AQUATIC RESEARCH

AQUATIC RESEARCH
E-ISSN 2618-6365

Aquatic Research 9(2), 148-165 (2026) • https://doi.org/10.3153/AR26013 Review Article

The role of *Eleutherine palmifolia* (Dayak Onion) as a natural immunostimulant for enhancing fish disease resistance in aquaculture systems

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Cite this article as:
Fahturohman, A.B., Takwin, B.A., Ristyaningrum, W., Muna, Z., Feska, S. (2026). The role of Eleutherine palmifolia (Dayak Onion) as a natural immunostimulant for enhancing fish disease resistance in aquaculture systems. Aquatic Research, 9(2), 148-165. https://doi.org/10.3153/AR26013

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Submitted: 26.12.2025
Revision requested: 02.02.2026
Last revision received: 09.02.2026
Accepted: 10.02.2026
Published online: 30.03.2026

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ABSTRACT

The global food crisis, driven by population growth, climate change, and natural resource degradation, has intensified the need for efficient, affordable, and environmentally sustainable sources of animal protein. In aquaculture, this demand has accelerated the exploration of alternative feed ingredients and natural additives that enhance immune responses, suppress pathogens, and promote fish growth. *Eleutherine palmifolia* (Dayak onion), an endemic plant from Kalimantan, has emerged as a promising candidate due to its strong antibacterial activity and safety profile without chemical residues. Using a PRISMA-guided systematic review, this study synthesises current evidence on the phytochemical composition, immunological mechanisms, antibacterial activity, and application potential of *E. palmifolia* in sustainable aquaculture. The extract exhibits anti-inflammatory effects by modulating pro-inflammatory cytokines, enhancing TNF-α expression while reducing IL-β levels, thereby supporting a balanced immune response without excessive oxidative stress. Both ethanol and aqueous extracts effectively inhibit *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae*. The plant's secondary metabolites, including flavonoids, naphthoquinones, polyphenols, tannins, and saponins, demonstrate broad biological activities that improve fish health, growth, and disease resistance. Overall, *E. palmifolia* holds strong potential as an immunostimulant, antioxidant, and phytobiotic agent for environmentally friendly aquaculture practices.

Keywords: *Eleutherine palmifolia*, Immunostimulant, Antibacterial activity, Fish health, Sustainable aquaculture

Introduction

The global food crisis, driven by population growth, climate change, and natural resource degradation, has intensified the need for innovation in sustainable food production systems (Qu et al., 2024; Said et al., 2025). Aquaculture plays a strategic role in addressing this challenge by providing efficient and environmentally friendly sources of animal protein. According to FAO (2025), global fish production reached 223.2 million tons in 2022, reflecting a pronounced transition from capture fisheries to aquaculture. This shift is particularly evident in Asia, which contributes nearly 90% of global aquaculture output and positions the region as a central driver of food security and low-emission fisheries development (Chan et al., 2024). Nevertheless, the long-term sustainability of aquaculture remains constrained by recurrent disease outbreaks that cause substantial production losses and economic impacts (Subhashreedevasena et al., 2022; Patil et al., 2025)

In response to these challenges, global research efforts increasingly emphasise the development of alternative feed ingredients and natural functional additives that enhance immune responses, suppress pathogenic infections, and improve fish growth performance (Idenyi et al., 2022; Van Doan et al., 2023; Banu et al., 2025). Among these strategies, phytobiotics, bioactive compounds derived from medicinal plants, have gained considerable attention due to their multifunctional biological activities, lack of antibiotic residues, and environmental compatibility (Mabrouk et al., 2025). Consequently, locally sourced herbal plants are increasingly recognised as viable and eco-friendly alternatives to antibiotics in modern aquaculture systems (Banu et al., 2025).

Various local herbal plants, including garlic (*Allium sativum*), pennywort (*Phyllanthus niruri*), turmeric (*Curcuma longa*), and temulawak (*Curcuma xanthorrhiza*), have been widely evaluated as natural immunostimulants in aquaculture (Rezaei et al., 2022; Khieokhajonkhet et al., 2023; Rosidi et al., 2025). However, the efficacy of phytobiotics strongly depends on dosage, extraction methods, and the stability of bioactive compounds during feed formulation and coating processes (Pudota et al., 2025). Moreover, many phytobiotic candidates still require dose optimisation and long-term toxicity assessments to ensure safe and effective application in intensive farming systems. By contrast, probiotics as biological additives often exhibit instability in microbial populations under fluctuating culture conditions, leading to inconsistent performance outcomes (Rahayu et al., 2024). Similarly, low-dose vaccines show limited efficacy, as they frequently fail to provide long-term protection against diverse pathogen strains, particularly in high-density aquaculture systems (Miccoli et al., 2021).

One of the endemic plants from Kalimantan that has gained increasing attention is *Eleutherine palmifolia*, commonly known as Dayak onion. This species offers distinct advantages over other medicinal plants due to its strong antibacterial activity, which mimics antibiotic mechanisms while remaining safe and residue-free (Arbain et al., 2022). This characteristic highlights the importance of exploring Indonesia's endemic flora, which possesses broad bioactivity and ecological relevance. *Eleutherine palmifolia* contains diverse bioactive compounds, including alkaloids, flavonoids, naphthoquinones, saponins, tannins, and triterpenoids, that exhibit antibacterial, anti-inflammatory, antioxidant, and immunomodulatory properties (Chabib et al., 2018; Harlita et al., 2018; Masfria & Tampubolon, 2019; Annisa et al., 2020; Hongthongkham et al., 2025). The presence of these compounds supports the potential application of *E. palmifolia* as a phytobiotic in modern aquaculture systems. Mechanistically, the bioactive constituents of Eleutherine palmifolia enhance fish disease resistance through multiple innate immune pathways. These effects include increased phagocytic activity, stimulation of lysozyme production, and activation of non-specific immune signalling against major aquaculture pathogens such as *Aeromonas hydrophila* and *Vibrio haryeyi* (Hasim et al., 2023).

Previous studies have demonstrated that dietary supplementation with Dayak onion extract enhances phagocytic activity, total leukocyte counts, and post-infection survival rates in fish challenged with pathogenic bacteria (Nur et al., 2020; Hasim et al., 2023). Its ethanol extract also exhibits strong antibacterial activity against *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* by disrupting bacterial cell membranes and inducing protein denaturation (Harlita & Aina, 2023; Sirajuddin et al., 2025). Beyond its immunological and antibacterial effects, the complex carbohydrates and polyphenols present in *Eleutherine palmifolia* function as natural prebiotics, supporting gut microbiota balance and enhancing nutrient absorption through fermentative activity and improved intestinal functionality (Andriani et al., 2024). The synergistic integration of immunostimulatory, antibacterial, antioxidant, and growth-promoting effects positions *E.*

palmifolia as a promising phytobiotic capable of partially replacing antibiotics and synthetic additives in aquaculture feeds.

