E-ISSN 2477-6939 P-ISSN 0216-0749
AQUACULTURA INDONESIANA
An International Journal of Indonesian Aquaculture Society

Aquacultura Indonesiana, 27(1), 2026
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On-line: https://aquacultura.id/index.php/ai/article/view/211

Screening of Herbal Extracts as Potential Phytobiotics for Crab Aquaculture: Phytochemical, Toxicity, and Antioxidant Evaluation

Yushinta Fujaya[1*], Siti Aslamyah[1], Asmi Citra Malina[1], Herlina Rasyid[2], Dwi Kesuma Sari[3], Herlinah[4]

[1] *Fisheries Department, Faculty of Marine Sciences and Fishery, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia*
[2] *Chemistry Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia*
[3] *Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia*
[4] *Technology in Aquaculture and Postharvest Study Program, Faculty of Vocational, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia*

Submitted: March 10th, 2026; Revised: May 19th, 2026; Accepted: May 21th, 2026; Available Online: May 25th, 2026

10.21534/ai.v27i1.211

Abstract

Phytobiotics have gained increasing attention in aquaculture as natural additives to improve the health and performance of cultured species, particularly crustaceans that are highly susceptible to oxidative stress. However, the identification of effective and safe herbal candidates remains limited due to the lack of integrative evaluation approaches. This study aimed to screen selected herbal extracts as potential phytobiotic candidates for crab aquaculture based on their phytochemical composition, toxicity, and antioxidant activity. Six herbal plants: Moringa oleifera, Amaranthus sp., Morus sp., Sargassum sp., Avicennia sp., and Apium graveolens were evaluated. Phytochemical screening was conducted using standard qualitative methods, toxicity was assessed using the Brine Shrimp Lethality Test (BSLT), and antioxidant activity was determined using the DPPH assay. All extracts contained various secondary metabolites, with flavonoids and steroids/terpenoids being the dominant compounds. Toxicity analysis indicated LC_{50} values of 786.69 µg/mL for M. oleifera, and 779.40 µg/mL for Morus sp., indicating safe to moderately toxic ranges. In comparison, Sargassum sp. (1355.56 µg/L) and Avicennia sp. (2199.20 µg/mL) exhibited the lowest toxicity. Antioxidant activity varied among species, with A.graveolens (IC_{50} = 324.24 ± 6.00 µg/mL), M. oleifera (234.14 ± 7.23 µg/mL), and Morus sp. (199.18 ± 1.58 µg/mL) showing strong activity. Scatter plot analyses and an integrative Phytobiotic Safety–Efficacy Map approach identified T. catappa, M. oleifera, and Morus sp. as the most promising phytobiotic candidates due to their strong antioxidant activity and acceptable safety levels. These findings highlight the potential of herbal phytobiotics to mitigate oxidative stress and support physiological processes in crab aquaculture.

Keywords: Antioxidant Activity; Crab Aquaculture; Herbal Extracts; Oxidative Stress; Phytobiotics; Toxicity; Phytochemical Screening;

1. Introduction

Aquaculture has emerged as one of the fastest-growing sectors in global food production, with crustaceans representing a high-value commodity that supports both local livelihoods and international trade (Ababouch *et al.,* 2022). Among crustaceans, crabs are of considerable economic importance in tropical regions. However, the sustainability of crab aquaculture is increasingly constrained by challenges such as physiological stress, disease outbreaks, and inconsistent growth performance, which are often associated with intensive culture systems and fluctuating environmental conditions (Apine *et al.,* 2023).

One of the key physiological issues affecting cultured crabs is oxidative stress (Paital & Chainy, 2012; Wang *et al.,* 2022), resulting from an imbalance between the production of reactive oxygen species (ROS) and the organism's antioxidant defense mechanisms. Elevated oxidative stress has been linked to impaired molting processes, reduced immune competence, and increased mortality in crustaceans (Xu

*) Corresponding author: yushinta.fujaya@unhas.ac.id

et al., 2020). In this context, the application of functional feed additives with antioxidant properties has gained significant attention as a strategy to enhance physiological resilience and improve aquaculture productivity (Hu *et al.,* 2025).

