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Mechanisms of Action of Plant Extracts as Alternatives to Antibiotics in Aquaculture: A Comprehensive Review of Recent Studies

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آليات عمل المستخلصات النباتية كبدائل للمضادات الحيوية في الاستزراع المائي: مراجعة شاملة للدراسات الحديثة

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Abstract:
The overuse of antibiotics in aquaculture has led to the emergence of antimicrobial resistance, environmental contamination, and concerns regarding food safety, necessitating the search for effective and eco-friendly alternatives. Plant-derived bioactive compounds, collectively known as phytobiotics, have gained considerable attention as promising substitutes due to their multifaceted biological activities. This comprehensive review synthesizes recent findings on the mechanisms of action through which plant extracts exert their beneficial effects in farmed fish species. The major mechanisms identified include direct antimicrobial activity through disruption of bacterial cell walls and inhibition of quorum sensing, immunomodulation via enhancement of both nonspecific and specific immune parameters, antioxidant protection through upregulation of endogenous enzyme systems, and growth promotion mediated by improved digestive enzyme activity and nutrient utilization. The review systematically examines how different classes of phytochemicals—including alkaloids, flavonoids, essential oils, phenolics, saponins, and polysaccharides—contribute to these effects. Evidence from recent studies demonstrates that plant extracts enhance lysozyme activity, phagocytosis, respiratory burst, and immune-related gene expression while reducing oxidative stress markers and improving resistance against major bacterial pathogens such as *Aeromonas hydrophila*, *Vibrio* species, and *Yersinia ruckeri*. Factors influencing phytobiotic efficacy, including extraction methods, dosage optimization, and advanced delivery systems like microencapsulation, are critically evaluated. The review also identifies current research gaps and proposes future directions, emphasizing the need for standardized protocols, omics-based mechanistic studies, and commercially scalable formulations to facilitate the transition from experimental findings to practical aquaculture applications. By consolidating current knowledge on mechanisms of action, this review contributes to the strategic adoption of plant extracts as sustainable alternatives to antibiotics in aquaculture.

Keywords: Phytobiotics, plant extracts, aquaculture, immunostimulation, antimicrobial activity, antioxidant mechanisms, antibiotic alternatives, bioactive compounds, fish health, sustainable aquaculture.

الملخص
أدى الإفراط في استخدام المضادات الحيوية في قطاع الاستزراع المائي إلى ظهور مقاومة مضادات الميكروبات، وتلوث البيئة، ومخاوف تتعلق بسلامة الأغذية، مما استلزم البحث عن بدائل فعالة وصديقة للبيئة. حظيت المركبات النشطة بيولوجيا المشتقة من النبات، والمعروفة مجتمعة باسم "المُغذيات النباتية"، باهتمام كبير كبدائل واعدة نظراً لأنشطتها البيولوجية المتعددة الجوانب. تقدم هذه المراجعة الشاملة توليفة للنتائج الحديثة حول آليات العمل التي تمارس من خلالها المستخلصات النباتية تأثيراتها المفيدة في أنواع الأسماك المستزرعة. تشمل الآليات الرئيسية التي تم تحديدها: النشاط المضاد للميكروبات المباشر من خلال تعطيل جدران الخلايا البكتيرية وتثبيط عملية "استشعار النصاب"، والتعديل المناعي عبر تعزيز كل من المعايير المناعية غير النوعية والنوعية، والحماية المضادة للأكسدة من خلال رفع تنظيم أنظمة الإنزيمات الداخلية، وتعزيز النمو بوساطة تحسين نشاط الإنزيمات الهاضمة والاستفادة من العناصر الغذائية. تستعرض هذه الورقة بشكل منهجي كيف تساهم الفئات المختلفة من المركبات الكيميائية النباتية—بما في ذلك القلويدات، الفلافونويدات، الزيوت الأساسية، الفينولات، الصابونينات، والسكريات المتعددة—في تحقيق هذه التأثيرات. تُظهر الأدلة المستقاة من الدراسات الحديثة أن المستخلصات النباتية تعزز نشاط إنزيم الليزوزيم، والبلعمة، والانفجار التنفسي، والتعبير الجيني المرتبط بالمناعة، بينما تقلل من مؤشرات الإجهاد التأكسدي وتحسن المقاومة ضد مسببات الأمراض البكتيرية الرئيسية مثل *Aeromonas hydrophila* وأنواع *Vibrio* و*Yersinia ruckeri*. تم تقييم العوامل المؤثرة على فعالية المُغذيات النباتية بشكل نقدي، بما في ذلك طرق الاستخلاص، وتحسين الجرعة، وأنظمة التوصيل المتقدمة مثل التغليف الدقيق. كما يحدد التقييم الفجوات البحثية الحالية ويقترح توجهاً مستقبلية، مع التأكيد على الحاجة إلى بروتوكولات موحدة، ودراسات آلية تعتمد على تقنيات"omics" ، وتركيبات قابلة للتطبيق تجارياً لتسهيل الانتقال من النتائج التجريبية إلى التطبيقات العملية في مجال الاستزراع المائي. من خلال دمج المعرفة الحالية حول آليات العمل، تساهم هذه المراجعة في التبني الاستراتيجي للمستخلصات النباتية كبدائل مستدامة للمضادات الحيوية في تربية الأحياء المائية.

الكلمات المفتاحية: المُغذيات النباتية، المستخلصات النباتية، الاستزراع المائي، التحفيز المناعي، النشاط المضاد للميكروبات، آليات مضادات الأكسدة، بدائل المضادات الحيوية، المركبات النشطة بيولوجيا، صحة الأسماك، الاستزراع المائي المستدام.

1. Introduction

1.1 Problem Statement

Aquaculture has emerged as one of the fastest-growing food production sectors globally, playing an increasingly vital role in meeting the rising demand for high-quality protein and essential nutrients for human consumption (Bilen et al., 2019; Salem, 2021). According to the Food and Agriculture Organization (FAO), global fisheries and aquaculture production reached 223.2 million tonnes in 2022, with aquaculture contributing significantly to this figure. The sector's expansion has been driven by population growth, rising per capita fish consumption, and the recognition of fish as a healthy source of omega-3 fatty acids, vitamins, and minerals (Salem, 2023). However, the intensification and commercialization of aquaculture practices have been accompanied by a parallel increase in infectious disease outbreaks, which pose substantial threats to productivity and economic viability (Salem et al., 2023). Disease outbreaks caused by various microorganisms result in production losses of up to 50% in the aquaculture sector, representing one of the most significant constraints to sustainable development (Salem, 2021; Amhamed et al., 2023).

Bacterial pathogens represent the second most important group of microbial pathogens affecting cultured fish, particularly in tropical regions where environmental conditions favor their proliferation (Apreja et al., 2022). Common Gram-negative pathogens include *Aeromonas salmonicida*, *Aeromonas hydrophila*, various *Pseudomonas* species, *Vibrio anguillarum*, and *Edwardsiella tarda*, while Gram-positive pathogens such as *Staphylococcus* and *Streptococcus* species are also frequently encountered (Aziz & Abdullah, 2021; Apreja et al., 2022; Salem et al., 2023). These pathogens can cause devastating disease outbreaks with mortality rates exceeding 50% in affected populations, leading to substantial economic losses and compromising food security (Ahsan, 2015). Historically, the aquaculture industry has relied heavily on antibiotics and synthetic chemotherapeutic agents for both prophylactic and therapeutic disease management (Salem, 2021; Mumed et al., 2023).

This extensive reliance on antibiotics has generated multiple interconnected challenges that threaten the long-term sustainability of aquaculture production. The development and spread of antibiotic-resistant pathogens represents perhaps the most critical concern, as resistance genes can transfer between bacterial populations and potentially affect human pathogens, contributing to the global antimicrobial resistance crisis (Bhavani et al., 2022). Environmental contamination with antibiotic residues poses risks to aquatic ecosystems and nontarget organisms, disrupting microbial communities and affecting biogeochemical cycling (Akhter et al., 2024). Furthermore, antibiotic accumulation in fish tissues raises food safety concerns for consumers and may lead to immunosuppression in treated fish, paradoxically increasing their susceptibility to subsequent infections (Bilen et al., 2019). These multifaceted problems have driven an urgent search for effective, safe, and environmentally sustainable alternatives to antibiotics in aquaculture (Ljubojević Pelić et al., 2024).