Despite growing interest in *Eleutherine palmifolia* as a natural immunostimulant in aquaculture, existing studies remain fragmented and largely descriptive. Most investigations emphasise isolated outcomes such as antibacterial activity, haematological responses, or survival rates without integrating these findings into a coherent mechanistic framework. Furthermore, immune modulation pathways are frequently inferred rather than experimentally validated, and standardised extraction methods, dosage regimes, and application strategies remain lacking. Notably, the performance of *E. palmifolia* in intensive aquaculture systems, including biofloc and recirculating aquaculture systems, has received limited attention. These knowledge gaps constrain the translational application of *E. palmifolia* as a phytobiotic alternative to antibiotics.

Accordingly, this article is designed as a PRISMA-guided systematic integrative review that synthesises existing evidence on the application of *Eleutherine palmifolia* as a phytobiotic in aquaculture systems. The novelty of this review resides in three principal contributions. First, unlike previous reviews that largely emphasise phytochemical identification or general pharmacological properties, this study systematically integrates aquaculture-specific evidence linking phytochemical profiles to functional immune, antibacterial, antioxidant, and growth-related outcomes in aquatic species. Second, this review explicitly distinguishes between direct experimental evidence and indirect mechanistic inference, thereby providing a critical evidence-weighted framework that clarifies the strength, limitations, and translational relevance of existing findings. Third, by contextualising current evidence within modern intensive aquaculture systems, including biofloc and recirculating aquaculture systems (RAS), this review extends applicability beyond laboratory-scale studies toward production-oriented scenarios. Through this integrative and critical approach, the present review aims to advance mechanistic understanding, identify methodological gaps, and support the rational development of *E. palmifolia* as a sustainable phytobiotic alternative to antibiotics in aquaculture.

Materials and Methods

Literature Search Strategy

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and reproducibility. A comprehensive literature search was performed using three major scientific databases: Scopus, Web of Science, and PubMed. The search was conducted between January 2016 and June 2025 using combinations of the following keywords: “Eleutherine palmifolia” OR “Dayak onion” AND “aquaculture” OR “immunostimulant” OR “phytobiotic” OR “antibacterial” OR "growth performance."

Study Selection Process

All retrieved records were imported into reference management software, and duplicate records were removed. Titles and abstracts were screened for relevance, followed by full-text assessment based on inclusion and exclusion criteria. The study selection process followed the PRISMA flow diagram, resulting in the final dataset used for qualitative synthesis. The study selection process is summarised in the PRISMA flow diagram (Figure 1).

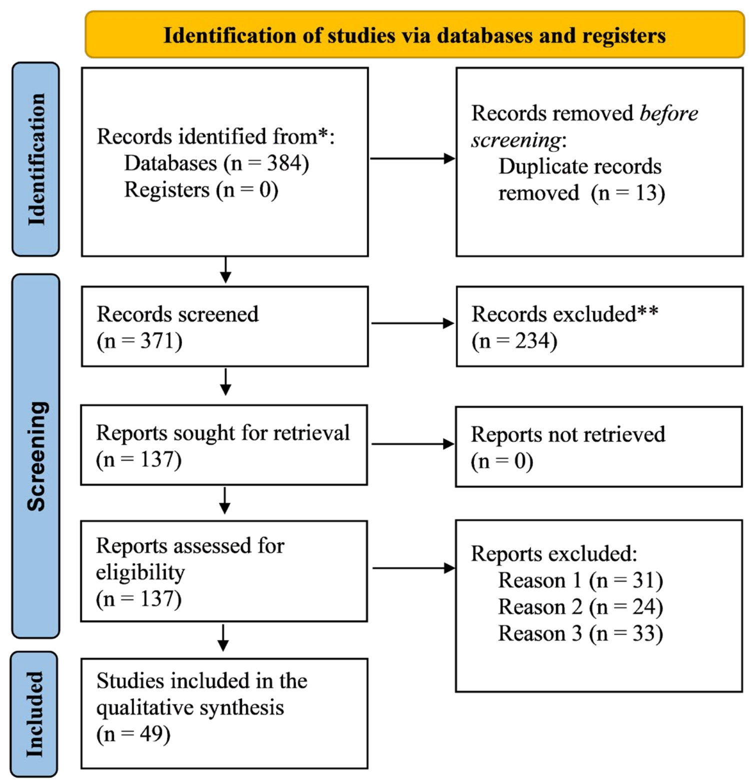

Figure 1. PRISMA flow diagram showing the literature search and selection process for studies on *Eleutherine palmifolia* in aquaculture applications

Due to the broad nature of keyword combinations, an initial title and abstract screening was applied to exclude records not directly related to *Eleutherine palmifolia* and aquaculture applications. All full-text articles considered relevant after title and abstract screening were successfully retrieved and assessed for eligibility. Full-text excluded with reasons (n= 88): not related to aquaculture species (n= 31), human studies only (n= 24), and insufficient experimental outcomes (n=33).

Inclusion and Exclusion Criteria

Inclusion criteria:

1. Original research articles or review papers
2. Studies evaluating Eleutherine palmifolia or its extracts
3. Research related to aquaculture species (fish or crustaceans)
4. Outcomes related to immunity, antibacterial activity, antioxidant capacity, growth performance, or stress resistance

Exclusion criteria:

1. Non-peer-reviewed articles, theses, or conference abstracts without full text
2. Studies focusing solely on human applications
3. Articles lacking clear experimental design or outcome parameters

Data Extraction and Synthesis

From each selected study, the following information was extracted: study type, aquaculture species, plant part and extraction method, dosage and application strategy, measured immune, antibacterial, antioxidant, or growth parameters, main outcomes, and reported limitations. Due to heterogeneity in experimental designs, species, and outcome measurements, a quantitative meta-analysis was not feasible. Therefore, a qualitative integrative synthesis was applied. Accordingly, this article is explicitly defined as a systematic-integrative review, combining a PRISMA-guided systematic literature selection process with an integrative analytical framework that connects phytochemical characteristics, immunological mechanisms, antibacterial actions, and physiological performance outcomes in aquaculture systems. This approach enables comprehensive evidence integration while maintaining methodological transparency and reproducibility.

