is known to contain bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and polyphenols, and high polysaccharide compounds, water-based extract, which function as natural antioxidants, antibacterial agents, and immunostimulants (Dolorosa *et al.*, 2017; Yudiati *et al.*, 2025). These compounds have the potential to be utilized as feed additives to enhance fish growth performance and resistance to environmental stress (Sofiana *et al.*, 2024).

Recent studies highlight *Sargassum* as a sustainable feed additive in aquaculture. Extracts of *Sargassum cristaefolium* show strong antioxidant activity and bioactive potential, supporting its use in functional aquafeeds (Rohim *et al.*, 2025). Other brown seaweeds, such as *Sargassum natans* and *Sargassum fluitans*, are also recognized as abundant carbohydrate sources suitable for tropical aquaculture feed formulations (Ahmad and Turkistani, 2024).

Feed is a major factor in fish farming, accounting for up to 70% of the total production cost (Salamah and Zulpikar, 2020). Dependence on expensive commercial feed poses a challenge for small-scale farmers. Efforts to reduce feed costs while improving fish quality can be achieved by supplementing with natural functional ingredients such as *Spirulina* sp., alginate, and seaweed extracts rich in antioxidants and nutrients (Yudiati *et al.*, 2024). Several studies have reported that seaweed-based supplements can improve growth rate, immunity, and stress resistance in fish (Siddik *et al.*, 2023). *Sargassum* sp., in particular, contains polysaccharides such as fucoidan and alginate, which strengthen the immune system and enhance nutrient absorption (Seo *et al.*, 2022).

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Zebrafish (*Danio rerio*) were selected as the test organism due to their short life cycle, clear physiological responses to stressors, and widespread use as a model organism, not only in aquaculture, but also in biomedical research due to similarity to humans (Chowdhury and Saikia, 2022). The pH shock test is used to assess fish tolerance to extreme environmental changes, particularly pH fluctuations that frequently occur in aquaculture systems in tropical areas, and recent findings show that extreme pH stress significantly affects zebrafish physiology and gene expression, reinforcing its relevance as a model for environmental tolerance studies (Das & Panigrahi, 2024). This study aimed to evaluate the effects of dietary supplementation with *Sargassum* sp. extract on the survival rate, growth performance, and pH stress tolerance of zebrafish.

MATERIALS AND METHODS

This research was conducted experimentally in a laboratory setting using a Completely Randomized Design (CRD) with treatments consisting of *Sargassum* sp. alkaline extract (four treatments and *Sargassum* sp. acidic extract (four treatments), each with three replications, including control. The feed used was a commercial fish feed (brand "Sae") mixed with *Sargassum* sp. extract. *Danio rerio* fish were maintained for 30 days to observe survival rate, absolute weight growth, and tolerance to pH shock.

Sargassum sp. samples were collected from Sadranan Beach, Gunung Kidul, during low tide using a random sampling method. Healthy, undamaged, and mud-free samples were selected, and thallus parts were cut without damaging the holdfast (Gazali *et al.*, 2017). The samples were cleaned, dried for three days, ground into fine powder, and stored in airtight containers until extraction (Erniati *et al.*, 2024). Extraction was carried out by maceration using acidic (HCl, pH 3) and basic (NaOH, pH 12) solutions to obtain bioactive compounds and polysaccharides (Nasruddin *et al.*, 2016). A total of 10 g of *Sargassum* sp. powder was dissolved in 500 mL of distilled water, heated at 70 °C for 3 hours with a stirring speed of 800 rpm (Yudiati *et al.*, 2020). The filtrate was then filtered and slowly evaporated until a viscous extract of 50 mL was obtained, which was subsequently used for LC₅₀ analysis.

Toxicity testing was conducted using 20 *Artemia* sp. nauplii aged 48 hours in test solutions with concentrations ranging from 0–125,000 ppm. Each treatment was replicated three times. After 48 hours, the number of dead larvae was counted to determine the percentage of mortality (%). The LC₅₀ value was calculated using the Brine Shrimp Lethality Test (BSLT) method by converting concentration to logarithmic form and mortality to probit values, followed by establishing a linear regression equation $Y = aX + b$, where $Y = 5$ was used to determine LC₅₀ (Reymon *et al.*, 2021).