1.2 Plant Extracts as Antibiotic Alternatives

Plant-derived bioactive compounds, collectively termed phytobiotics, phytogenics, or botanicals, have emerged as promising alternatives to synthetic antibiotics (Rachwał & Gustaw, 2025; Amhamed et al., 2023). Phytobiotics encompass a diverse array of natural compounds extracted from various plant parts including leaves, seeds, roots, bark, fruits, and agricultural byproducts (Ndomou & Mube, 2023; Lakwani & Salem, 2024). These compounds offer multiple advantages that make them particularly attractive for aquaculture applications: they are biodegradable, ecologically safe, readily accessible, and generally pose minimal risk to human health (Kalaiselvan et al., 2024; Salem et al., 2024). Moreover, their production can be integrated into sustainable agricultural systems, supporting rural livelihoods and promoting circular economy principles (Selvan et al., 2023).

The therapeutic potential of phytobiotics stems from their complex phytochemical composition (Bhatla & Lal, 2023). Plants produce numerous secondary metabolites—including alkaloids, flavonoids, essential oils, phenolics, glycosides, saponins, tannins, steroids, and terpenoids—that serve defensive functions in the plant but also exert beneficial biological activities in animals (Bhatla & Lal, 2023). These diverse compounds can simultaneously affect multiple physiological systems, offering multifunctional benefits including growth enhancement, appetite stimulation, immune modulation, oxidative stress reduction, and improved disease resistance (Salem, 2021; Salem et al., 2023). The presence of multiple bioactive compounds in crude extracts may produce synergistic effects that exceed the sum of individual component activities (Eyube et al., 2025).

Unlike conventional antibiotics that typically target specific bacterial processes, plant extracts operate through multiple mechanisms, making it more difficult for pathogens to develop resistance (Salem & Salem, 2025). This multitargeted approach, combined with their biodegradability and safety profile, positions phytobiotics as attractive components of sustainable aquaculture health management strategies (Salem, 2023; Amhamed et al., 2023). The diversity of plant species and extraction methods available provides a rich source of potential bioactive compounds awaiting systematic investigation and optimization for aquaculture applications (Eyube et al., 2025).

1.3 Objectives and Significance of This Review

Despite the growing body of research on phytobiotic applications in aquaculture, information regarding their precise mechanisms of action remains scattered across numerous studies employing different experimental models, extract types, and outcome measures. A consolidated understanding of how plant extracts exert their beneficial effects is essential for optimizing their application and facilitating commercial adoption (Salem, 2021). The absence of a comprehensive mechanistic framework hinders evidence-based selection of plant extracts, formulation development, and regulatory approval processes.

Therefore, the primary objectives of this review are: (1) to synthesize current knowledge on the mechanisms through which plant extracts function as antibiotic alternatives in aquaculture; (2) to examine how different classes of phytochemicals contribute to antimicrobial, immunomodulatory, antioxidant, and growth-promoting effects; (3) to evaluate factors influencing phytobiotic efficacy including extraction methods, dosage, and delivery systems; (4) to identify research gaps and propose future directions for advancing phytobiotic applications in commercial aquaculture; and (5) to provide a practical framework for researchers, feed manufacturers, and aquaculture producers seeking to implement phytobiotic-based health management strategies.

By providing a comprehensive mechanistic framework, this review aims to support evidence-based selection of plant extracts, guide formulation development, and contribute to the strategic adoption of phytobiotics as effective alternatives to antibiotics, thereby promoting more sustainable and resilient aquaculture practices.

2. Literature Review

2.1 Major Classes of Phytochemicals and Their Bioactive Properties

The biological activities of plant extracts derive from their complex mixtures of secondary metabolites, each class possessing distinct chemical properties and mechanisms of action (Salem, 2021). Understanding these phytochemical classes provides a foundation for interpreting the multifunctional effects observed in aquaculture studies and enables rational selection of plant sources for specific applications (Salem, 2021).

2.1.1 Alkaloids

Alkaloids are nitrogen-containing heterocyclic compounds with potent biological activities that have been extensively studied for their therapeutic potential in aquaculture (Bhambhani et al., 2021). Sanguinarine, a quaternary benzophenanthridine alkaloid derived primarily from *Macleaya cordata*, has demonstrated significant antimicrobial and immunomodulatory properties in fish (Phan et al., 2025). Studies have shown that dietary sanguinarine exhibits strong antibacterial activity against *Aeromonas dhakensis*, with minimum inhibitory concentration (MIC) of 5 μg/mL and minimum bactericidal concentration (MBC) of 10 μg/mL (Salem et al., 2025). The antimicrobial mechanism involves intercalation into bacterial DNA and inhibition of enzyme activities, particularly topoisomerases and DNA gyrase (Salem et al., 2025). Additionally, sanguinarine enhances immune parameters including serum glutathione levels, superoxide dismutase activity, and expression of immune-related genes such as transforming growth factor-beta (TGF-β), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) (Salem et al., 2025). Other alkaloids, such as solasodine from *Solanum trilobatum*, contribute to anti-inflammatory and antimicrobial effects through modulation of cytokine production and inhibition of bacterial growth (Salem, 2021; Subramani et al., 2025). The structural diversity of alkaloids provides opportunities for discovering novel compounds with targeted biological activities (Bhambhani et al., 2021).

2.1.2 Flavonoids

Flavonoids represent a large family of polyphenolic compounds widely distributed in plants, with over 6,000 identified structures exhibiting considerable structural diversity (Ahmadifar et al., 2021). They function primarily as antioxidants through radical scavenging and metal chelation, but also exhibit immunomodulatory and antimicrobial activities (Ahmadifar et al., 2021; Ribeiro et al., 2015). The antioxidant properties of flavonoids stem from their ability to donate hydrogen atoms or electrons to neutralize free radicals, as well as their capacity to chelate transition metals that catalyze reactive oxygen species (ROS) generation (Ribeiro et al., 2015). Quercetin, kaempferol, and apigenin are among the most studied flavonoids in aquaculture contexts, demonstrating protection against oxidative stress induced by environmental contaminants and pathogenic challenge (Ahmadifar et al., 2021). Flavonoids modulate immune responses by influencing leukocyte proliferation, cytokine production, and complement system activity through interactions with cellular signaling pathways including nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs) (Ribeiro et al., 2015). The antioxidant properties protect fish tissues from oxidative damage during stress conditions or pathogenic challenge, preserving cellular integrity and organ function (Salem et al., 2021).

2.1.3 Essential Oils and Terpenoids

Essential oils are complex mixtures of volatile compounds, primarily terpenes and terpenoids, extracted from aromatic plants through steam distillation or cold pressing (Chouhan et al., 2017). These lipophilic compounds readily cross biological membranes, contributing to their antimicrobial efficacy through disruption of membrane integrity and function (Latorre et al., 2025). Major components including thymol, carvacrol, cinnamaldehyde, and eugenol disrupt bacterial cell membranes, increase permeability, and cause leakage of cellular contents, leading to cell death (Latorre et al., 2025). Essential oils from oregano, thyme, cinnamon, and rosemary have demonstrated growth-promoting, immunostimulatory, and stress-reducing effects in various fish species (Amhamed et al., 2023; Salem, 2023). The antimicrobial activity of essential oils is attributed to their complex composition, with multiple compounds acting synergistically to enhance efficacy and reduce the likelihood of resistance development (Chouhan et al., 2017). Their volatility and hydrophobicity, however, present formulation challenges that advanced delivery systems such as microencapsulation and nanoemulsions can address to improve stability and bioavailability in aquaculture feeds (Masoomi Dezfooli et al., 2019).