Study Quality Assessment

To evaluate the methodological robustness of the included studies, a qualitative study quality assessment was conducted. Each study was appraised based on predefined criteria, including clarity of experimental design, description of extraction and application protocols, sample size adequacy, presence of control groups, and relevance of outcome parameters related to immunity, antibacterial activity, growth performance, or physiological responses. Studies were not excluded based on quality scores; instead, variations in methodological rigour were considered during evidence interpretation and synthesis. Given the heterogeneity in species, experimental durations, exposure routes, and measured endpoints, a formal quantitative scoring system was not applied. This approach aligns with integrative review methodologies, which emphasise contextualised interpretation of evidence rather than exclusionary quality thresholds, while still allowing critical evaluation of study reliability and comparability.

Critical Appraisal

A narrative critical appraisal was applied to identify methodological strengths, limitations, inconsistencies, and knowledge gaps across studies, with particular emphasis on dosage standardisation, molecular validation, and applicability in modern intensive aquaculture systems.

Risk of Bias and Limitations

Several sources of potential bias were identified across the reviewed studies. Selection bias was evident in studies employing single-dose designs or limited sample sizes, which may restrict the generalizability of reported outcomes. Performance and detection biases were also observed, as many in vivo experiments relied predominantly on haematological or survival parameters without molecular validation of immune signalling pathways. In addition, variability in extraction methods, solvent types, and plant parts used introduces heterogeneity that may influence bioactive compound composition and biological efficacy.

Publication bias cannot be excluded, as studies reporting positive effects of *Eleutherine palmifolia* were more prevalent than those documenting neutral or negative outcomes. Furthermore, most experiments were conducted under controlled laboratory conditions or short-term trials, limiting extrapolation to intensive aquaculture systems such as biofloc or recirculating aquaculture systems (RAS). These limitations were explicitly considered during evidence synthesis and highlight the need for standardised methodologies, long-term evaluations, and system-based studies to strengthen the translational applicability of *E. palmifolia* as a phytobiotic alternative to antibiotics.

Results and Discussion

Dayak Onion (*Eleutherine palmifolia*)

Dayak onion belongs to the genus *Eleutherine*, which is native to tropical America but has long been introduced and cultivated throughout Asia. A key botanical record dates to 1912, when Merrill documented its presence in the Philippines and classified it as *Eleutherine palmifolia* L. Merr., later recognised as a botanical synonym of *Sisyrinchium palmifolium* L. Over time, this species adapted successfully to Southeast Asian environments and acquired distinct cultural significance. In Kalimantan, its tubers have been used for generations by the Dayak people as traditional medicine to treat various health conditions. This close association with ethnomedical practices led to the widespread use of the local name "Bawang Dayak," which was subsequently adopted in scientific literature and is now commonly used to refer to this species in Indonesia. Figure 2. Morphological characteristics of Dayak onion, showing the whole plant, bright red bulbs, and dried bulb slices.

Dayak Onion Phytochemical Content

Local communities have traditionally used dayak onion to treat various health conditions, including hypertension, hypercholesterolemia, diabetes, gastric disorders, constipation, and stroke, and is also consumed as a postnatal herbal drink. In addition, Dayak onion exhibits antifungal and antioxidant activities (Harlita et al., 2018). Phytochemical analyses have shown that Dayak onion extracts contain diverse secondary metabolites, including flavonoids, naphthoquinones, anthraquinones, alkaloids, saponins, tannins, triterpenoids, and steroids.

Anthraquinones are naturally occurring organic compounds belonging to the phenolic quinone derivatives and are widely distributed in medicinal plants. Anthraquinone extracts derived from *Rheum officinale* Baill. contain bioactive constituents such as emodin, chrysophanol, and rhein, and have long been used as immunostimulants (Huang et al., 1995). More recent studies have demonstrated that anthraquinone extracts significantly enhance immune function, improve stress resistance, and promote growth performance in common carp and giant freshwater prawn (Xie et al., 2008). Findings by Liu et al. (2012) further revealed that dietary supplementation with 0.1% anthraquinone extract from *R. officinale* Baill. effectively increased the resistance of *Megalobrama amblycephala* to infection by the pathogenic bacterium *Aeromonas hydrophila*. This enhanced resistance was mediated through strengthened nonspecific immune responses, including increased lysozyme and alkaline phosphatase activities, reduced physiological stress responses (cortisol, AST, and ALT), and improved hepatic antioxidant capacity, as indicated by elevated catalase and superoxide dismutase activities accompanied by reduced malondialdehyde levels. In addition, the induction of hepatic *HSP70* gene expression before and during the early phase of infection highlights the role of anthraquinones in maintaining cellular homeostasis and stress tolerance during pathogenic challenge.

Saponins are glycoside-based secondary metabolites widely distributed in plants and are well known for their broad biological activities, including antibacterial, immunomodulatory, and antioxidant effects. In aquaculture, saponins have gained attention as phytogenic feed additives and non-antibiotic alternatives for disease control, particularly amid growing concerns about antibiotic resistance and chemical residues in aquatic products. Nevertheless, their efficacy and safety depend heavily on source, dosage, and application method, necessitating careful empirical evaluation. Boran et al. (2015) assessed the antibacterial activity of green tea (*Camellia sinensis*) seed extracts and their secondary metabolite saponin against five major fish pathogens in rainbow trout (*Oncorhynchus mykiss*). The results demonstrated that both saponin and watered tea seed (WTS) exhibited in vitro antibacterial activity, with statistically significant inhibition particularly against *Listonella anguillarum*. In vivo, fish fed saponin-containing diets showed significantly increased survival following challenge with *L. anguillarum*. However, mild and non-vital histopathological alterations were observed in the gills and liver, emphasising that the application of saponins requires careful consideration of dosage and safety.