In recent years, phytobiotics derived from herbal plants have been increasingly explored as natural alternatives to synthetic additives in aquaculture systems (Bhanja *et al.,* 2023). Herbal extracts are known to contain diverse bioactive compounds, including flavonoids, phenolics, alkaloids, and terpenoids, which exhibit antioxidant, antimicrobial, and immunostimulatory activities (Dar *et al.,* 2023). These properties make phytobiotics particularly promising for supporting health and performance in cultured species. Moreover, the use of plant-based bioactives is generally considered environmentally friendly and sustainable, aligning with current trends toward eco-friendly aquaculture practices (Kolygas *et al.,* 2025).

However, current approaches remain empirical. Many studies focus solely on biological activity without adequately addressing toxicity, a crucial factor in determining the suitability of herbal extracts for aquaculture applications. Preliminary phytochemical screening is often underutilized as a tool to provide insight into the class of compounds that may contribute to the observed biological activity. Therefore, a more integrated approach is needed; a systematic screening approach that combines phytochemical characterization, antioxidant activity assessment, and toxicity evaluation is essential for identifying safe and effective herbal candidates for aquaculture use. Such an approach would enable the identification of promising plant extracts while providing a scientific basis for their potential application as phytobiotics in crustacean culture systems. In this study, we aimed to screen selected herbal extracts as potential phytobiotic candidates for crab aquaculture by evaluating their phytochemical composition, toxicity levels, and antioxidant activities. The results of this study are expected to contribute to the development of natural, safe, and sustainable bioactive inputs to support improved health and productivity in crab aquaculture.

2. Materials And Methods

2.1. Plant Materials and Preparation of Extracts

Six herbal plants: *Moringa oleifera*, *Amaranthus* sp., *Morus* sp., *Sargassum* sp., *Avicennia* sp., and *Terminalia catappa*, were collected from South Sulawesi, Indonesia (Figure 1). The plant materials were washed with distilled water, air-dried at room temperature, and ground into fine powder. Extraction was performed using the maceration method with [solvent, e.g., 70% ethanol] at a ratio of [1:10 (w/v)] for [48 h] under continuous agitation. The extracts were filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40–45°C under reduced pressure. The crude extracts were then stored at 4°C until further analysis.

Figure 1. Six herbal plants were evaluated as potential phytobiotic candidates in this study: *Moringa oleifera*, *Amaranthus sp*, *Morus sp*., *Sargassum sp*., *Avicennia sp*., and *Apium graveolens*

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2.2. Phytochemical Screening

Preliminary phytochemical screening was conducted to identify the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids, following standard qualitative procedures. Alkaloids were detected using Dragendorff's, Mayer's, and Wagner's reagents, flavonoids were identified using natrium hydroxide (NaOH 10%) solution, tannins were detected using ferric chloride ($FeCl_3$ 10%) solution, saponins were determined by the foam test with hot distilled water, steroids and terpenoids were identified using acetate anhydride + H_2SO_4 reaction. The presence or absence of each compound group was recorded qualitatively.

2.3. Toxicity Assay

Toxicity of the herbal extracts was evaluated using the Brine Shrimp Lethality Test (BSLT) with *Artemia salina* nauplii. Artemia cysts were hatched in artificial seawater (salinity 30–35 ppt) under continuous aeration and illumination for 24–48 h. Active nauplii were collected and transferred into test vials containing different concentrations of each extract ([e.g., 10, 50, 100, 250, 500, and 1000 µg/mL]). Each treatment was performed in triplicate with 10 nauplii per vial. After 24 h exposure, the number of dead nauplii was recorded, and LC_{50} values were calculated using probit analysis.

Table 1. Toxicity levels were categorized as follows:

Toxicity Level	LC_{50} (µg/mL)
High toxicity	< 100
Moderate toxicity	100 - 500
Low toxicity	500 - 1000
Non-toxic	> 1000

2.4. Antioxidant Activity Assay

2.4.1. DPPH Radical Scavenging Activity

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A DPPH solution (0.1 mM) was prepared in methanol. Various concentrations of each extract were mixed with DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage of inhibition was calculated as:

% DPPH Inhibition = [Abs Vit C – Abs Sample / Abs Control]x 100%

IC_{50} values were determined from the inhibition curve. Ascorbic acid was used as a positive control.