2.1.4 Phenolic Acids and Polyphenols

Phenolic compounds, including phenolic acids, tannins, and lignans, contribute substantially to the antioxidant capacity of plant extracts and represent one of the most abundant classes of phytochemicals in plant tissues (Serrano et al., 2009). These compounds neutralize reactive oxygen species and upregulate endogenous antioxidant enzyme systems through activation of nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathways (Tonev & Momchilova, 2023). Gallic acid, caffeic acid, and ferulic acid are common phenolic acids with demonstrated bioactivity in fish species (Abasubong et al., 2023). Tannins additionally exhibit protein-precipitating properties that can influence digestive processes and antimicrobial activity through complexation with bacterial surface proteins and enzymes (Serrano et al., 2009). The polymeric nature of tannins enables multiple interactions with biological macromolecules, contributing to their diverse biological effects (Serrano et al., 2009).

2.1.5 Saponins and Glycosides

Saponins are steroid or triterpenoid glycosides characterized by their membrane-active properties and amphiphilic nature, which enables them to interact with both aqueous and lipid environments (Salem, 2023; Pikhtirova et al., 2023). They enhance immune responses by facilitating antigen uptake and presentation, stimulating antibody production, and activating macrophages and lymphocytes (Pikhtirova et al., 2023). The adjuvant properties of saponins have been exploited in vaccine development, and their inclusion in fish feeds may enhance responses to both natural pathogen exposure and vaccination (Pikhtirova et al., 2023). Saponins also exhibit direct antimicrobial effects through membrane disruption and cholesterol binding in bacterial and fungal membranes (Pikhtirova et al., 2023). However, high concentrations may negatively affect growth by reducing nutrient absorption through interactions with intestinal epithelial cells and formation of indigestible complexes with nutrients, emphasizing the importance of dosage optimization for aquaculture applications (Isibor et al., 2024; Lakwani & Salem, 2024).

2.1.6 Polysaccharides

Plant-derived polysaccharides have gained increasing attention as functional feed additives due to their immunomodulatory and prebiotic properties. These complex carbohydrates modulate immune function through interaction with pattern recognition receptors (PRRs) on immune cells, including toll-like receptors (TLRs) and dectin-1, stimulating phagocytosis, cytokine production, and complement activation. Characteristics including low molecular weight and specific monosaccharide profiles (galactose, arabinose, mannose) correlate with immunomodulatory efficacy (Salem, 2021). Polysaccharides also function as prebiotics, promoting beneficial gut microbiota that contribute to disease resistance through competitive exclusion of pathogens, production of short-chain fatty acids, and modulation of intestinal immune function (Kadak, and Salem, 2020).). The structural complexity of polysaccharides enables diverse biological activities that complement those of other phytochemical classes.

2.2 Antimicrobial Mechanisms

The direct antimicrobial activity of plant extracts involves multiple mechanisms that collectively reduce pathogen loads and decrease disease incidence in cultured fish populations (Kadak, and Salem, 2020). Unlike conventional antibiotics that typically target specific bacterial structures or metabolic pathways, phytochemicals often exert pleiotropic effects that complicate resistance development and provide broad-spectrum protection (Salem, 2025; Ustun-Argon, 2025).

2.2.1 Cell Membrane Disruption

Many phytochemicals, particularly essential oil components and saponins, disrupt bacterial cell membrane integrity through multiple complementary mechanisms (Amhamed et al., 2023; Nourbakhsh et al., 2022). The lipophilic nature of terpenoids and phenolic compounds enables their intercalation into the phospholipid bilayer, altering membrane fluidity and permeability through expansion and destabilization of the membrane structure (Gugleva et al., 2021; Salem, 2023). This disruption leads to leakage of ions and cellular contents, dissipation of proton motive force, and ultimately cell death through loss of essential metabolites and energy failure (Salem et al., 2023). Thymol and carvacrol, major components of oregano and thyme oils, exemplify this mechanism, causing rapid membrane damage at concentrations achievable through dietary supplementation (Wink,& Schimmer., 2010). The membrane-active properties of saponins result from their amphiphilic structure, which enables insertion into membranes and formation of pores or disruption of lipid organization (Verstraeten, 2020). Electron microscopy studies have confirmed morphological damage to bacterial cells exposed to plant extracts, including cell wall disruption, membrane blebbing, and cytoplasmic leakage (Salem et al., 2024; Sana et al., 2025).

2.2.2 Inhibition of Quorum Sensing and Virulence Factors

Beyond direct bactericidal activity, subinhibitory concentrations of plant extracts can attenuate pathogenicity by interfering with quorum sensing systems—bacterial cell-to-cell communication mechanisms that regulate virulence factor expression in response to population density. By disrupting quorum sensing, phytochemicals reduce biofilm formation, decrease production of toxins and hydrolytic enzymes, and render pathogens more susceptible to host immune clearance (Khanashyam, 2023). This antivirulence approach represents a promising strategy for disease management with reduced selective pressure for resistance, as it targets pathogenicity rather than essential survival functions (Salem & Salem, 2025). Studies have demonstrated that extracts from various plants can inhibit quorum sensing-regulated behaviors in fish pathogens, including *Aeromonas hydrophila* and *Vibrio* species, reducing their virulence without affecting growth (Dickey et al, 2017). The potential of plant extracts to modulate quorum sensing offers opportunities for developing novel disease control strategies that complement conventional approaches (Salem & Salem, 2025).

2.2.3 Enzyme Inhibition and Metabolic Disruption

Alkaloids and phenolic compounds can inhibit essential bacterial enzymes through multiple mechanisms, including competitive inhibition, non-competitive inhibition, and irreversible modification of active sites. Sanguinarine, for example, inhibits bacterial DNA gyrase and topoisomerase activities through intercalation into DNA and direct enzyme binding, interfering with DNA replication and supercoiling. Phenolic compounds may denature microbial enzymes through protein precipitation or metal chelation at active sites, disrupting metabolic pathways essential for bacterial survival and proliferation.These metabolic disruptions compound membrane damage to achieve antimicrobial effects through multiple parallel mechanisms that collectively overwhelm bacterial defense systems. The multiplicity of enzyme targets affected by complex extract mixtures reduces the likelihood of resistance development through single mutations (Kadak, and Salem, 2020).

2.2.4 Minimum Inhibitory Concentration and Bactericidal Activity

Quantitative assessment of antimicrobial potency typically employs MIC and MBC determinations, which provide essential information for dosage optimization in aquaculture applications (Salem et al., 2024; Sana et al., 2025). Recent studies demonstrate that purified phytochemicals can achieve potent activity comparable to conventional antibiotics. Sanguinarine exhibited MIC of 5 μg/mL and MBC of 10 μg/mL against *A. dhakensis*, with time-kill curves confirming concentration- and time-dependent bactericidal effects (Salem et al., 2025). Similarly, essential oils from various plants have demonstrated MIC values ranging from 0.1 to 10 mg/mL against common fish pathogens, with Gram-positive bacteria generally showing greater susceptibility than Gram-negative organisms due to differences in cell envelope structure (Iman Daw Amhamed et al., 2023; Salem, 2023). Crude extracts generally show higher MIC values but remain effective at dietary inclusion levels achievable in practical aquaculture, with the advantage of containing multiple bioactive compounds that may act synergistically (Salem, 2021; Salem, 2023). The relationship between *in vitro* antimicrobial activity and *in vivo* efficacy is influenced by factors including bioavailability, tissue distribution, and interactions with host immune responses (Salem, 2023).

2.3 Immunomodulatory Mechanisms

The immunostimulatory properties of plant extracts represent perhaps their most valuable contribution to disease resistance in aquaculture, providing broad-spectrum protection against diverse pathogens through enhancement of host defense mechanisms. Phytochemicals enhance both nonspecific (innate) and specific (adaptive) immune mechanisms, creating a state of heightened immune readiness that improves resistance to infection (Bilen et al., 2019; Salem et al., 2021).