The supplementation level of *Sargassum* sp. extract was determined based on the results of the BSLT to identify the optimal threshold for feed application. The dosage preparation procedure began by setting the LC₅₀ value as the highest concentration treatment, followed by three lower concentration levels prepared through serial dilution (two-fold reduction) from the highest concentration (Reymon *et al.*, 2021). This yielded four total test concentrations. For the alkaline extraction method, the concentrations were 1,375 ppm, 2,750 ppm, 5,500 ppm, and 11,000 ppm, while for the acid extraction method, the concentrations were 1,875 ppm, 3,750 ppm, 7,500 ppm, and 15,000 ppm.

Sargassum sp. extract was added to commercial feed according to the above treatments. The liquid extract was thoroughly mixed with the feed, dried until it adhered completely, and then stored in labeled containers (Agung *et al.*, 2021).

Fish survival rate (SR) was counted at the beginning and the end of the rearing period, using this formula:

$$SR = \frac{Nt}{N0} \times 100\%$$

N_t is Number of fish alive at the end of the experiment and N_0 is Number of fish at the beginning of the experiment.

Fish weight growth was measured at the beginning and the end of the rearing period using an analytical balance (0.01 gram). Absolute weight growth was calculated according to Dewantari *et al.* (2024) using the following formula:

$$W_m = W_t - W_0$$

W_m is Absolute growth (g); W_t is Final biomass weight of the test fish (g), and W_0 is Initial biomass weight of the test fish (g).

The pH shock test was conducted by preparing aquarium water media at pH 3, adjusted gradually using an HCl solution until the desired pH was reached. The prepared stock solution (10 L) was then used to fill 500 mL of test water in small aquaria. Ten *Danio rerio* individuals were placed into each aquarium according to the treatment (including control). Fish were observed for 2 hours to record mortality, which was monitored and recorded every 10 minutes. Mortality rate was calculated using the formula by Muchlisin *et al.* (2016):

$$\text{Mortality} = \frac{N_0 - N_t}{N_0} \times 100\%$$

M is Mortality rate (%); N_t is Number of fish alive at the end of the observation and N_0 is Number of fish at the beginning of the observation.

Data on growth, survival rate, and mortality were analyzed using SPSS software. Data normality and homogeneity were tested before analysis. Statistical differences among treatments were determined using Analysis of Variance (ANOVA) at a 5% significance level. When significant differences ($P < 0.05$) were detected, Duncan's Multiple Range Test was performed to identify the best treatment.

RESULT AND DISCUSSION

The toxicity test was conducted to determine the LC_{50} value within 24 hours. The LC_{50} value was obtained from the regression between log concentration (log X) and log probit (log Y). Based on probit calculations, the 24-hour LC_{50} value for *Sargassum* sp. extracts using the acidic extraction method was 15,117.18 ppm, while the alkaline extraction method yielded an LC_{50} of 11,493.77 ppm. Both values fall under the non-toxic category. According to Kawung *et al.* (2023), substances with LC_{50} values < 30 ppm are categorized as highly toxic, 30–100 ppm as toxic, 100–250 ppm as moderately toxic, 250–1000 ppm as mildly toxic, and > 1000 ppm as non-toxic.

Based on these toxicity test results; both extracts were classified as non-toxic and suitable for use as feed supplement formulations for zebrafish. These results are consistent with the findings of Bareta *et al.* (2023), who reported that *Sargassum duplicatum* extract exhibited low toxicity activity against *Artemia salina*, with an LC_{50} value of 140 ppm. This indicates that *Sargassum* extract at low concentrations can be used as a feed supplement ingredient; however, higher concentrations may pose a risk of material toxicity.