Table 2. Category of antioxidant activity strength in vitro against DPPH

Intensity of IC_{50}	Value (µg/mL)
Very active	< 50
Active	50 - 100
Medium	101 - 250
Weak	250 - 500
Inactive	> 500

2.5. Data Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). LC_{50} values were calculated using probit regression analysis. IC_{50} values were determined using nonlinear regression analysis. A scatter plot was used to visualize the relationship between toxicity and antioxidant activity among phytobiotic candidates. In this study, the integrated Phytobiotic Safety-Effectiveness Map approach was used to identify the most promising phytobiotic candidates due to their strong antioxidant activity and acceptable safety level (Table 3).

Table 3. Phytobiotic Safety-Effectiveness Map approach

Zone	characteristics	interpretation
A	High antioxidant + low toxicity	Ideal phytobiotic
B	High antioxidant + high toxicity	Potent but risky
C	Low antioxidant + low toxicity	Limited bioactivity
D	Low antioxidants + high toxicity	Restricted application

3. Results

3.1. Phytochemical Composition of Herbal Extracts

Phytochemical screening revealed the presence of various secondary metabolites across all tested herbal extracts, with notable variation in composition among species (Table 3). Flavonoids were the most consistently detected compounds, particularly in Morus sp. and Sargassum sp., which contain high levels of flavonoids.

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Table 3. Phytochemical screening of herbal extracts

Plant	Alkaloid			Steroid/terpenoid	Tanin	Flavonoid	Saponin
	Dragendorf	Mayer	Wagner				
Moringa oleifera	(+)	(+)	(+)	(++)	(-)	(++)	(+++)
Morus sp.	(-)	(-)	(-)	(+++)	(-)	(+++)	(-)
Amaranthus sp.	(-)	(-)	(-)	(+++)	(-)	(+)	(+)
Sargassum sp.	(-)	(-)	(-)	(+++)	(-)	(+++)	(++)
Avicennia sp.	(-)	(+)	(-)	(+)	(-)	(+)	(-)
Apium graveolens	(-)	(+)	(-)	(+++)	(-)	(++)	(-)

Note: (–) not detected; (+) low; (++) medium; (+++) high

3.2. Toxicity of Herbal Extracts

The toxicity of the herbal extracts varied considerably, as indicated by LC_{50} values obtained from the BSLT assay (Table 4).

Table 4. Toxicity of Herbal Extracts

Plant	LC_{50} (µg/mL)	Toxicity Level
Moringa oleifera	786.69	Low toxicity
Morus sp.	779.4	Low toxicity
Amaranthus sp.	13.05	High toxicity
Sargassum sp.	1355.56	Non-toxic
Avicennia sp.	2199.2	Non-toxic
Apium graveolens	26.3	High toxicity

3.3. Antioxidant Activity of Herbal Extracts

The antioxidant activity of the extracts, expressed as IC_{50} values, showed significant variation among species (Table 5).

Table 5. Antioxidant Activity of Herbal Extracts

Plant	IC_{50} (µg/mL)	Antioxidant Strength
Moringa oleifera	234.14 ± 7.23	medium
Morus sp.	199.18 ± 1.58	medium
Amaranthus sp.	660.18 ± 4.33	In active
Sargassum sp.	>1000	in active
Avicennia sp.	377.08 ± 2.65	weak
Apium graveolens	324.24 ± 6.00	weak

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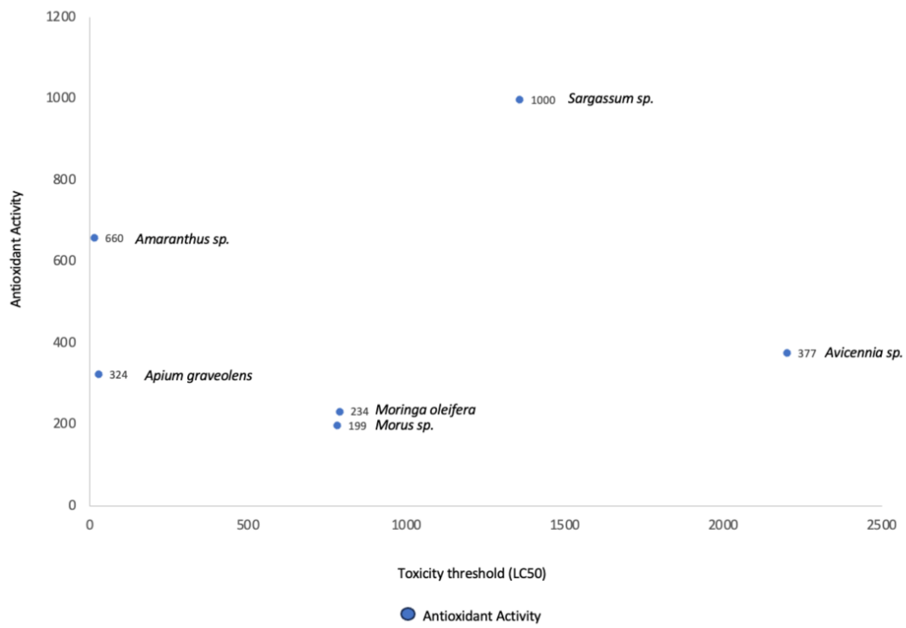