2.3.1 Enhancement of Humoral Innate Immunity

Humoral innate immune parameters consistently respond to phytobiotic supplementation, providing soluble factors that limit pathogen proliferation and facilitate clearance (Diaz, 2025). Lysozyme, an antimicrobial enzyme that cleaves bacterial peptidoglycan, increases in serum following administration of various plant extracts, enhancing the capacity to eliminate both Gram-positive and Gram-negative pathogens (Bilen et al., 2019). *Solanum trilobatum* fractions significantly elevated serum lysozyme activity in tilapia, with the hydrophobic hexane-soluble fraction showing greater efficacy than the water-soluble fraction, indicating the importance of compound polarity for immunostimulatory activity (Subramani et al., 2025). Similarly, antiprotease activity, which inhibits bacterial proteases and limits pathogen spread through tissues, increased in extract-fed fish, providing protection against pathogen-derived enzymes that facilitate invasion and immune evasion (Salem et al., 2023).

Complement system activity, measured through alternative pathway hemolytic activity, also responds to phytobiotic stimulation, enhancing both direct lysis of bacterial pathogens and opsonization that facilitates phagocytic. Enhanced complement activity improves the efficiency of pathogen recognition and elimination through multiple convergent mechanisms (Diaz, 2025). The magnitude of humoral immune enhancement depends on factors including extract composition, dosage, and duration of administration, with optimal protocols required to achieve maximum benefit (Salem, 2021).

2.3.2 Cellular Innate Immune Enhancement

Phagocytic cells, including neutrophils and macrophages, represent the front line of cellular defense against invading pathogens, and their functional capacity is consistently enhanced by phytobiotic supplementation (Salem et al., 2023). Plant extracts enhance phagocytic activity, increasing both the proportion of phagocytic cells actively engaged in pathogen uptake and the rate of pathogen engulfment per cell (Bilen et al., 2019). Respiratory burst activity, measured through reactive oxygen species (ROS) and reactive nitrogen intermediate (RNI) production, also increases following phytobiotic administration, enhancing the oxidative killing capacity that eliminates intracellular pathogens (Salem et al., 2023; Subramani et al., 2025). This enhanced oxidative burst results from upregulation of NADPH oxidase and inducible nitric oxide synthase (iNOS) activities, providing potent antimicrobial effector mechanisms.

Myeloperoxidase (MPO) activity, which generates hypochlorous acid from hydrogen peroxide and chloride, further contributes to antimicrobial defense through production of highly reactive oxidizing agents (Subramani et al., 2025). Hexane-soluble *S. trilobatum* fractions significantly increased MPO production in tilapia peripheral blood leukocytes, demonstrating the capacity of hydrophobic phytochemicals to enhance this important antimicrobial pathway (Subramani et al., 2025). The coordinated enhancement of multiple cellular effector mechanisms provides comprehensive protection against diverse pathogen types and infection routes (Salem, 2023).

2.3.3 Modulation of Immune-Related Gene Expression

Molecular studies reveal that phytochemicals modulate immune responses through effects on gene expression, providing sustained enhancement of immune capacity through transcriptional regulation (Salem et al., 2025). Dietary sanguinarine upregulated expression of key immune-related genes in largemouth bass spleen, including transforming growth factor-beta (TGF-β), interleukin-10 (IL-10), myeloid differentiation primary response 88 (MYD88), tumor necrosis factor-alpha (TNF-α), toll-like receptor 2 (TLR2), and immunoglobulin M (IgM). This coordinated upregulation of both pro-inflammatory and regulatory cytokines indicates sophisticated immunomodulation rather than simple immune stimulation, maintaining balance between effective pathogen clearance and avoidance of immunopathology (Salem et al., 2025). Similar gene expression changes have been observed with other plant extracts, suggesting conserved mechanisms of immunomodulation across phytochemical classes (Salem, 2023). The transcriptional effects of phytochemicals are mediated through interactions with cellular signaling pathways, including NF-κB, MAPK, and Nrf2, which coordinate immune and antioxidant gene expression.

2.3.4 Influence of Extract Polarity on Immunostimulatory Efficacy

The physicochemical properties of phytochemicals substantially influence their immunostimulatory efficacy, with compound polarity emerging as a key determinant of biological activity (Salem, 2021; Subramani et al., 2025). Comparative studies of water-soluble versus hydrophobic fractions demonstrate superior activity of nonpolar compounds in multiple immune assays (Subramani et al., 2025). Hexane-soluble *S. trilobatum* fractions, containing approximately 40% aromatic compounds and 11% phytosterols, produced greater enhancement of ROS, MPO, and antibody responses than water-soluble fractions containing low-molecular-weight alcohols and carbonyls (Subramani et al., 2025). This difference likely reflects greater bioavailability and tissue retention of hydrophobic compounds, which persist longer in biological systems and accumulate in cellular membranes where they can interact with signaling complexes (Salem, 2021; Subramani et al., 2025). The implications for formulation development are significant, suggesting that extraction and delivery systems should prioritize retention of hydrophobic bioactives for optimal immunostimulatory efficacy (Salem, 2023).

2.4 Antioxidant Mechanisms

Oxidative stress from ROS imbalance damages fish tissues during infection and stress. Plant extracts counter this through four key mechanisms. Phenolic compounds (flavonoids, phenolic acids) neutralize ROS via hydrogen/electron donation, protecting lipids, proteins, and DNA. Antioxidant capacity correlates with phenolic content, with efficacy influenced by molecular structure (hydroxyl groups, double bonds). Phytochemicals activate Nrf2 pathways, boosting endogenous enzymes (SOD, CAT, GPx, GST). Sanguinarine increased GSH and SOD in bass, providing sustained protection beyond direct scavenging (Salem et al., 2025; Bilen et al., 2019). Flavonoids and phenolic acids chelate iron/copper, preventing Fenton-driven ROS generation. This complements radical scavenging and reduces metal availability to pathogens Enhanced antioxidants improve resilience to high density, temperature changes, and infection. Essential oils reduced lipid peroxidation and protein damage during stress, preserving organ function and immunity (Iman Daw Amhamed et al., 2023; Salem, 2025).

2.5 Growth-Promoting Mechanisms

The growth-enhancing effects of phytobiotics involve multiple physiological mechanisms that improve nutrient utilization and metabolic efficiency, contributing to improved production performance and economic returns. Understanding these mechanisms enables optimization of supplementation strategies for maximum growth benefit.

2.5.1 Digestive Enzyme Enhancement

Plant extracts stimulate secretion and activity of digestive enzymes, improving nutrient breakdown and absorption through effects on pancreatic and intestinal enzyme systems (Bilen et al., 2019; Keriman et al., 2018). Enhanced protease, amylase, and lipase activities have been documented in various fish species fed phytobiotic-supplemented diets, leading to improved protein digestibility, carbohydrate utilization, and lipid absorption (Mohamed Omar Abdalla Salem et al., 2022; Lakwani & Salem, 2024). Improved protein digestibility translates to better growth performance and feed conversion ratios, reducing production costs and environmental nutrient loading (Salem & Moammer, 2024). The mechanisms underlying enzyme enhancement may include direct stimulation of pancreatic secretion, increased enzyme synthesis, and improved intestinal health that supports digestive function (Salem, 2022). Studies with *Vitex agnus-castus* extract demonstrated significant enhancement of digestive enzyme activities in rainbow trout, contributing to improved growth performance (Salem & Mohamed, 2025; Salem & Barkah, 2025).

2.5.2 Appetite Stimulation and Palatability

Certain phytochemicals enhance feed intake by improving diet palatability through stimulation of olfactory and gustatory receptors (Salem, 2021; Mousa et al., 2024). Essential oils and aromatic compounds stimulate sensory receptors, increasing appetite and feed consumption through neural and endocrine pathways that regulate feeding behavior (Iman Daw Amhamed et al., 2023; Salem et al., 2024). Garlic, oregano, and peppermint extracts have demonstrated palatability-enhancing effects in multiple fish species, though high doses may conversely inhibit feed intake due to sensory overstimulation or aversive taste properties, emphasizing the importance of dosage optimization (Salem, 2021; Salem & Lakwani, 2024). The palatability effects of phytochemicals are species-specific, reflecting differences in sensory physiology and feeding ecology, necessitating empirical optimization for each target species (Salem, 2022).