Figure 2. Dayak onion: (a) whole plant, (b) bright-red bulbs, and (c) dried bulb slices (Harlita et al., 2018)

Recent evidence indicates that plant-derived polyphenols, such as tannic acid, can significantly enhance antioxidant capacity, improve liver and intestinal health, and increase fish survival, particularly under nutritional or metabolic stress conditions. Dietary supplementation with appropriate levels of tannic acid has been shown to elevate catalase activity, reduce malondialdehyde accumulation, decrease hepatic glycogen deposition, and alleviate histopathological alterations in the liver and intestine, thereby improving overall physiological resilience without endocrine manipulation (Zhang et al., 2022). Supporting this concept, Wang et al. (2024), reported that dietary tannic acid supplementation at 200–400 mg/kg in a high-carbohydrate diet enhanced antioxidant capacity, reduced oxidative stress, and improved liver and intestinal health in *Micropterus salmoides*, as evidenced by decreased MDA levels, reduced hepatocyte vacuolation, and improved intestinal morphology. However, higher supplementation levels suppressed growth performance due to inhibition of digestive enzyme activity

Triterpenoids are increasingly recognised as functional phytogenic compounds in aquaculture due to their immunomodulatory, antioxidant, and antimicrobial properties. Dietary supplementation with triterpenoid-rich plant extracts has been shown to enhance innate immune responses, improve epithelial and gut integrity, and increase resistance to bacterial infections in fish. Notably, triterpenic acid– and polyphenol-rich extracts from *Olea europaea* significantly enhanced systemic immunity and protection against furunculosis in Atlantic salmon smolts, demonstrating clear in vivo efficacy in aquaculture settings (Salomón et al., 2021). In addition, triterpenoid compounds such as glycyrrhizic and glycyrrhetinic acids from *Glycyrrhiza glabra* have been reported to exert immunomodulatory, antioxidant, anti-inflammatory, and antimicrobial effects, contributing to improved health status and disease resistance in aquatic animals (Abasubong et al., 2024).

Steroid compounds have historically been used in aquaculture to enhance fish growth and reproduction; however, growing evidence indicates that their application may exert broad adverse effects, including suppression of immune function (reduced T-cell activity and B-cell proliferation), disruption of metabolic and digestive processes, impairment of reproductive systems, and detrimental effects on the hematopoietic system. These concerns raise serious issues regarding food safety and ecosystem health (Islam et al., 2024).

The phytochemical profile of *Eleutherine palmifolia* indicates the presence of multiple bioactive compounds with significant biological activities. These metabolites contribute to the antibacterial, antioxidant, immunomodulatory, and anti-inflammatory properties of the extract. Several studies have reported that naphthoquinones and anthraquinones represent the dominant metabolite groups in Dayak onion bulbs, while flavonoids, alkaloids, tannins, and triterpenoids/steroids have been identified through qualitative phytochemical screening (Kamarudin et al., 2021; Mukti et al., 2023). Collectively, these findings highlight Dayak onion as a promising source of bioactive metabolites with potential applications in health-related fields and sustainable aquaculture. Table 1. Summary of major bioactive compounds identified in Dayak onion.

Table 1. Dayak Onion Phytochemical Content

Compound Name	References
Naphthoquinone	(Mutiah et al., 2020; Kamarudin et al., 2021)
Naphthalene	(Mutiah et al., 2020; Kamarudin et al., 2021)
Polyphenols	(Mutiah et al., 2020; Qureshi & Javed, 2022; Wahdaningsih et al., 2024)
Alkaloid	(Harlita et al., 2018)
Saponin	(Harlita et al., 2018)
Tannin	(Harlita et al., 2018)
Isoliquiritigenin	(Mutiah et al., 2020)
Sitosterol	(Saputra et al., 2016)
Glicoside	(Mukti et al., 2023)
Triterpenoid	(Gunawan et al., 2020)
Eleutherol	(Muti'ah et al., 2020)
Eleutherin	(Muti'ah et al., 2020)
Isoeleuterin	(Muti'ah et al., 2020)
Hongconin	(Muti'ah et al., 2020)
Elecanacin	(Muti'ah et al., 2020)
Isoeleutherol	(Muti'ah et al., 2020)
Dihydroeleuterinol	(Muti'ah et al., 2020)
1,3,6-trihidroksi-8-metilantrakuinon	(Muti'ah et al., 2020)
Eleuterinosida A	(Muti'ah et al., 2020)
6,8-dihidroksi-3,4- dimetoksi-1-metilantraquin-on-2-asam karboksilat metal ester	(Muti'ah et al., 2020)
Oxyresveratrol	(Muti'ah et al., 2020)
Isoliquiritigenin	(Muti'ah et al., 2020)
4-hidroksi-eleuterin	(Muti'ah et al., 2020)

The Role of Dayak Onion (Eleutherine palmifolia) as a Phytobiotic in Functional Fish Feed

Figure 3. Schematic illustration of the mechanisms by which *Eleutherine palmifolia* (Dayak onion) extract enhances physiological performance, immune responses, and fish growth. Bioactive compounds, including flavonoids, saponins, and tannins, act as natural antioxidants and immunostimulants that strengthen host defence systems. The synergistic antioxidant and immunomodulatory effects of Dayak onion extract increase phagocytic activity and total leukocyte counts, key indicators of enhanced non-specific immunity (Yuanita et al., 2023; Sirajuddin et al., 2025). Enhanced phagocytic function subsequently improves haematological parameters, including erythrocyte count, haemoglobin concentration, and hematocrit values, reflecting increased resilience to environmental stress. In addition, phenolic compounds from Dayak onion exhibit antibacterial activity against pathogenic bacteria, reducing the risk of secondary infections that impair productivity and indirectly improving feed efficiency and growth performance (Hasim et al., 2023).

The anti-inflammatory activity of Dayak onion extract is reflected in the modulation of pro-inflammatory cytokines, characterised by increased TNF-α and reduced IL-β expression, indicating balanced immune regulation without inducing excessive oxidative stress (Chabib et al., 2018; Mutiah et al., 2020). Its bioactive compounds also reduce malondialdehyde (MDA) levels and enhance the activity of key antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT) (Susilowati & Setiawan, 2020). The reciprocal interaction between enhanced antioxidant defences and improved immune function provides a strong scientific rationale for the use of Dayak onion extract as a functional feed additive (Fransira et al., 2020). Overall, Figure 3 illustrates that Dayak onion extract represents a promising natural alternative to synthetic antibiotics in aquaculture feeds, improving growth performance and disease resistance while supporting sustainable and environmentally friendly farming practices. The utilisation of local plant resources, such as Dayak onion, therefore, constitutes an important strategy for reducing reliance on synthetic antibiotics in aquaculture systems (Phaik Sim et al., 2019).

Research on Dayak Onion in Aquaculture

Studies evaluating Dayak onion as a bioactive feed ingredient have been conducted in several aquaculture species, including carp, tilapia, and tiger shrimp. These studies consistently demonstrate the potential of Dayak onion as an immunostimulant, antioxidant, and enhancer of physiological performance. Table 2**.** Summarises representative studies investigating the application of Dayak onion in aquaculture systems.