The supplementation of *Sargassum* sp. extract in zebrafish feed using the alkaline extraction method resulted in a relatively high and consistent survival rate across treatments and control groups (Figure 2). In contrast, the *Sargassum* sp. extract obtained through the acidic extraction method showed a decreasing trend in survival rate with increasing dosage. Sunaryo *et al.* (2024) stated that extreme acidic conditions can affect the stability of other components in the extract, leading to the degradation of active compounds.

Overall, the survival rate between the treatment and control groups showed no significant difference in the same extract method, with an average of 95%, and the highest value observed at the lowest alkaline concentration (1,375 ppm). The highest acidic concentration (15,000 ppm) showed the lowest survival rate at 73%. However, the survival rate of all fish across all treatments is still considered good, as stated by Irmadiati et al. (2021) that a survival rate of $\geq 50\%$ is considered good, a survival rate of 30-50% is considered moderate, and less than 30% is considered poor. Several factors, such as feed, genetics, water quality, and the presence of parasites, influence the survival rate of fish. This is supported by Shoimah et al. (2022), who stated that survival is determined by both internal and external factors. Internal factors include age, genetic traits, feed utilization efficiency, and resistance to diseases. Meanwhile, external factors involve the physical and chemical conditions of the water, available swimming space, as well as the quality and quantity of feed in the environment. This is further reinforced by Dewantari et al. (2024), who stated that maintaining water quality is an essential factor for the success of fish cultivation and overall fish health.

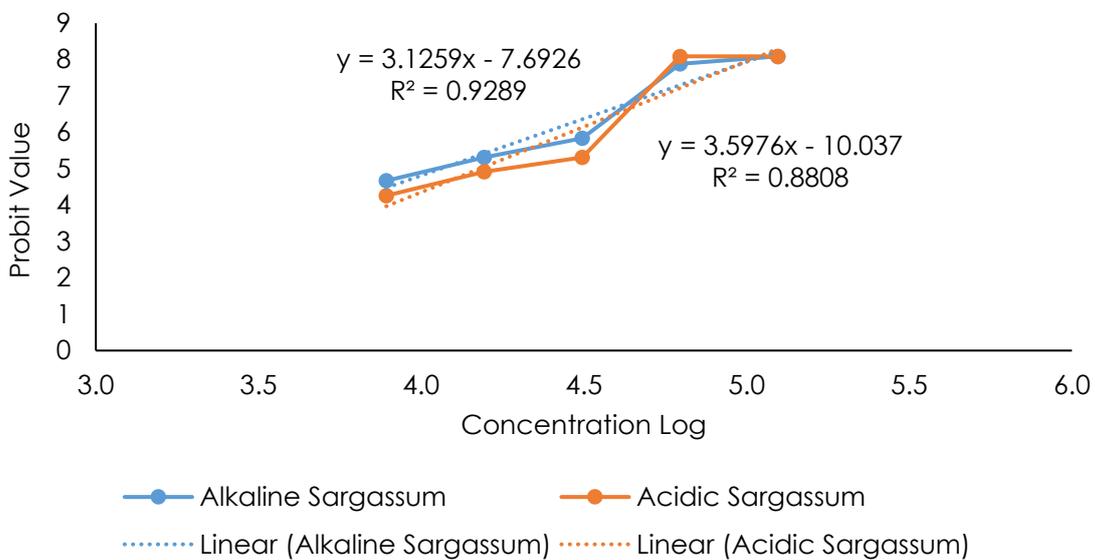


Figure 1. Regression Analysis of Log Concentration and Probit Mortality (%) of Acidic and Alkaline *Sargassum* sp. Extracts