2.5.3 Intestinal Health and Microbiota Modulation

Phytobiotics positively influence intestinal structure and function through multiple mechanisms that enhance nutrient absorption and reduce inflammatory burden (Salem, 2021; Lakwani & Salem, 2024). Enhanced villus height, increased absorptive surface area, and improved epithelial integrity facilitate nutrient absorption and reduce energy costs associated with intestinal maintenance (Salem & Moammer, 2024). Additionally, plant extracts modulate intestinal microbiota composition, suppressing potential pathogens while promoting beneficial bacteria that contribute to nutrient processing, vitamin synthesis, and short-chain fatty acid production (Salem, 2023; Kolygas et al., 2025). Polysaccharide fractions particularly exhibit prebiotic effects, selectively stimulating beneficial microbial populations including *Lactobacillus* and *Bifidobacterium* species that contribute to intestinal health and immune function (Salem, 2021; Subramani et al., 2025). The modulation of intestinal microbiota by phytochemicals represents an important mechanism linking dietary supplementation to systemic health benefits (Salem, 2023).

2.5.4 Metabolic Hormone Modulation

Emerging evidence suggests phytochemicals influence metabolic hormone axes that regulate growth and nutrient partitioning, providing additional mechanisms for growth enhancement (Salem, 2023; Salem, 2026). Thyroid hormone modulation, including effects on thyroxine (T4) and triiodothyronine (T3) production and conversion, may influence metabolic rate and protein synthesis (Salem, 2022; Salem & Alhadad, 2026). Insulin-like growth factor (IGF-1) stimulation enhances protein accretion and cellular proliferation, contributing to tissue growth (Salem, 2025; Salem & Lakwani, 2025). Effects on growth hormone secretion and receptor expression may amplify growth-promoting signals through multiple endocrine pathways (Salem, 2023; Subramani et al., 2025). While these mechanisms require further investigation in fish species, preliminary evidence suggests that phytochemicals can influence the growth hormone-IGF axis through effects on gene expression and receptor sensitivity (Salem, 2026; Mohamed Omar Abdalla Salem et al., 2025).

3. Methodology

3.1 Search Strategy and Databases

This comprehensive review was conducted through systematic searches of peer-reviewed literature published between 2015 and 2026, with emphasis on studies from 2020-2026 to ensure currency and relevance. The following electronic databases were searched: PubMed/MEDLINE, ScienceDirect, Scopus, Web of Science, Google Scholar, FAO AGRIS database, and regional scientific databases including African Journals Online (AJOL) and Arab Journals Platform. Search terms included combinations of: "phytobiotics," "plant extracts," "herbal extracts," "essential oils," "phytochemicals," "aquaculture," "fish," "antibiotic alternatives," "immunostimulation," "antimicrobial mechanisms," "antioxidant," "growth promotion," "Aeromonas," "Vibrio," "Streptococcus," and "disease resistance". Boolean operators (AND, OR, NOT) were employed to refine search results and ensure comprehensive coverage of relevant literature. Reference lists of retrieved articles were manually searched to identify additional relevant studies.

3.2 Inclusion and Exclusion Criteria

Studies were included in this review if they met the following criteria: (1) investigated plant-derived extracts, fractions, or purified compounds in fish species; (2) reported original research data on antimicrobial, immunomodulatory, antioxidant, or growth-promoting effects; (3) included mechanistic information relevant to antibiotic alternative applications; (4) were published in peer-reviewed journals or academic conference proceedings; (5) were written in English, Turkish, or Arabic with English abstracts; and (6) were published between January 2015 and March 2026, with emphasis on studies from 2020-2026 to ensure currency .

Studies were excluded if they: (1) examined terrestrial livestock only without fish applications; (2) used inorganic compounds or synthetic chemicals exclusively; (3) lacked mechanistic data relevant to understanding modes of action; (4) were duplicate publications of the same data; (5) were conference abstracts without full data availability; or (6) were opinion pieces or non-peer-reviewed sources.

3.3 Data Extraction and Synthesis

From each included study, the following information was extracted using standardized data collection forms: fish species and size, plant source and extraction method, phytochemical composition (when available), dosage and administration route, experimental duration, measured outcomes (antimicrobial, immunological, antioxidant, growth parameters), and proposed mechanisms of action. Data were organized according to the major mechanistic categories (antimicrobial, immunomodulatory, antioxidant, growth-promoting) and synthesized to identify patterns, consistencies, and knowledge gaps across studies. Studies were grouped by fish species, phytochemical class, and outcome measure to facilitate comparative analysis and identification of species-specific or compound-specific effects. Results are presented narratively with supporting tables summarizing key findings.

4. Results and Discussion

4.1 Synthesis of Mechanistic Evidence

The accumulated evidence from studies conducted over the past decade demonstrates that plant extracts function through interconnected mechanisms that collectively enhance fish health and disease resistance (Kolygas et al., 2025; Pudota et al., 2025). The integration of antimicrobial, immunomodulatory, antioxidant, and growth-promoting effects provides comprehensive protection that exceeds the sum of individual mechanism contributions (Salem et al., 2025; Liu et al., 2025).

The synthesis of recent studies reveals consistent patterns across different plant sources and fish species, suggesting conserved mechanisms of action that transcend taxonomic boundaries (Fadaei et al., 2025; Pudota et al., 2025). Direct antimicrobial activity reduces pathogen loads, decreasing the infectious challenge facing the host and providing first-line defense against colonization and invasion (Ma et al., 2025; Kolygas et al., 2025). Simultaneous immunostimulation enhances both cellular and humoral defenses, improving pathogen clearance when infections occur and providing protection through modulation of adaptive immunity (Salem et al., 2025; Fadaei et al., 2025). Enhanced antioxidant capacity protects immune cells and tissues from oxidative damage during inflammatory responses, preserving functional capacity and preventing immunopathology (Liu et al., 2025; Ma et al., 2025). Improved digestive function and nutrient utilization support the metabolic demands of enhanced immune activity while promoting growth, creating positive feedback between health and production performance (Fadaei et al., 2025; Salem et al., 2025).

The temporal dynamics of these effects are important for practical application. Antimicrobial effects occur rapidly following exposure, providing immediate protection against pathogens present at the time of supplementation (Ma et al., 2025; Kolygas et al., 2025). Immunomodulatory effects develop over days to weeks as gene expression changes and cellular responses accumulate, providing sustained protection that persists beyond the immediate presence of phytochemicals (Salem et al., 2025; Fadaei et al., 2025). Antioxidant enhancement occurs through both rapid direct radical scavenging and slower enzyme upregulation, providing immediate and sustained protection (Liu et al., 2025; Pudota et al., 2025). Growth-promoting effects require extended supplementation to manifest as improved weight gain and feed conversion, reflecting cumulative improvements in nutrient utilization (Fadaei et al., 2025; Salem et al., 2025). These temporal considerations inform optimal supplementation protocols for different production objectives (Kolygas et al., 2025; Pudota et al., 2025).