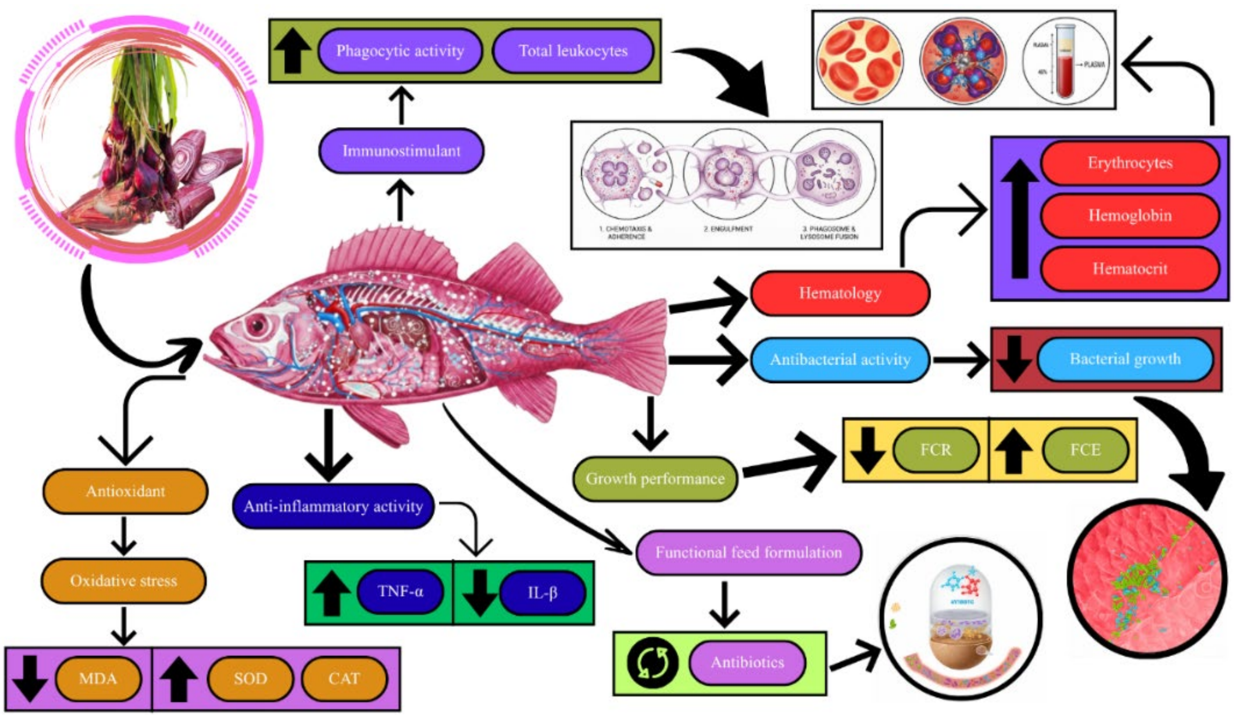

Figure 3. Mechanisms by which Dayak onion (*Eleutherine palmifolia*) extract enhances physiological and immunological performance in fish

Table 2. Research on the use of dayak onions in aquaculture

Biota	Plant Parts	Treatment	Result	Reference
Carp (*Cyprinus carpio*)	Extract+soaking	50, 60, 70 and 80 ppm	A crude extract of Dayak onion at a dose of 80 ppm was shown to improve tissue damage in carp infected with *Aeromonas hydrophila* in a dose-dependent manner.	(Maftuch, H. Suprastyani, et al., 2018)
Tilapia	Powder+feed	powders of 5% (P5), 10% (P10), and 15% (P15), while the extract was 0.5% (E05)	The addition of 15% powder and 0.5% crude Dayak onion extract significantly improved the immunity and blood of the fish. SR 50%, after being infected with *A. hydrophilla*	(Fauzi et al., 2024)
Tilapia	Extract+soaking	30 ppm extract (A), 50 ppm extract (B), 70 ppm extract (C)	Treatment C (70 ppm) SR 86.67%	(Fransira et al., 2023)
Tilapia	Extract+ application to wounds	(A: 10 ppt/ind), (B: 20 ppt/ind),	Treatment B (20 ppt/ind) SR 100%	(Akbar, 2017)

Notably, several studies reported enhanced haematological and immune-related parameters without a proportional increase in survival rate (SR), particularly under bacterial challenge conditions. This apparent discrepancy suggests that improved innate immune indicators alone may not be sufficient to guarantee survival, especially when infection pressure, pathogen virulence, or environmental stressors exceed physiological buffering capacity. Short experimental durations, single-dose designs, and the absence of molecular validation of immune signalling pathways may further obscure the relationship between immunological enhancement and survival outcomes. Moreover, suboptimal timing of phytobiotic administration relative to pathogen exposure could limit protective efficacy despite elevated immune indices.

Evidence Synthesis of Eleutherine palmifolia Applications in Aquaculture

The main characteristics, experimental designs, and key outcomes of representative studies evaluating Eleutherine palmifolia in aquaculture systems are summarised in Table 3.

As shown in Table 3, most in vivo studies report positive immunological and growth-related outcomes; however, methodological limitations such as short experimental duration, single-dose designs, and limited molecular validation remain prevalent. Despite the consistently positive biological responses reported across studies, considerable variability remains in the effective doses of *Eleutherine palmifolia* applied to different aquaculture species. This interspecific variation can be attributed to differences in digestive physiology, metabolic rate, immune system sensitivity, and routes of administration. Fish species with faster metabolic turnover and higher feeding rates may require lower dietary inclusion levels to achieve immunostimulatory effects. In contrast, immersion-based applications often demand higher concentrations to ensure sufficient bioactive uptake through the gill and skin surfaces. In addition, species-specific tolerance thresholds and differences in gut microbiota composition may influence the bioavailability and efficacy of phytochemical compounds, resulting in divergent optimal dose ranges across taxa. Beyond their antibacterial effects, onion-based herbal therapeutics, including *Allium cepa* and *Allium sativum*, have also been shown to exhibit strong antiparasitic activity against major protozoan parasites in aquaculture, particularly *Ichthyophthirius multifiliis* and *Trichodina* spp (Özil, 2023; Gadallah et al., 2024). These findings suggest that onion-related phytochemicals may provide broader protective benefits encompassing both bacterial and parasitic disease management and support the potential antiparasitic relevance of *E. palmifolia*. However, direct experimental evidence remains limited and warrants further investigation.