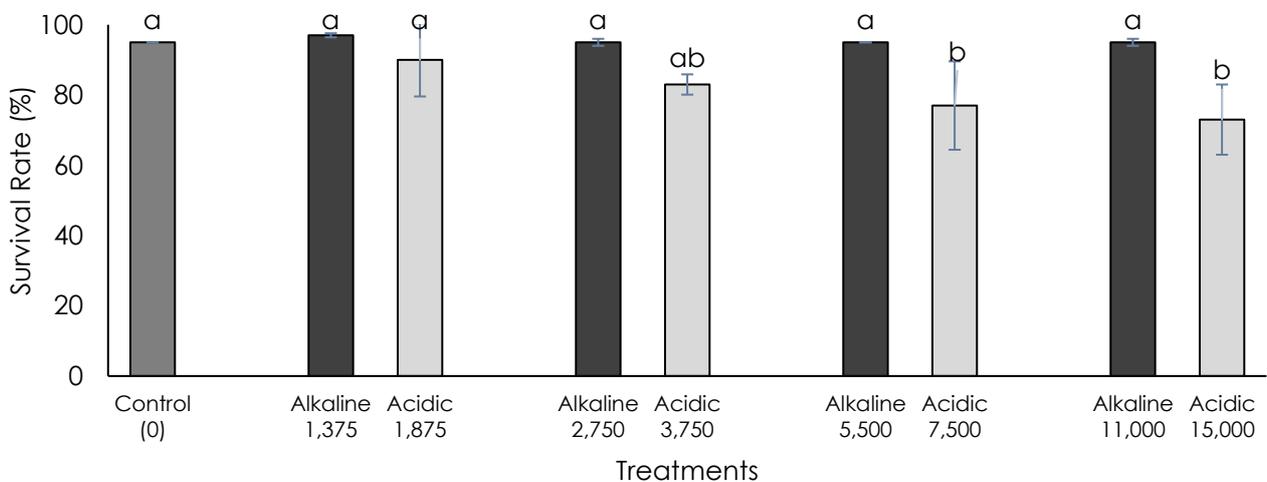


Figure 2. Survival Rate (%) of zebrafish (*Danio rerio*) after 30 Days of Rearing

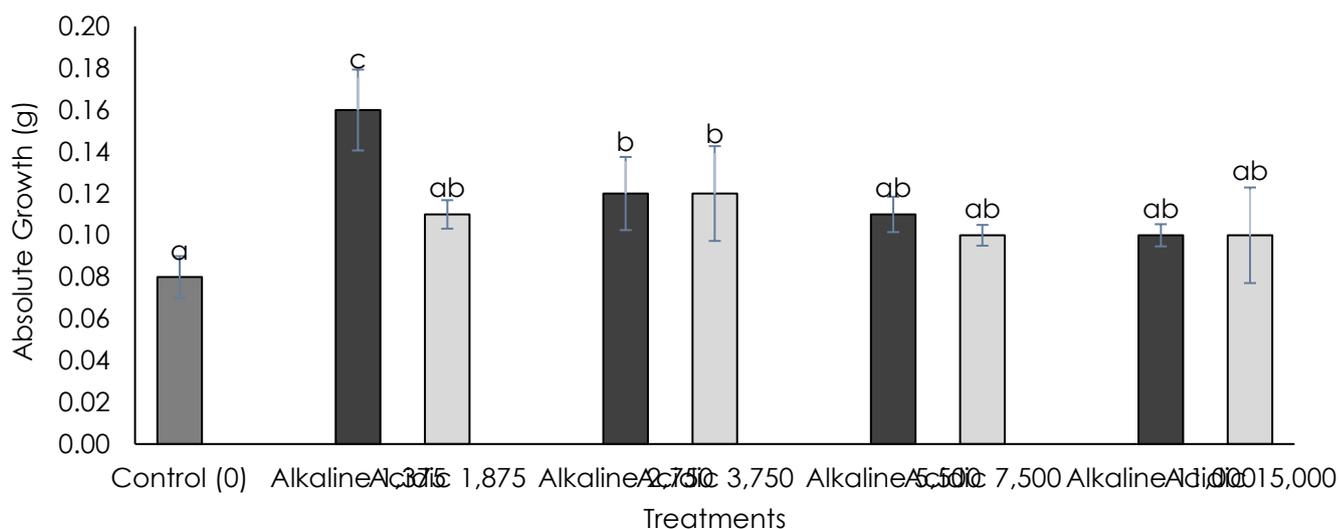


Figure 3. Absolute Weight Growth of zebrafish (*Danio rerio*) after 30 Days of Rearing