4.2 Factors Influencing Phytobiotic Efficacy

4.2.1 Extraction Method and Solvent Polarity

The extraction method fundamentally determines phytochemical composition and consequently biological activity, representing a critical control point for phytobiotic development and quality assurance (Baeshen et al., 2023; Balde et al., 2018). Polar solvents (water, methanol, ethanol) extract hydrophilic compounds including glycosides, polysaccharides, and some phenolics, while nonpolar solvents (hexane, dichloromethane, ethyl acetate) recover hydrophobic compounds such as essential oils, terpenoids, and phytosterols (Baeshen et al., 2023; Balde et al., 2018). Comparative studies demonstrate that hydrophobic fractions often exhibit superior immunostimulatory activity, likely reflecting greater bioavailability and tissue retention (Baeshen et al., 2023). The choice of extraction method also affects yield, with factors including temperature, extraction time, and solvent-to-material ratio influencing recovery of target compounds (Balde et al., 2018). Advanced extraction technologies including ultrasound-assisted, microwave-assisted, supercritical fluid, and pulsed electric field extraction offer advantages in yield, efficiency, and preservation of bioactivity compared to conventional maceration or Soxhlet extraction (Baeshen et al., 2023). These methods reduce extraction time, solvent consumption, and thermal degradation of sensitive compounds, producing extracts with enhanced biological activity (Balde et al., 2018).

The phytochemical profile of extracts should be characterized using appropriate analytical methods including high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and spectrophotometric assays for total phenolics, flavonoids, and other compound classes (Baeshen et al., 2023; Elbahnaswy et al., 2025). Chemometric characterization enables quality control and reproducibility, essential for commercial applications and regulatory approval (Kamble et al., 2026). Studies reporting detailed phytochemical characterization provide greater value for mechanistic interpretation and formulation development than those using crude extracts without compositional analysis (Fadaei et al., 2025).

4.2.2 Dosage Optimization

The dose-response relationship for phytobiotics is typically nonlinear, with optimal ranges producing maximum benefit and excessive doses potentially causing adverse effects through toxicity, immunosuppression, or reduced feed intake (Elbahnaswy et al., 2025; Tadese et al., 2022). Immunostimulatory effects generally increase with dosage within a therapeutic window, but supraoptimal doses may suppress immune responses through feedback inhibition or cellular stress (Kamble et al., 2026). Studies on Nile tilapia demonstrated that intermediate doses of commercial phytobiotic/probiotic mixtures (200-400 mg/kg) produced optimal growth and immune enhancement, while higher doses (600 mg/kg) showed diminished returns (Elbahnaswy et al., 2025). Similar dose optimization has been reported for essential oils and other extract types, with optimal doses typically ranging from 0.5-2.0% of the diet depending on extract potency and fish species (Tran et al., 2024; Fadaei et al., 2025).

Species-specific optimization remains essential, as metabolic rates, digestive physiology, and tolerance vary among fish species (Tadese et al., 2022). Salmonids may show different dose-responses compared to cyprinids or cichlids, reflecting differences in metabolic rate and immune system organization (Kamble et al., 2026). Life stage also influences optimal dosage, with larvae and juveniles generally requiring lower inclusion rates than adults due to higher metabolic rates and greater sensitivity to dietary components (Tadese et al., 2022). The therapeutic window should be established through dose-response studies measuring both efficacy and safety endpoints, including growth performance, immune parameters, and histopathological examination of key organs (Elbahnaswy et al., 2025).

4.2.3 Delivery Systems and Formulation

The development of advanced delivery systems addresses challenges associated with phytobiotic stability, bioavailability, and palatability, facilitating translation from laboratory findings to commercial applications (Kamble et al., 2026; Dawood et al., 2022). Microencapsulation technology protects sensitive compounds from degradation during feed processing, storage, and passage through the gastrointestinal tract, preserving bioactivity until delivery to target sites (Kamble et al., 2026). Encapsulation enables targeted release in specific intestinal regions, optimizing absorption and minimizing degradation by gastric acid and digestive enzymes (Dawood et al., 2022). Microencapsulated phytogenic feed additives significantly reduced mortality caused by bacterial pathogens in various fish species, with excellent feed acceptance confirming palatability (Kolygas et al., 2025). Water-stable formulations ensure retention of bioactive compounds in aquatic environments prior to consumption, reducing waste and ensuring consistent intake (Tran et al., 2024).

Nanoemulsions and nanoparticle formulations offer advantages including enhanced bioavailability through improved solubility and cellular uptake, sustained release profiles, and protection from degradation (Kamble et al., 2026; Salem et al., 2025). The use of nanoparticles from plant derivatives represents an emerging approach with potential applications in aquaculture for targeted delivery of bioactive compounds (Dawood et al., 2022). These advanced formulations may enable dose reduction while maintaining efficacy, improving cost-effectiveness and reducing environmental loading (Kamble et al., 2026). The selection of appropriate delivery systems should consider the physicochemical properties of target compounds, the production system characteristics, and regulatory requirements for feed additives (Ferreira et al., 2025).

4.2.4 Species-Specific Responses

Fish species differ in their responses to phytobiotic supplementation, reflecting variations in digestive physiology, metabolic capacity, immune system organization, and evolutionary history (Tadese et al., 2022; Dawood et al., 2022). Tilapia and other cichlids generally respond favorably to a wide range of plant extracts, showing consistent enhancement of immune parameters and disease resistance across multiple studies (Elbahnaswy et al., 2025; Fadaei et al., 2025). Salmonids including rainbow trout show more variable responses, with some extracts producing robust effects while others show limited efficacy, possibly reflecting differences in intestinal physiology and immune system organization (Fadaei et al., 2025). Cyprinids such as common carp and goldfish demonstrate intermediate responses, with many extracts showing efficacy at appropriate doses (Tadese et al., 2022).

These species-specific differences underscore the importance of empirical validation for each target species rather than extrapolating results across diverse taxonomic groups (Kamble et al., 2026). Factors contributing to species-specific responses include differences in gastrointestinal pH affecting compound stability, variations in digestive enzyme profiles influencing compound metabolism, differences in immune cell populations and receptor expression, and variations in metabolic rate affecting compound turnover and clearance (Dawood et al., 2022). Understanding these factors will enable prediction of species responses based on physiological characteristics, reducing the need for empirical optimization for each new species (Ferreira et al., 2025).

4.3 Challenges and Limitations

Despite substantial progress in understanding phytobiotic mechanisms and demonstrating efficacy in experimental settings, several challenges impede the widespread adoption of phytobiotics in commercial aquaculture (Kamble et al., 2026; Ferreira et al., 2025). Addressing these challenges is essential for realizing the potential of plant extracts as sustainable alternatives to antibiotics (Tadese et al., 2022).

4.3.1 Standardization and Quality Control

Plant extracts exhibit inherent variability due to genetic factors, growing conditions, harvest timing, processing methods, and storage conditions, complicating standardization and quality assurance for commercial production (Baeshen et al., 2023; Ferreira et al., 2025). This variability affects batch-to-batch consistency of biological activity, presenting challenges for regulatory approval and commercial reliability (Kamble et al., 2026). Chemometric characterization using chromatographic and spectroscopic methods enables quality control, but implementation adds cost and complexity to production processes (Baeshen et al., 2023). Development of reference standards, validated analytical protocols, and acceptance criteria for key bioactive markers remains a priority for the phytobiotic industry (Ferreira et al., 2025). Good agricultural and collection practices (GACP) and good manufacturing practices (GMP) should be implemented to minimize variability and ensure product quality (Kamble et al., 2026).

4.3.2 Regulatory Frameworks

Clear regulatory frameworks for phytobiotic feed additives remain underdeveloped in many jurisdictions, creating uncertainty for manufacturers and users (Ferreira et al., 2025; Kamble et al., 2026). Classification as feed ingredients, functional feeds, or veterinary medicines affects registration requirements, market access, and permitted claims (Ferreira et al., 2025). The complex composition of plant extracts presents challenges for regulatory evaluation, as traditional paradigms developed for single-compound drugs are poorly suited to multicomponent mixtures (Kamble et al., 2026). Harmonized international standards and guidance documents would facilitate commercialization and trade, supporting the global aquaculture industry (Ferreira et al., 2025). Engagement with regulatory authorities during product development can streamline approval processes and ensure compliance with applicable requirements (Tadese et al., 2022).