FIGURE 2. Scatter plot of antioxidant versus toxicity threshold (LC50) s for six plant extracts.

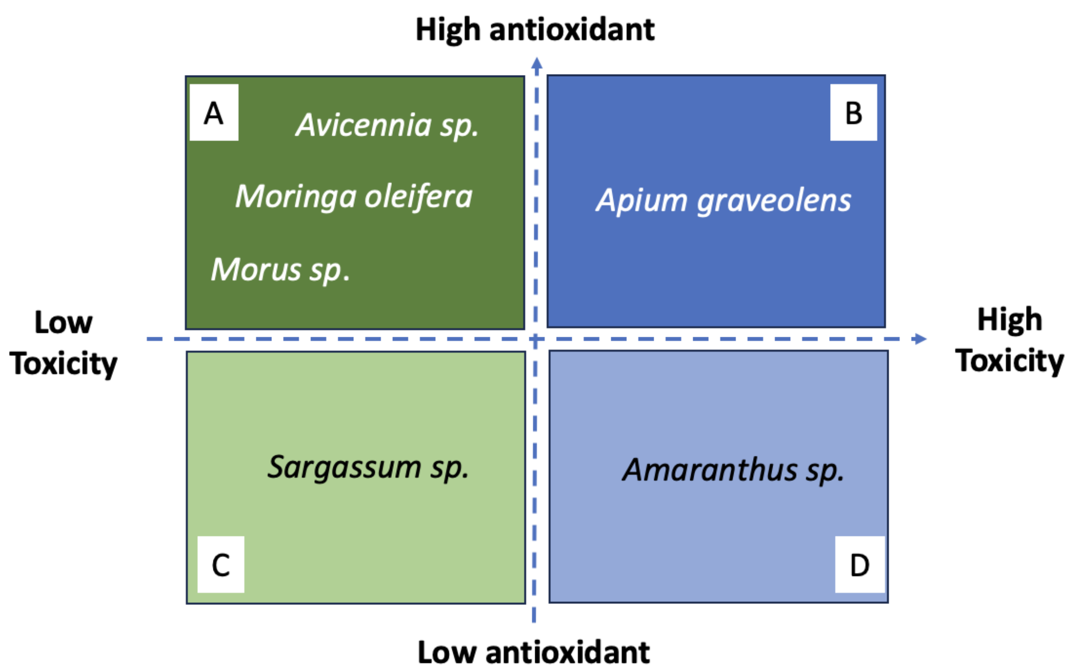

Figure 3. Phytobiotic Safety–Effectiveness map of six plant extracts**.** Zona A, Ideal phytobiotic, Zona B, Potent but risky, Zona C, Weak activity, Zona D, Undesirable.

3.4. Integrated Evaluation of Phytobiotic Potential

To identify the most promising candidates, an integrated evaluation was conducted by considering toxicity and antioxidant activity. Scatter plot analyses (Figure 2) and using an integrative Phytobiotic Safety–Effectiveness Map approach identified *Morus sp*. *M. oleifera*, and *Avicennia sp*. as the most promising phytobiotic candidates due to their strong antioxidant activity and low toxicity (Figure 3). For simplification of the phytobiotic screening framework, antioxidant activity was categorized into two levels: high (medium to high) and low (inactive to low) antioxidant capacity based on IC_{50} threshold values

3.2. Discussion

The present study demonstrated that the tested herbal extracts exhibit diverse phytochemical compositions, toxicity levels, and antioxidant activities, which collectively determine their potential as phytobiotic candidates for crab aquaculture. The integration of these parameters is essential, as effective phytobiotics must not only provide biological benefits but also ensure safety for cultured organisms.