The results of *Sargassum* sp. extract supplementation in zebrafish feed using both the alkaline and acidic extraction methods showed significant differences among treatments ($P < 0.05$). Alkaline extraction method showed a positive growth response of *D. rerio* at low to moderate concentrations, with the highest absolute weight recorded at the lowest dose of alkaline extract (1.375 ppm). At higher doses, growth performance decreased, likely due to overstimulation or excessive bioactive compound concentration (Figure 3). This result is consistent with the findings of Nazarudin *et al.* (2020), who reported that optimal doses of *Sargassum* supplements promote fish weight gain, while excessive supplementation may inhibit growth, possibly due to the presence of toxic iron (Fe) content in *Sargassum*.

The acidic extraction method, on the other hand, showed significantly higher average weights compared to the control group. However, no statistically significant differences were found among the extract treatment groups. This finding is supported by Phomkaivon *et al.* (2024) and He *et al.* (2016), who stated that acidic extraction (pH 3) effectively breaks algal cell walls to release sulfated polysaccharides while preventing degradation of bioactive compounds sensitive to alkaline conditions, although this process may also extract unwanted cellular materials.

The improvement in fish growth performance may also be attributed to enhanced digestive efficiency, as reported by Zeraatpisheh *et al.* (2018). The bioactive compounds in *Sargassum* have the potential to enhance enzymatic function and intestinal microflora health, both essential for optimal nutrient absorption. Khanzadeh *et al.* (2024) demonstrated that growth parameters of oscar fish, including Weight Gain (WG), Feed Conversion Ratio (FCR), and Specific Growth Rate (SGR), improved significantly after fucoidan supplementation derived from *S. ilicifolium* compared to the control group. Similar to this present research, those findings indicate that polysaccharides derived from water-based extract and other active components in *Sargassum* extracts contribute to improved feed efficiency, likely supported by the stimulation of the fish's growth.

The test results showed that the rearing of zebra fish *D. rerio* with treatment exhibited a significant difference compared to the control. The lowest resistance level of zebra fish *D. rerio* was observed in the control group, where the test subjects died at the 40th minute. In contrast, the alkaline extract at 1,375 ppm and the acidic extract at 1,875 ppm demonstrated strong resistance to extreme pH decline, surviving up to the 80th minute. Further analysis of the pH shock test on *D. rerio* after feed supplementation with *Sargassum* sp. extract at the 40th minute revealed clearer differences between treatments.