4.3.3 Cost-Effectiveness and Scalability

Economic viability depends on achieving consistent efficacy at costs competitive with conventional alternatives, including antibiotics and synthetic chemotherapeutic agents (Tran et al., 2024; Dawood et al., 2022). Agricultural and food processing byproducts offer sustainable, low-cost sources of phytochemicals that can improve economic competitiveness while supporting circular economy principles (Tran et al., 2024). Efficient extraction technologies, including enzyme-assisted, ultrasound-assisted, and microwave-assisted methods, can improve yields and reduce production costs compared to conventional extraction (Baeshen et al., 2023). Optimized formulation strategies, including use of synergistic combinations and advanced delivery systems, can enhance efficacy and enable dose reduction, improving cost-effectiveness (Kamble et al., 2026). Scale-up from laboratory to commercial production requires process validation, quality systems, and investment in production infrastructure, representing barriers for small-scale producers (Dawood et al., 2022).

4.3.4 Environmental Interactions

The environmental fate of phytochemicals and their effects on nontarget organisms require investigation to ensure ecological safety of large-scale applications (Ferreira et al., 2025; Kamble et al., 2026). While plant-derived compounds are generally biodegradable and less persistent than synthetic chemicals, their ecological impacts in aquatic systems receiving effluent deserve assessment (Ferreira et al., 2025). Interactions with environmental microbiomes and potential effects on nutrient cycling, microbial community structure, and ecosystem function represent knowledge gaps requiring systematic investigation (Kamble et al., 2026). The potential for phytochemical accumulation in sediments and aquatic organisms should be evaluated through environmental fate studies (Ferreira et al., 2025). Life cycle assessment of phytobiotic production and use can identify environmental hotspots and guide sustainable development (Tadese et al., 2022).

4.3.5 Knowledge Gaps in Mechanistic Understanding

Despite progress in understanding phytobiotic mechanisms, significant knowledge gaps remain that limit rational formulation and optimization (Fadaei et al., 2025; Kamble et al., 2026). The specific molecular targets of many phytochemicals in fish cells remain unidentified, limiting understanding of structure-activity relationships and species-specific effects (Kamble et al., 2026). Interactions between phytochemicals and the complex microbial communities of fish intestines are incompletely characterized, limiting understanding of prebiotic effects and microbiome-mediated health benefits (Dawood et al., 2022). The fate of phytochemicals in fish tissues, including metabolism, tissue distribution, and elimination, requires investigation to optimize dosing regimens and assess food safety implications (Ferreira et al., 2025). Addressing these knowledge gaps through targeted research will support evidence-based development of phytobiotic products (Fadaei et al., 2025).

5. Future Directions

5.1 Mechanistic Studies Using Omics Technologies

Advanced molecular approaches offer opportunities to elucidate phytobiotic mechanisms with unprecedented resolution, providing systems-level understanding of how complex extract mixtures produce integrated physiological effects. Transcriptomics, proteomics, and metabolomics can reveal genome-wide responses to phytochemical exposure, identifying pathways and networks affected by supplementation and revealing unexpected mechanisms of action . Integration of multi-omics data through bioinformatic approaches enables construction of mechanistic models that predict responses to different extracts and identify optimal combinations targeting specific pathways. Metagenomic analysis of intestinal microbiota can reveal effects on microbial community structure and function, linking microbiome modulation to host health outcomes . Genomic and multi-omics methods have successfully identified health-associated microbes and metabolites, such as Vibrio-dominated dysbiosis markers in shrimp and butyrate-mediated immunity, providing biomarkers for targeted intervention strategies . These systems-level approaches will accelerate discovery and optimization of phytobiotic formulations for aquaculture applications.

5.2 Synergistic Combinations

Combinations of phytochemicals may produce synergistic effects exceeding those of individual compounds through complementary mechanisms of action and enhanced bioavailability. Rational design of phytobiotic blends based on complementary mechanisms could achieve enhanced efficacy at reduced dosages, improving cost-effectiveness and reducing potential for adverse effects. Recent studies demonstrate that compound herbal formulations exhibit superior advantages compared to single-herb formulations, with multi-component synergy providing comprehensive disease control through multi-target mechanisms that reduce the risk of pathogen resistance. Integration with other functional feed additives including probiotics, prebiotics, organic acids, and immunostimulants represents a promising strategy for comprehensive health management that addresses multiple aspects of fish health simultaneously. Systematic evaluation of combinations using factorial designs and response surface methodology can identify optimal mixtures and quantify synergistic interactions. Research on large yellow croaker demonstrated that combining Astragalus membranaceus polysaccharides with Forsythia suspensa extract synergistically boosted antioxidant defenses via Nrf2 activation, enhanced digestive enzymes, and mitigated inflammation-induced apoptosis. Similarly, combinations with Eleutherococcus senticosus extract achieved optimal growth and feed efficiency through improved intestinal structure and digestive enzyme activity . The development of commercial products containing optimized phytobiotic combinations offers opportunities for product differentiation and improved performance.

5.3 Sustainable Sourcing and Circular Economy Approaches

Agricultural and food processing byproducts represent underutilized sources of phytochemicals that could be valorized as aquaculture feed additives, supporting sustainable development and circular economy principles . Fruit and vegetable processing wastes, oilseed meals, pruning residues, and other agricultural byproducts contain bioactive compounds that could be extracted and incorporated into fish feeds. Olive mill waste, grape pomace, citrus processing residues, and other byproducts have demonstrated potential as sources of phenolic compounds with antioxidant and antimicrobial activities . This approach addresses waste management challenges while providing cost-effective phytobiotic sources, creating value from materials that would otherwise require disposal. Integration of phytobiotic production with existing agricultural and food processing industries can create new value chains and support rural development . The valorization of agri-food biowaste aligns with sustainable production goals and contributes to the protection of aquatic ecosystems by reducing waste streams and providing environmentally friendly alternatives to synthetic inputs .

5.4 Commercial-Scale Validation

Translation of experimental findings to commercial production requires validation under practical farming conditions that account for the complexity and variability of real-world production systems. Large-scale trials assessing growth performance, disease resistance, feed efficiency, and economic returns under commercial stocking densities, feeding practices, and environmental conditions will support industry adoption by providing evidence of practical benefits . Recent collaborative research demonstrated that microencapsulated phytogenic feed prototypes achieved statistically significant reductions in mortality due to *Yersinia ruckeri*—by 26.3% and 29.3%, respectively, compared to controls, with high feed acceptance rates confirming palatability and application feasibility in commercial aquaculture systems . These trials should include multiple production cycles, varied environmental conditions, and representative disease challenges to demonstrate robustness and reliability of effects. Economic analysis should assess cost-effectiveness and return on investment under commercial conditions, providing information essential for producer decision-making. Collaboration between researchers, feed manufacturers, and aquaculture producers is essential for successful commercial validation .

5.5 Development of Standardized Protocols

The development of standardized protocols for phytobiotic evaluation would facilitate comparison across studies and accelerate progress toward commercial applications . Consensus on experimental designs, outcome measures, and reporting standards would enable meta-analysis and systematic review, strengthening the evidence base for phytobiotic efficacy. Harmonized protocols for extract characterization, including identification and quantification of major bioactive compounds, would support quality assurance and regulatory approval. Standardized challenge models using reference pathogens and standardized conditions would enable comparison of efficacy across studies and identification of most promising extracts . International collaboration through scientific societies and industry associations can facilitate development and adoption of standardized protocols, as has been achieved in probiotic research where FAO/WHO guidelines provide frameworks for evaluation despite the complexities introduced by environmental and host species diversity .

6. Conclusion

This comprehensive review synthesizes current understanding of the mechanisms through which plant extracts function as alternatives to antibiotics in aquaculture, drawing on recent studies conducted between 2015 and 2026. The evidence demonstrates that phytobiotics operate through interconnected antimicrobial, immunomodulatory, antioxidant, and growth-promoting mechanisms that collectively enhance fish health and disease resistance, providing a multifaceted approach to health management that contrasts with the single-target action of conventional antibiotics.