3.2.1 Phytochemical Basis of Antioxidant Activity

The presence of high levels of flavonoids indicates a strong antioxidant capacity (Table 1). The strong antioxidant activity observed in *Morus* sp., *M. oleifera,* and *Avicennia sp*. is likely associated with their high flavonoid content identified during phytochemical screening. These phenolic compounds are well known for donating hydrogen atoms or electrons, thereby neutralizing reactive oxygen species (ROS) and preventing oxidative damage (Jevas, 2016; Jomova *et al.,* 2023). In crustaceans, excessive ROS production is a common physiological response to environmental stressors such as fluctuating salinity, temperature changes, and high stocking density (Hapsari *et al.,* 2025). Without adequate antioxidant defenses, ROS accumulation can damage cellular components, including lipids, proteins, and DNA, ultimately impairing physiological functions (Jevas, 2016).

Steroids/terpenoids were also widely distributed across all tested samples, especially in *Morus* sp., *Amaranthus* sp., *Sargassum* sp., and *Apium graveolens*. Steroids/terpenoids are important for molting and growth. Several previous studies reported the effect of administering phytoecdysteroids, namely ecdysteroid hormone mimics found in plants, which have the potential to stimulate molting and growth in crustaceans (Sorach *et al.,* 2013; Ahmad *et al.,* 2015; Rosmiati *et al.,* 2016; Fujaya *et al.,* 2018; Sutrisno *et al.,* 2025). The diversity of phytochemical constituents observed in this study highlights the potential of these herbal plants as sources of bioactive compounds for aquaculture applications.

3.2.2. Relevance to Oxidative Stress in Crab Physiology

Antioxidants are usually associated with anti-stress. Oxidative stress plays a central role in determining the health and performance of cultured crabs. Elevated ROS levels have been linked to reduced immune response, increased susceptibility to pathogens, and decreased survival rates (Spooner & Yilmaz_2011; Li *et al.,* 2021). Therefore, the application of antioxidant-rich phytobiotics offers a promising strategy to enhance the endogenous defense system of crabs. The low IC_{50} values observed in *M. oleifera*, *Morus* sp., and *A. graveolens* extracts indicate strong radical scavenging capacity, suggesting their potential to mitigate oxidative stress in aquaculture systems. This is particularly relevant in intensive farming conditions, where crabs are frequently exposed to suboptimal environmental conditions that trigger oxidative imbalance.

3.2.3. Link Between Antioxidants and Molting Process

Molting is a critical physiological process in crabs, governing growth, regeneration, and reproduction (Hosamani et al_2017; Yuan *et al.,* 2023). This process is tightly regulated by hormonal and biochemical pathways, and is highly sensitive to oxidative status. During molting, crabs undergo extensive metabolic and structural changes, including exoskeleton degradation and synthesis, which are energetically demanding and prone to oxidative stress. Excessive ROS during this phase can disrupt endocrine signaling, delay molting cycles, and increase mortality risk. (Mykles, 2021). In this context, herbal extracts with strong antioxidant properties, such as *T. catappa*, *M. oleifera*, and *Morus* sp may play a supportive role in stabilizing oxidative balance during molting. By reducing oxidative damage, these phytobiotics may help maintain physiological homeostasis, thereby promoting successful molting and improving growth performance. This mechanism may involve the modulation of endocrine pathways associated with ecdysteroid regulation.

3.2.4. Importance of Toxicity Screening for Aquaculture Application

While antioxidant activity is desirable, safety remains a critical consideration in the application of herbal extracts. The present study revealed that some extracts, such as *Amaranthus* sp., exhibited high toxicity despite containing important bioactive compounds. This finding highlights a key trade-off in phytobiotic selection: compounds with strong bioactivity may also exert toxic effects at certain concentrations. Therefore, toxicity screening is essential to define safe dosage ranges and avoid adverse impacts on cultured species (Parasuraman, 2011). In contrast, *M. oleifera* and *Morus sp*. extracts demonstrated low toxicity, indicating their suitability for application in aquaculture systems. The combination of low toxicity and strong antioxidant activity makes *M. oleifera* and *Morus sp*. particularly promising as a phytobiotic candidate.

