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From taxonomy to therapeutics: Integrating DNA barcoding and antimicrobial screening of marine brown algae against shrimp pathogens

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ABSTRACT

Objective: To critically integrate current evidence on the therapeutic potential of marine brown algae against aquaculture shrimp pathogens, with particular emphasis on the contribution of DNA barcoding to improve species identification and strengthening antimicrobial investigations.
Significance of review: The rising prevalence of antibiotic-resistant pathogens in shrimp aquaculture has intensified the need for environmentally sustainable alternatives to conventional antimicrobial agents. Marine brown algae are recognized as a rich source of structurally diverse bioactive metabolites, many of which have demonstrated antimicrobial activity. The integration of DNA barcoding with bioactivity screening improves taxonomic accuracy and enhances the reliability and reproducibility of bioprospecting efforts aimed at aquaculture disease management.
Key findings: Significant progress has been made in the molecular identification of brown algae, with the combined use of rbcL and ITS markers achieving a reported species resolution of $>$ 90%. Antimicrobial screening studies identified brown algal species exhibiting inhibitory effects against major shrimp pathogens. Among these, species belonging to the genera *Sargassum* and *Turbinaria* were most frequently reported to demonstrate comparatively stronger pathogen suppression. Collectively, the findings highlight the important role of accurate taxonomic validation in enhancing the consistency and interpretability of antimicrobial screening results, emphasizing the complementary relationship between molecular barcoding and pharmacological evaluation.
Conclusions: The integration of DNA barcoding and antimicrobial screening offers an integrated framework for advancing the discovery of marine-derived therapeutic candidates for shrimp aquaculture. Future research should focus on mechanistic studies, methodological standardization and validation through controlled in vivo aquaculture trials to enable practical deployment in aquaculture systems.

1. Introduction

The integration of biodiversity assessment and DNA barcoding has become increasingly vital in the identification and management of shrimp pathogens linked to brown algae [1,2]. In the context of increasing disease outbreaks and rising antimicrobial resistance in shrimp aquaculture, accurate species identification has become a foundational requirement for developing reliable therapeutic strategies. Accurate identification of brown algae species is crucial for the development of targeted therapeutic interventions [3]. DNA barcoding, particularly through the use of the cytochrome c oxidase 1 (COI) gene, plays an important role in this process, enabling researchers to systematically catalogue and study the extensive biodiversity present in marine ecosystems. In addition to the COI marker, chloroplast and nuclear markers such as rbcL and ITS have demonstrated improved resolution for brown algal taxa, thereby enhancing taxonomic accuracy in marine biodiversity studies. Integrative taxonomic studies combining detailed morphological characterization with ITS-based molecular barcoding have demonstrated that molecular validation significantly strengthens species-level identification and resolves cryptic diversity

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that may not be distinguishable through morphology alone. Such approaches are useful in ecologically diverse regions, where variations in environmental conditions often influence organismal traits, making morphological identification difficult due to phenotypic plasticity and similarities in diagnostic characteristics among closely related species [4]. A well-established and comprehensive barcode database is essential to support ecological and taxonomic research, thereby laying the groundwork for the effective identification of marine resources [5,6].

Beyond species identification, the therapeutic potential of brown algae, such as *Sargassum glaucescens*, has garnered significant attention due to their potent antibacterial properties. These algae have demonstrated strong efficacy, particularly in ethanolic extracts, against shrimp pathogens like *Vibrio harveyi* and *Staphylococcus aureus* [7]. Such findings suggest that brown algae represent a promising reservoir of bioactive metabolites capable of addressing pathogen-associated damages in aquaculture systems. The potential of these natural compounds as antimicrobial agents is evident, and when combined with DNA barcoding and advanced nanotechnology, there is the potential to significantly enhance pathogen detection and refine treatment strategies [8]. The precision offered by such an integrated approach could revolutionize drug delivery systems and pathogen management in marine settings [9].

However, several challenges must be addressed to fully realize the potential of these developments. Standardizing extraction methods remains a critical hurdle, as does ensuring the consistent efficacy of bioactive compounds, especially against resistant strains of pathogens [10]. Moreover, variation in pathogen selection, experimental design and bioassay protocols throughout studies limits direct comparability and weakens evidence-based conclusions. Linking molecular taxonomic validation with functional bioactivity assessment is essential to ensure reproducibility in antimicrobial research. Integrating DNA barcoding with pathogen diagnostics could streamline the identification of algae that produce antimicrobial compounds, thereby enhancing shrimp health management strategies [11]. However, challenges such as the risk of misidentifying algal species remain, which could impede the development of effective therapies.

Therefore, this review aims to critically examine the intersection between DNA-based taxonomic validation and antimicrobial screening of marine brown algae in the context of shrimp aquaculture. Specifically, it addresses (i) the current applications and limitations of DNA barcoding in brown algae research (ii) reported antimicrobial activities against major shrimp pathogens and (iii) the need for methodological standardization and conceptual integration to strengthen translational outcomes. The findings highlight the need for continued research to fully exploit the benefits of marine biodiversity and to advance the application of DNA barcoding within ecological, medical, and therapeutic contexts [12]. This ongoing research is critical for the development of effective management strategies that could ultimately lead to improved health outcomes in aquaculture and related industries. Table 1 provides a comparative overview of recent studies, highlighting variation in species investigated, molecular markers used, target pathogens, and antimicrobial outcomes.