Direct antimicrobial activity, including membrane disruption, quorum sensing inhibition, and enzyme inactivation, reduces pathogen loads and decreases infection pressure through multiple parallel mechanisms that complicate resistance development. Immunomodulation enhances both humoral and cellular innate defenses while modulating adaptive immune responses through effects on gene expression and cellular function, improving pathogen clearance capacity and providing sustained protection. Antioxidant mechanisms, including direct radical scavenging, upregulation of endogenous enzyme systems, and metal chelation, protect tissues from oxidative damage during immune responses and environmental stress, preserving functional capacity and preventing immunopathology. Growth-promoting effects, including digestive enzyme enhancement, improved intestinal health, appetite stimulation, and metabolic hormone modulation, improve nutrient utilization and feed efficiency, supporting the metabolic demands of enhanced immune activity while promoting production performance.

The diversity of phytochemical classes—including alkaloids, flavonoids, essential oils, phenolics, saponins, and polysaccharides—provides a rich source of bioactive compounds with complementary mechanisms of action, enabling formulation of optimized blends targeting specific health challenges or production objectives. Factors influencing phytobiotic efficacy include extraction methods that determine phytochemical composition, optimized dosages that balance efficacy and safety, advanced delivery systems that improve stability and bioavailability, and species-specific responses that require tailored formulations for different cultured species.

Despite substantial progress in understanding phytobiotic mechanisms and demonstrating efficacy in experimental settings, several challenges impede widespread commercial adoption. Standardization and quality control remain challenging due to inherent variability in plant materials, requiring implementation of good agricultural and collection practices, validated analytical methods, and acceptance criteria for key bioactive markers. Regulatory frameworks for phytobiotic feed additives remain underdeveloped in many jurisdictions, creating uncertainty for manufacturers and users. Cost-effectiveness and scalability require development of efficient extraction technologies, utilization of agricultural byproducts as low-cost feedstocks, and optimized formulation strategies that enhance efficacy while reducing production costs. Environmental interactions and potential effects on nontarget organisms require investigation to ensure ecological safety of large-scale applications.

Future research should employ omics technologies to elucidate molecular mechanisms with unprecedented resolution, providing systems-level understanding of phytochemical effects. Exploration of synergistic combinations of phytochemicals and integration with other functional feed additives offers opportunities for enhanced efficacy and comprehensive health management. Development of sustainable sourcing strategies using agricultural byproducts supports circular economy principles while improving cost-effectiveness. Commercial-scale validation under practical farming conditions is essential for demonstrating practical benefits and supporting industry adoption. Development of standardized protocols for phytobiotic evaluation would facilitate comparison across studies and accelerate progress toward commercial applications.

By consolidating current mechanistic knowledge and identifying strategic research priorities, this review aims to accelerate the development and adoption of plant-based alternatives to antibiotics, contributing to more sustainable, resilient, and environmentally responsible aquaculture practices. The transition from antibiotic-dependent disease management to phytobiotic-based health promotion represents a fundamental shift in aquaculture health management that aligns with global goals for antimicrobial resistance reduction, environmental protection, and sustainable food production. Realizing this vision requires continued investment in research, development, and technology transfer, supported by enabling policies and regulatory frameworks that recognize the unique characteristics and potential of plant-derived bioactive compounds.

7. Recommendations

Based on the findings of this comprehensive review, the following recommendations are proposed for researchers, feed manufacturers, aquaculture producers, regulatory authorities, and funding agencies:

7.1 For Researchers

1. **Prioritize mechanistic studies** using standardized extracts with detailed phytochemical characterization, enabling identification of active compounds and elucidation of molecular mechanisms through which they exert biological effects.
2. **Investigate dose-response relationships** across relevant fish species and life stages, establishing therapeutic windows and safety margins for practical application.
3. **Employ omics technologies** (transcriptomics, proteomics, metabolomics, metagenomics) to elucidate molecular pathways and microbial community changes affected by phytobiotic supplementation, providing systems-level understanding of effects.
4. **Explore synergistic combinations** of complementary phytochemicals and integration with other functional feed additives, using factorial designs and response surface methodology to identify optimal formulations.
5. **Conduct commercial-scale validation trials** under practical farming conditions, including assessment of growth performance, disease resistance, economic returns, and environmental impacts.
6. **Investigate species-specific responses** to identify physiological and genetic factors determining efficacy, enabling prediction of responses across cultured species.
7. **Develop standardized protocols** for phytobiotic evaluation, including extract characterization, experimental designs, outcome measures, and reporting standards, facilitating comparison across studies.
8. **Study environmental fate and effects** of phytochemicals in aquatic systems, including biodegradation, accumulation, and impacts on nontarget organisms.

7.2 For Feed Manufacturers

1. **Invest in quality control systems** ensuring consistent phytochemical composition through implementation of validated analytical methods and acceptance criteria for key bioactive markers.
2. **Develop advanced delivery systems** including microencapsulation and nanoformulations to improve stability, bioavailability, and palatability of phytobiotic products.
3. **Collaborate with researchers** on commercial-scale validation trials to demonstrate efficacy and safety under practical conditions.
4. **Establish sustainable supply chains** for plant materials, including utilization of agricultural and food processing byproducts, supporting circular economy principles.
5. **Invest in efficient extraction technologies** that improve yields, reduce production costs, and preserve bioactivity of sensitive compounds.
6. **Develop optimized phytobiotic blends** based on complementary mechanisms of action, targeting specific health challenges or production objectives.
7. **Engage with regulatory authorities** during product development to ensure compliance with applicable requirements and streamline approval processes.

8. **Provide clear product information** to users, including recommended inclusion rates, expected benefits, and guidance for integration with existing health management practices.

7.3 For Aquaculture Producers

1. **Implement phytobiotic supplements** as components of integrated health management programs, combining them with good husbandry practices, biosecurity measures, and vaccination where applicable.
2. **Monitor responses under farm conditions** and adjust inclusion rates based on observed outcomes, maintaining records of disease incidence, growth performance, and treatment costs.
3. **Maintain detailed records** of disease incidence, treatment costs, and production performance to document economic benefits and support continuous improvement.
4. **Engage with extension services and researchers** to access current information on effective products and practices, participating in on-farm trials where possible.
5. **Source phytobiotic products** from reputable manufacturers with quality control systems and demonstrated efficacy through commercial-scale validation.
6. **Consider species-specific requirements** when selecting phytobiotic products, recognizing that optimal formulations may differ among cultured species.
7. **Integrate phytobiotic use** with other health management strategies, including probiotics, prebiotics, and improved nutrition, for comprehensive health promotion.

7.4 For Regulatory Authorities

1. **Develop clear regulatory frameworks** for evaluating and approving phytobiotic feed additives, recognizing the unique characteristics of multicomponent natural products.
2. **Establish standards for quality, safety, and efficacy** that accommodate the inherent complexity of plant-derived products while ensuring consumer and environmental protection.
3. **Facilitate harmonization of international regulations** to support global trade and reduce barriers to market access for phytobiotic products.
4. **Provide guidance documents** for manufacturers regarding data requirements, testing protocols, and approval processes for phytobiotic feed additives.
5. **Support research on regulatory science** for natural products, including development of validated analytical methods and assessment approaches appropriate for complex mixtures.
6. **Consider tiered approval pathways** that recognize different levels of risk and allow for innovation while ensuring safety.

7.5 For Funding Agencies

1. **Prioritize research on phytobiotic alternatives** to antibiotics in aquaculture, recognizing the global importance of antimicrobial resistance and sustainable food production.
2. **Support collaborative research programs** bringing together researchers, industry partners, and regulatory experts to address translational challenges.
3. **Fund commercial-scale validation studies** that demonstrate practical efficacy and economic viability under real-world conditions.
4. **Invest in capacity building** for phytochemical analysis, formulation development, and quality control in regions with significant aquaculture production.
5. **Support development of standardized protocols** and reference materials that facilitate comparison across studies and accelerate progress toward commercial applications.
6. **Fund research on sustainable sourcing** and valorization of agricultural byproducts, supporting circular economy approaches to phytobiotic production.

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