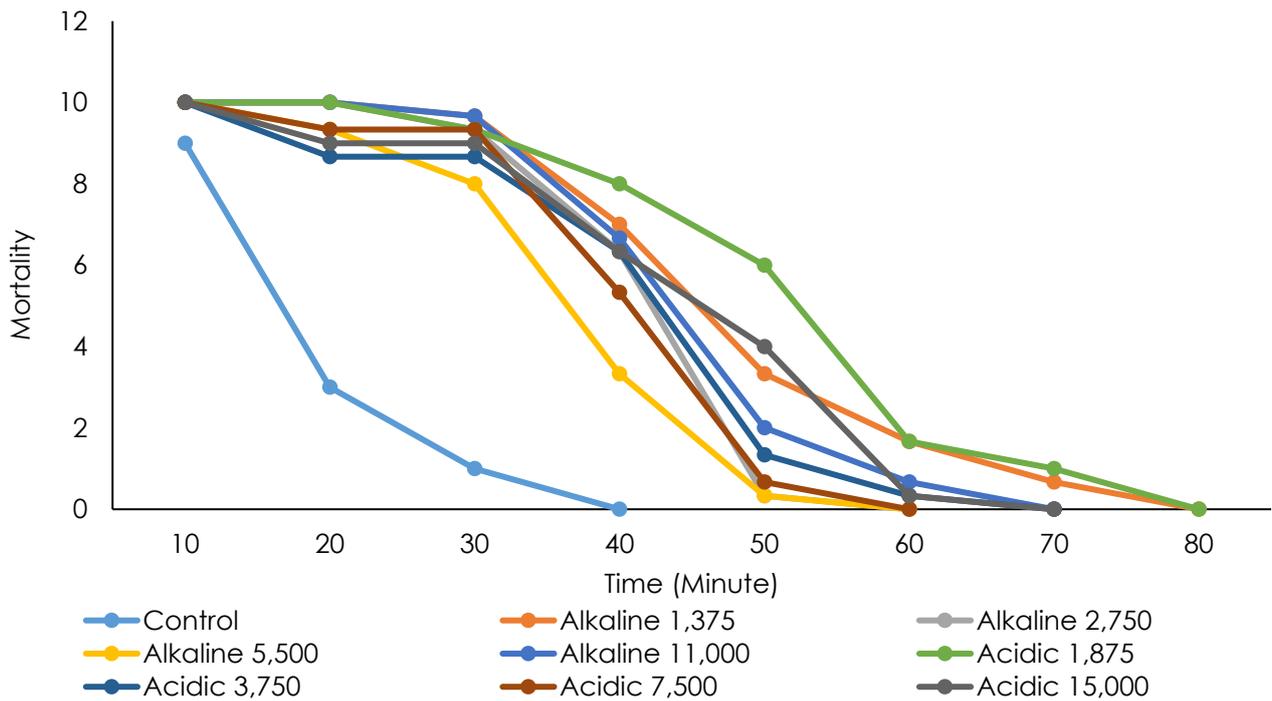


Figure 4. Resistance Level of zebrafish *Danio rerio* after Exposure to pH Shock Test

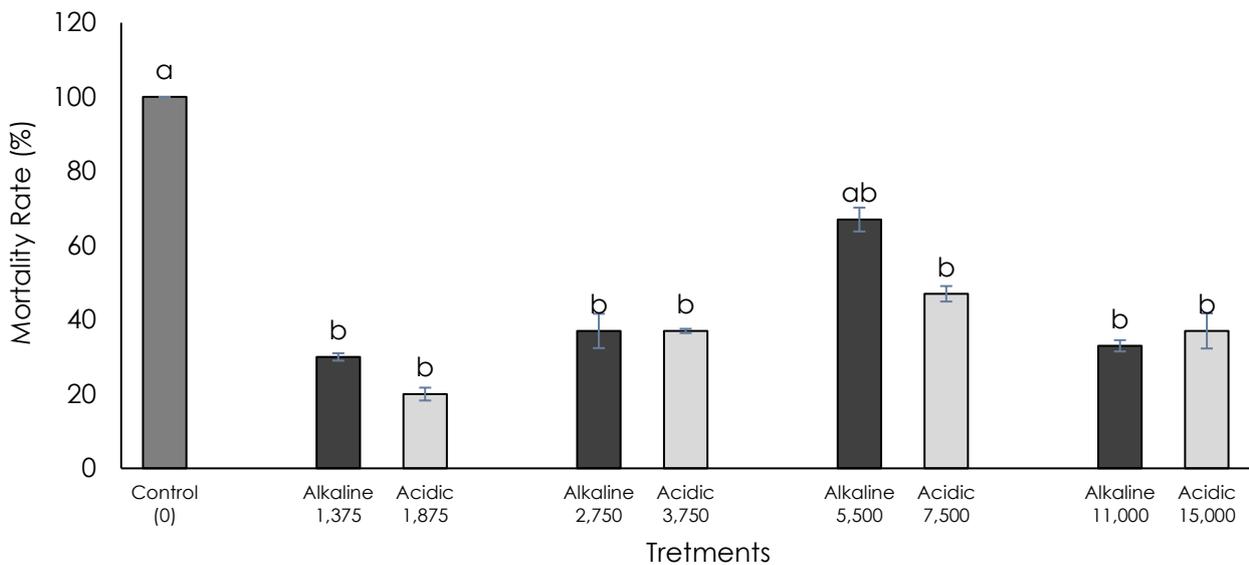


Figure 5. Mortality Rate (%) of zebrafish *Danio rerio* under Acidic pH Shock Test After 40 Minutes Exposure