Amaranthus sp. extracts showed comparatively higher toxicity, which may be associated with the presence of bioactive compounds such as phenolics and terpenoids known for their defensive roles in natural environments. In contrast, *Sargassum sp*. and Avicennia sp extracts demonstrated low toxicity levels, indicating their potential suitability as safe phytobiotic candidates. These findings emphasize the importance of toxicity screening to ensure that selected herbal extracts do not pose harmful effects when applied in aquaculture systems. In its application, the dose is considered based on the toxicity level. Plant extracts with high toxicity levels can still be used, but at a lower dose. Conversely, plant extracts with lower toxicity can be administered with higher doses.

3.2.5. Integrated Selection of Phytobiotic Candidates

The integration of phytochemical composition, antioxidant activity, and toxicity provides a more comprehensive basis for selecting phytobiotic candidates compared to evaluating single parameters. Scatter plot analysis and the Phytobiotic Safety-Effectiveness Map further support this approach by clearly separating extracts based on their overall bioactivity profiles. *Morus sp*., *M. oleifera*, and *Avicennia sp*. consistently emerged as the most favorable candidates (Figure 3), combining strong antioxidant properties with acceptable safety. Apium graveolens also exhibits high antioxidant potential; however, its high toxicity suggests that dosage optimization is necessary. Typically, only a small amount of extract is needed to elicit the required response from this group of plants. For example, the toxic tuba root can still be used medicinally with a high degree of caution (Pandey *et al.,* 2018). Similarly, the use of *Amarantus sp*. These extracts, despite being detected to have high levels of toxicity, can be used to stimulate molting in shrimp and crabs using low doses (Fujaya *et al.,* 2011; Sahare, 2018; Sutrisno *et al.,* 2025; Redzuari *et al.,* 2026). Although some extracts exhibit relatively high toxicity and low antioxidant performance, they may still contain condition-specific bioactive compounds (e.g., phytoecdysteroids) relevant to aquaculture applications. Therefore, these extracts are categorized in the Limited Application Zone (Table 3). Furthermore, in addition to antioxidants, which are the primary consideration, several active ingredients in plants (e.g., *Sargassum sp*.) need to be considered. *Sargassum* is a brown algae rich in bioactive compounds. Although this study detected *Sargassum* as being in Zone C, which is low antioxidant and low toxicity, *Sargassum* is rich in fucoxanthin, carotene pigment, and minerals that are important for health and disease prevention (Alfreen *et al.,* 2023).

3.2.6. Implications for Sustainable Crab Aquaculture

The use of herbal-based phytobiotics aligns with the growing demand for sustainable and environmentally friendly aquaculture practices. By reducing reliance on synthetic chemicals and enhancing the natural defense mechanisms of cultured species, phytobiotics offer a viable pathway toward more resilient aquaculture systems. In crab aquaculture, the use of selected herbal extracts may improve stress tolerance, enhance molting success, and enhance survival and growth performance. However, further studies are required to validate these effects under in vivo conditions, including feeding trials and physiological assessments.

4. Conclusion

This study demonstrated that herbal extracts exhibit distinct phytochemical profiles, antioxidant capacities, and toxicity levels that collectively influence their suitability as phytobiotic candidates for crab aquaculture. Using the Phytobiotic Safety–Effectiveness Map approach, the study successfully identified candidate extracts by integrating antioxidant activity and toxicity assessment.

Among the plants tested, Moringa oleifera, Morus sp., and Avicennia sp. showed the greatest potential as phytobiotics due to their relatively strong antioxidant activity and low toxicity levels. These properties indicate their potential application in reducing oxidative stress and supporting physiological functions in farmed crabs. However, A. graveolens and Amaranthus sp. also have potential, although they require dosage optimization due to their high toxicity profiles. Sargassum sp., despite having low antioxidant activity, is rich in fucoxanthin, beta-carotene pigment, and minerals that are important for health and disease prevention.

Overall, the findings emphasize the importance of integrating phytochemical characterization, antioxidant evaluation, and toxicity screening in the early selection of herbal resources for aquaculture. The proposed framework provides a practical and scientifically grounded strategy for identifying safe and effective phytobiotics in crustacean aquaculture. Further in vivo studies are needed to validate their effects on growth, molting performance, and stress tolerance, as well as to clarify their underlying mechanisms of action.

5. Acknowledgment

This work was supported by the National Research and Innovation Agency (BRIN), Research and Innovation for a Progressive Indonesia, Year 1st (Grant no. 48/II.7/HK/2025).

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