Table 3. Summary of representative studies evaluating *Eleutherine palmifolia* in aquaculture systems

Study type	Species	Dose/ Application	Main Outcome	Limitation	Reference
In vivo immersion trial	Nile tilapia (*Oreochromis niloticus*)	30,50,70 mg/L (immersion)	Improved haematological parameters: ↑ erythrocytes, lymphocytes, Hb, Ht: ↓ leucocytes, monocytes, neutrophils	No molecular immune markers; no growth or feed data	(Fransira et al., 2020)
In vitro antibacterial assay	*Pseudomonas fluorescens*	Disk diffusion, MIC not stated	Inhibition zone formed; confirmed antibacterial activity of phenol, flavonoid, tannin, and saponin	No MIC quantification; no comparison with antibiotics	(Fransira et al., 2020)
In vivo immersion trial	Common carp (*Cyprinus carpio*)	50, 60, 70, 80 ppm (immersion)	Dose-dependent histopathological recovery in the gills, kidney, liver, and muscle; reduced necrosis, oedema, and congestion	No molecular immune markers; no haematology or survival data	(Maftuch, H Suprastyani, et al., 2018)
In vitro antibacterial assay (preliminary)	*Aeromonas hydrophilla*	Not specified	Confirmed antibacterial activity of Dayak onion extract	No MIC or inhibition zone data; not detailed in this article	(Maftuch, H Suprastyani, et al., 2018)
In vivo feeding & challenge trial	Tiger shrimp (*Penaeus monodon*)	6 ppm, 12 ppm, 18 ppm (extract applied in culture water; shrimp fed 4 x daily)	Improved growth (absolute weight & length), best survival at 12 ppm (43.34%); antibacterial effect against *V. harveyi*)	Short duration (1 month); limited immune/ hematology markers; survival rates are still relatively low	(Jumadi, 2019)
In Vitro protozoan fish parasite assay	*Ichthyophthirius multifiliis*	0.1, 0.25, and 0.50 ml/L use onion and garlic	Onion essential oil caused 94% mortality, while garlic essential oil caused 92% mortality after 60 minutes of exposure to the parasite *Ichthyophthirius multifiliis*	The results are based on short-term in vitro assays and require in vivo validation.	(Özil, 2023)

Antibacterial Activity of Bawang Dayak

Dayak onion contains a range of bioactive compounds with inherent antibacterial properties, including alkaloids, flavonoids, steroids, and tannins, which play key roles in inhibiting and eliminating pathogenic bacteria. Previous studies reported that a 100% ethanol extract of Dayak onion produced an inhibition zone of 12.33 ±1.61 mm against *Staphylococcus aureus* (Warsiti et al., 2018). Similarly, ethanol and aqueous extracts effectively inhibited the growth of *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae*. These findings were further supported by Hidayah et al., (2021), who demonstrated that the K3M1 treatment yielded the largest inhibition zones, measuring 15.47 mm against *Salmonella* spp. and 13.40 mm against *E. coli*.

The antibacterial activity of Dayak onion against aquaculture-related pathogens has also been investigated. Safratilofa & Sugihartono (2018) evaluated several extraction methods, including infusion, decoction, maceration with 96% alcohol, and maceration with 96% ethanol, against *Aeromonas hydrophila*. Among these methods, alcoholic maceration produced the largest inhibition zone (3.5 mm). In addition, Fransira et al. (2019) reported that the minimum inhibitory concentration (MIC) of Dayak onion extract against *Pseudomonas fluorescens* was 100 ppm, representing the lowest effective concentration for bacterial growth inhibition.

Another research study. Extracts of *Eleutherine bulbosa* exhibit broad antibacterial activity attributed to diverse secondary metabolites, which effectively inhibit major aquatic pathogens such as *Vibrio harveyi, Aeromonas hydrophila, Vibrio parahaemolyticus*, and *Pseudomonas fluorescens (Munaeni et al., 2021).* In previous studies, we reported that crude extracts of *Eleutherine americana* exhibited antibiofilm and anti–quorum sensing activities against *Streptococcus pyogenes*. Furthermore, a partially purified fraction effectively inhibited methicillin-resistant *Staphylococcus aureus* (MRSA). The extract also demonstrated strong antibacterial activity against foodborne *S. aureus* isolates, with minimum inhibitory concentration (MIC) values ranging from 0.06 to 1.00 mg/mL (Mahabusarakam et al., 2010).

Mechanistically, phenolic compounds, quinones, and flavonoids disrupt bacterial cells by damaging cell walls and membranes, leading to protein denaturation and cell lysis (Haq et al., 2018). Increased phenolic content in *E. palmifolia* bulb extracts correlates positively with total phenol levels, thereby enhancing antibacterial efficacy. These phenolic constituents interact with microbial cell wall structures, reduce membrane permeability, and ultimately compromise bacterial survival.

Effects of Dayak Onion on Growth Performance

Fish growth is a critical determinant of aquaculture productivity. The application of Dayak onion as an immunostimulant is closely associated with improved growth performance, as its bioactive compounds enhance fish physiological responses and metabolic efficiency. Faramudhita (2024) reported that dietary supplementation with Dayak onion *simplicia* significantly improved growth parameters in Nile tilapia, including absolute length and weight gain, specific growth rate, feed intake, and feed conversion ratio. These improvements are primarily attributed to bioactive components in Dayak onion, particularly oligosaccharides and phenolic compounds, which function as prebiotics and immunostimulants. These compounds enhance digestive enzyme activity, modulate gut microbiota composition, and stimulate non-specific immune responses, including phagocytosis, lysozyme activity, and immune cell proliferation. Collectively, these mechanisms improve nutrient utilisation efficiency and promote accelerated growth. However, excessive inclusion levels may negatively affect performance. Oligosaccharide concentrations above optimal thresholds can disrupt feed formulation balance, as higher proportions of additives reduce the availability of essential nutrients such as proteins, lipids, carbohydrates, vitamins, and minerals. This imbalance lowers overall feed nutritional value, ultimately impairing growth performance and feed conversion efficiency. This outcome aligns with fundamental principles of feed nutrition, which emphasise that non-nutritive additives must be incorporated within limits that do not compromise primary nutrient composition.

Beyond fish, the growth-promoting effects of Dayak onion have also been documented in crustaceans. In tiger shrimp (*Penaeus monodon*), dietary administration of 18 ppm Dayak onion extract increased growth by up to 4.58 g. The underlying mechanism parallels that observed in fish, involving immune stimulation, improved digestive tract function, and more efficient energy allocation toward growth (Jumadi, 2019). *E. bulbosa* extracts also contain functional oligosaccharides, including FOS, raffinose, inulin, and GOS, that promote probiotic growth and support gut microbiota balance, indicating their potential role as natural prebiotics. Furthermore, the presence of xanthones, naphthoquinones, and anthraquinones confers strong antioxidant capacity, as reflected by low IC_{50} values in DPPH assays. Collectively, these properties highlight *E. bulbosa* as a multifunctional phytotherapeutic agent with promising applications in sustainable aquaculture (Munaeni et al., 2021).