pH shock test results on zebrafish supplemented with *Sargassum* sp. extract, both using alkaline and acidic extraction methods, showed a nearly similar trend, with total fish mortality occurring at the 80th minute across all treatments (Figure 4). Furthermore, the ANOVA test conducted at the 40th minute revealed statistically significant differences ($P < 0.05$) with similar graphical patterns. The use of supplementation demonstrated a positive response compared to the control group, suggesting its potential to enhance fish performance. This finding is supported by Wang *et al.* (2021), who reported that polysaccharides from *Sargassum fulvellum* improved the survival of zebrafish under oxidative stress induced by AAPH by reducing reactive oxygen species (ROS) levels, preventing cell death, and decreasing lipid peroxidation.

Table 1. Average Observations of Water Quality Parameters during zebrafish (*Danio rerio*) Rearing

Time	Parameter		
	Temperature (°C)	pH	DO (mg/L)
Morning	27.8	7.7	4.6
Afternoon	30.5	7.8	4.8

The increased stress tolerance of the fish can be attributed to the bioactive compounds in the aqueous extract of *Sargassum*, which are rich in sulfated polysaccharides (including fucoidan, alginate, and laminarin), polyphenols, and carotenoid pigments known to enhance fish immunity (Seo *et al.*, 2022). The difference between the two extraction methods was observed at the 50th minute, where the acidic extraction method exhibited a lower mortality rate compared to the base extraction method. In the alkaline extraction group, the lowest mortality rate and the longest survival time were observed at a dose of 1,375 ppm (30% mortality), while in the acidic extraction group, the best resistance was found at 1,875 ppm (20% mortality). This indicates that at lower doses, the bioactive components in the extract can be absorbed and utilized optimally by the fish.

Water quality is one of the key factors influencing the survival and growth of *D. rerio*. Based on observations during the 30-day rearing period, the water temperature ranged from 27.8°C to 30.5°C, pH values were within 7.7–7.8, and dissolved oxygen (DO) levels ranged between 4.6 and 4.8 mg/L. According to Dewantari *et al.* (2024), *D. rerio* can tolerate temperatures up to 30°C as long as stability is maintained, with an optimal pH range of 7.0–8.0, indicating that the observed conditions remained within a safe threshold. The DO concentration in this study was considered moderate, as supported by Khosim *et al.* (2023), who stated that zebrafish can survive at a minimum DO level of 4 mg/L in the absence of other environmental stressors, although optimal growth is achieved at DO levels between 6–8 mg/L. The presence of a stable aeration system during rearing helped maintain adequate oxygen supply and prevented hypoxic stress. Moreover, stable water quality conditions play a more crucial role than slight deviations in absolute values, as stability allows fish to perform optimal physiological adaptation.

CONCLUSION

The findings of this study indicate that *Sargassum* sp. extracts, both acidic (pH 3) and alkaline extraction (pH 12), are non-toxic (LC₅₀ > 1,000 ppm). The main results demonstrate that supplementation of the extract significantly (P<0.05) enhances the absolute weight gain and tolerance of *D. rerio* to low-pH stress, although it has no significant effect (P>0.05) on survival rate during the experiment. Supplementation of low alkaline *Sargassum* extract (1,375 ppm) reached the highest absolute growth. The lowest acidic extract (1,875 ppm) improved the tolerance to extreme pH stress. However, its effectiveness decreased at higher doses, indicating a narrow optimal dosage range. Furthermore, the potential co-extraction of undesired cellular materials, particularly with the acidic extraction method, may limit performance. Therefore, future studies should focus on identifying the specific active bio-compounds responsible, optimizing extraction methods, and validating their effectiveness at commercial aquaculture scales.

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