Dayak Onion as an Immune System Booster

Fish immune competence can be enhanced through dietary supplementation with immunostimulants that strengthen innate immune cell activity, regulate immune signalling pathways, and improve resistance to bacterial infections and environmental stressors (Citarasu, 2010; (Reverter et al., 2014). In aquaculture systems, such dietary strategies are particularly relevant because cultured fish are frequently exposed to pathogenic challenges and suboptimal environmental conditions that compromise immune performance and physiological stability.

Several studies have demonstrated the immunostimulatory potential of Dayak onion in aquaculture. Fauzi et al. (2024) reported that dietary inclusion of crude and extract forms of Dayak onion significantly enhanced haematological parameters, respiratory burst activity, and immune-related gene expression, including *IL-1β* and *TNF-α*, in tilapia challenged with *Aeromonas hydrophila*. These findings indicate that *E. palmifolia* stimulates both cellular and molecular components of innate immunity during bacterial infection. Consistent evidence was provided by Faramudhita. (2024), who observed increased survival rates, improved haematological indices, enhanced phagocytic activity, and elevated intestinal lactic acid bacteria populations in fish receiving Dayak onion supplementation, suggesting a linkage between immune enhancement and gut microbiota modulation.

From a mechanistic standpoint, the immunological benefits of *E. palmifolia* are largely attributed to its bioactive compounds, particularly flavonoids, tannins, saponins, and terpenoids, which contribute to erythrocyte stability, antioxidant defence, and immune resilience (Izzah et al., 2022). Flavonoids have been shown to enhance macrophage and lymphocyte activity and support erythropoiesis through their antioxidant properties, thereby strengthening systemic immune capacity (Izzah et al., 2022). In addition, Dayak onion extracts have been reported to modulate pro-inflammatory cytokine expression, maintaining immune homeostasis and preventing excessive inflammatory responses that may impair growth and physiological performance (Moustafa et al., 2020).

A study by Supomo et al. (2019), demonstrated that *E. palmifolia* exhibits strong antioxidant activity, as evaluated using DPPH radical scavenging and Brine Shrimp Lethality Test (BSLT) assays. Among the tested fractions, the chloroform fraction showed very high antioxidant capacity**,** with an IC_{50} value of 20.29 ppm, which is categorised as a strong antioxidant and comparable to standard antioxidants. In addition, the BSLT assay indicated a moderate toxicity level ($LC_{50} \approx 527$ ppm), suggesting notable bioactivity while remaining within an acceptable safety range. These findings provide direct experimental evidence supporting *E. palmifolia* as a potent plant-based antioxidant source, reinforcing its relevance as a functional phytogenic additive for mitigating oxidative stress in aquaculture systems.

These studies indicate that *E. palmifolia* acts as a multi-pathway immunostimulant, exerting its effects through haematological improvement, cellular immune activation, cytokine regulation, antioxidant protection, and microbiota support, rather than through a single immune mechanism. Such integrated immunomodulatory action aligns with current concepts of sustainable aquaculture, where long-term immune resilience is prioritised over short-term immune overstimulation (Citarasu, 2010).

Molecular Mechanism of Dayak Onion (Eleutherine palmifolia) Phytobiotics

Phytobiotics derived from the bioactive compounds of Dayak onion (*Eleutherine palmifolia*) have emerged as a key focus in the development of functional aquaculture feeds. Major constituents, including flavonoids, naphthoquinones, polyphenols, and tannins, exhibit multifaceted mechanisms that enhance non-specific immune responses, reinforce intestinal mucosal integrity, and inhibit pathogenic bacterial colonisation (Chabib et al., 2018; Harlita et al., 2018; Masfria & Tampubolon, 2019; Hongthongkham et al., 2025). A detailed mechanistic understanding of how these compounds act at cellular and tissue levels is essential for elucidating their collective contributions to fish health and disease resistance (Affandi et al., 2025). Figure 4 presents a schematic overview of the molecular interactions between Dayak onion bioactive compounds, pathogenic bacteria, and fish immune cells.

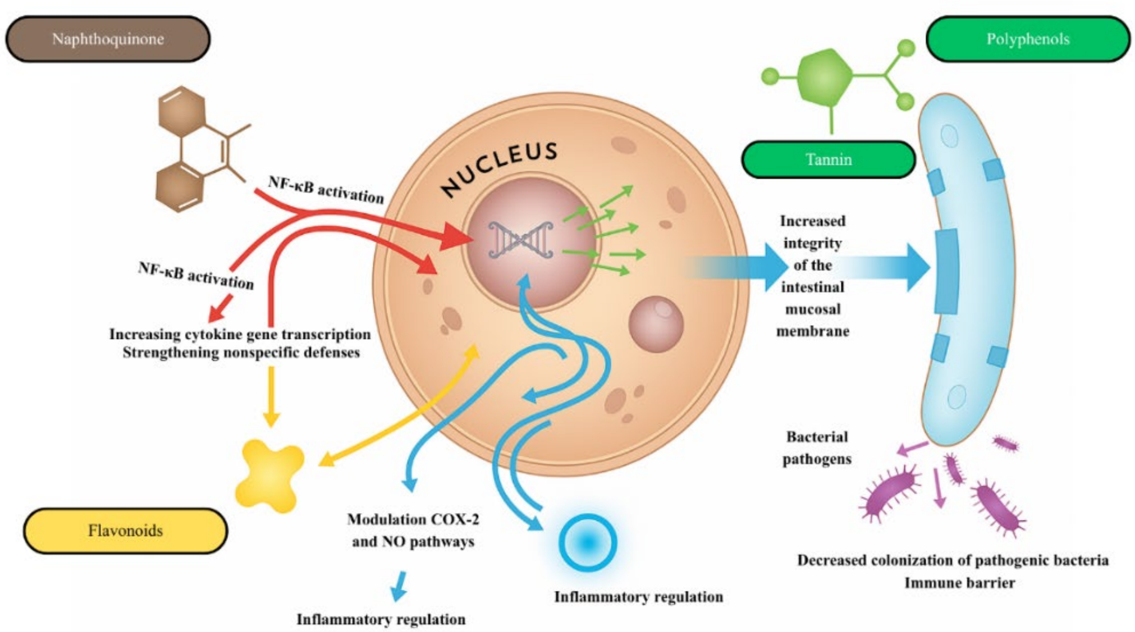

Figure 4. Molecular mechanisms by which bioactive compounds from *Eleutherine palmifolia* enhance fish immune responses

Figure 4 illustrates the mechanisms of action of major bioactive compounds in *E. palmifolia* that contribute to immune enhancement and protection against pathogenic infections in fish. On the left side of the schematic, naphthoquinones are depicted as key activators of the nuclear factor kappa B (NF-κB) signalling pathway. Activation of this pathway induces the transcription of cytokine-related genes, thereby strengthening non-specific immune defences and enhancing phagocytic activity and the production of protective immune molecules (Firmino et al., 2021; Affandi et al., 2025). Flavonoids modulate inflammatory signalling by regulating cyclooxygenase-2 (COX-2) expression and the nitric oxide (NO) pathway, thereby preventing excessive inflammation, reducing tissue damage, and maintaining physiological stability during infection and environmental stress (Maleki et al., 2019; Ysrafil et al., 2023; Kari, 2025; Sahakyan, 2025).

Polyphenols and tannins enhance intestinal mucosal integrity by strengthening the epithelial barrier, thereby limiting pathogenic bacterial adhesion and colonisation (Molino et al., 2022; Andriani et al., 2024). Improved barrier function reduces infection risk and supports digestive health and nutrient absorption in fish (Mutiah et al., 2020; Kanika et al., 2025). Overall, Figure 4 highlights the synergistic interactions among Dayak onion bioactive compounds acting at molecular, cellular, and tissue levels to promote immune competence and disease resistance in aquaculture species.

Dayak Onion as an Anti-Stress Agent

Several bioactive compounds in Dayak onion exhibit potential as anti-stress agents during fish transportation. One dominant constituent is quercetin, a flavonoid known to reduce stress responses by inhibiting intestinal motility and decreasing capillary permeability in the peritoneal cavity. This mechanism physiologically reduces faecal excretion, thereby limiting the accumulation of toxic metabolites in the transport medium. In addition to quercetin, Dayak onion contains essential oil components that may lower fish metabolic rates, suppress digestive activity, and reduce waste production. Similar effects have been reported for lemongrass (*Cymbopogon citratus*) essential oil, which induces metabolic suppression during transport. Reduced metabolic activity resembles mild anaesthetic effects and has been shown to improve fish survival during transportation. A comparable response was observed in 3–5 cm botia fish treated with 1 mL/L lemongrass essential oil, which exhibited increased opercular movement, reduced activity, and diminished responsiveness to external stimuli, ultimately achieving a survival rate of 76% (Izzah et al., 2022). These findings support the potential application of Dayak onion-derived compounds as natural anti-stress agents to enhance fish survival under transportation-related stress.

Development Prospects

The development of Dayak onion (*Eleutherine palmifolia*) as a phytobiotic offers a promising strategy to reduce antibiotic dependence in aquaculture. Accumulating evidence indicates that Dayak onion functions as an effective immunostimulant and improves intestinal health, supporting its potential application as a functional feed additive. Owing to its complex and synergistic bioactivities, Dayak onion can be developed as a key component of immunological and performance-enhancing feeds for various fish and shrimp species in intensive farming systems.

Several strategic factors support its future development. Agronomically, Dayak onion exhibits strong environmental adaptability, allowing scalable cultivation to meet feed industry demands. Technologically, advances in extraction and processing techniques enable the targeted isolation of bioactive metabolite fractions, resulting in products with improved stability and consistent biological efficacy. From an industrial and regulatory perspective, the global shift toward natural and antibiotic-free inputs creates substantial opportunities for the commercialisation of Dayak onion-based phytobiotics. Moreover, formulation strategies such as microencapsulation and controlled-release systems offer promising approaches to enhance bioavailability and protect active compounds during feed processing.

From a research standpoint, further studies should focus on elucidating molecular mechanisms, including interactions with gut microbiota, immune signalling pathways, and oxidative stress responses. Integrating Dayak onion into sustainable aquaculture models—such as biofloc systems, recirculating aquaculture systems (RAS), and ecosystem-based aquaculture—also presents significant innovation potential. With robust standardisation, multidisciplinary collaboration, and strengthened local supply chains, Dayak onion has the potential to emerge as a flagship Indonesian phytobiotic, contributing to improved fish health, production efficiency, and the long-term sustainability of the aquaculture industry.

Conclusion

Eleutherine palmifolia (Dayak onion) represents a promising phytobiotic for sustainable aquaculture due to its rich phytochemical profile and broad spectrum of biological activities, including immunomodulatory, antibacterial, antioxidant, anti-stress, and growth-promoting effects. Accumulating evidence demonstrates that dietary supplementation with *E. palmifolia* enhances non-specific immune responses, improves haematological and physiological parameters, suppresses pathogenic bacterial infections, and increases survival and feed efficiency across diverse aquaculture species.

These beneficial effects are primarily mediated through the modulation of immune-related signalling pathways, reinforcement of intestinal mucosal integrity, enhancement of antioxidant defences, and inhibition of harmful microbial colonisation.

From a novelty perspective, this review represents the first PRISMA-guided systematic integrative synthesis that critically differentiates between experimentally validated outcomes and mechanistic inferences regarding the application of *Eleutherine palmifolia* in aquaculture. By consolidating fragmented evidence into a unified, mechanism-oriented framework and aligning biological effects with practical aquaculture contexts, this work moves beyond descriptive compilation toward evidence-informed interpretation. By integrating dispersed findings into a coherent analytical structure, this review advances current understanding of *E. palmifolia* as a functional feed additive. It provides a clearer basis for evaluating its translational potential in sustainable aquaculture.

Nevertheless, existing research remains constrained by fragmented experimental designs, inconsistent extraction and dosage protocols, and limited molecular validation, particularly under intensive aquaculture conditions. By integrating dispersed findings into a coherent, mechanism-oriented framework, this review advances current understanding of *E. palmifolia* as a functional feed additive and identifies critical knowledge gaps that must be addressed before large-scale application. Future research should prioritise standardised methodologies, molecular-level investigations, and system-based evaluations to fully realise the potential of *E. palmifolia* as an environmentally friendly alternative to antibiotics, thereby supporting resilient, efficient, and sustainable aquaculture production systems. From a methodological perspective, the PRISMA-guided systematic integrative approach employed in this review enables comprehensive synthesis while acknowledging variability and bias across existing studies, thereby providing a balanced and evidence-informed foundation for future experimental and applied aquaculture research.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures, with no animal experiments.

Data availability: All data generated or analysed during this study are included in this published article and its references.

Funding disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements: The authors would like to express their sincere gratitude to all researchers whose studies contributed to this review. No external funding was received for this study.

Disclosure: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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