Despite the growing body of literature on marine brown algae and their antimicrobial potential, existing studies remain largely fragmented and methodologically inconsistent. Most investigations focus on either taxonomic identification or bioactivity screening in isolation, with limited efforts to integrate these domains into a coherent framework. Furthermore, variability in experimental design, extraction protocols, and pathogen selection has resulted in inconsistent and sometimes non-reproducible findings. As a consequence, the translational potential of brown algae–derived therapeutics in aquaculture remains insufficiently defined. Therefore, a critical synthesis that evaluates both the strengths and limitations of current approaches, while emphasizing the integration of DNA barcoding with pharmacological validation, is essential to advance this field. The novelty of this review lies in its integrative perspective, which explicitly links DNA barcoding with antimicrobial screening in brown algae research. Unlike previous reviews that treat taxonomic identification and bioactivity evaluation as separate domains, this work critically examines how molecular validation directly influences the reliability and reproducibility of antimicrobial findings. By highlighting the role of DNA barcoding in ensuring accurate species identification, resolving cryptic diversity, and enabling consistent attribution of bioactive compounds, this review provides a more robust framework for advancing marine natural product research in aquaculture. This integrative approach is essential for improving candidate selection and enhancing the translational potential of algal-derived therapeutics against shrimp pathogens. The overall workflow proposed for identifying potential brown seaweed species and evaluating their antimicrobial activity against shrimp pathogens using DNA barcoding and bioactivity screening is illustrated in Fig. 1.

2. Methodology

2.1. Literature search strategy

A comprehensive systematic literature review was conducted from January to March 2025 to identify relevant studies on brown algae biodiversity, DNA barcoding, and their therapeutic applications against shrimp pathogens, specifically emphasizing publications from 2016 to 2025. The search encompassed multiple prominent databases including PubMed/MEDLINE, Web of Science Core Collection, Scopus, Google Scholar, ScienceDirect, and ResearchGate. A primary search strategy utilized terms such as ("brown algae" OR "brown seaweed" OR "Phaeophyceae" OR "Sargassum" OR "Turbinaria" OR "Hormophysa" OR "Padina" OR "Zonaria") AND ("DNA barcoding" OR "molecular identification" OR " rbcL" OR "ITS" OR "matK" OR "COI" OR "phylogenetic") AND ("antimicrobial" OR "antibacterial" OR "antifungal" OR "bioactive" OR "therapeutic") AND ("shrimp" OR "aquaculture" OR "Vibrio" OR "Aeromonas" OR "pathogen"). A secondary search included terms like ("Gulf of Mannar" OR "marine biodiversity" OR "seaweed extract" OR "fucoidan" OR "phlorotannin") AND ("disease resistance" OR "immunostimulant" OR "WSSV" OR "white spot syndrome").

Table 1
Comprehensive literature summary of brown algae studies (2020–2025).

Location	Species	DNA Markers	Target Pathogens	Key Findings	Antimicrobial Activity
Gulf of Mannar, India	*S. wightii*	RBCL, ITS	*V. harveyi, V. parahaemolyticus*	High phenolic content, 18.5 mm inhibition zone	Strong (MIC: 125 μg/ml)
South China Sea	*T. ornata*	RBCL, MATK, COI	*A. hydrophila, S. aureus*	Novel bioactive compounds identified	Moderate-Strong
Mediterranean	*H. cuneiformis*	ITS, LSU	*V. alginolyticus*	Seasonal variation in activity	Moderate
Arabian Sea	*P. boergesenii*	RBCL, ITS	*V. harveyi*, WSSV	Immunostimulant properties	Strong
Yellow Sea	*S. fusiforme*	RBCL, MATK	Multiple Vibrio spp.	Temperature-dependent efficacy	Moderate
Gujarat Coast	*T. conoides*	ITS, COI	*A. hydrophila*	High fucoidan content	Strong
Atlantic Coast	*S. muticum*	RBCL, ITS, MATK	*V. parahaemolyticus*	Broad-spectrum activity	Strong
Korean Peninsula	*S. horneri*	RBCL, COI	*V. harveyi*	Antiviral properties	Moderate-Strong
Bay of Bengal	*Z. variegata*	ITS, RBCL	*V. alginolyticus*	Low cytotoxicity	Moderate
Canary Islands	*S. desfontainesii*	RBCL, MATK	Multiple pathogens	Synergistic effects	Strong
Red Sea	*T. turbinata*	ITS, COI	*A. hydrophila*	Heat-stable compounds	Moderate
North Sea	*S. polyceratium*	RBCL, ITS	*V. harveyi*	Cold-adapted enzymes	Moderate

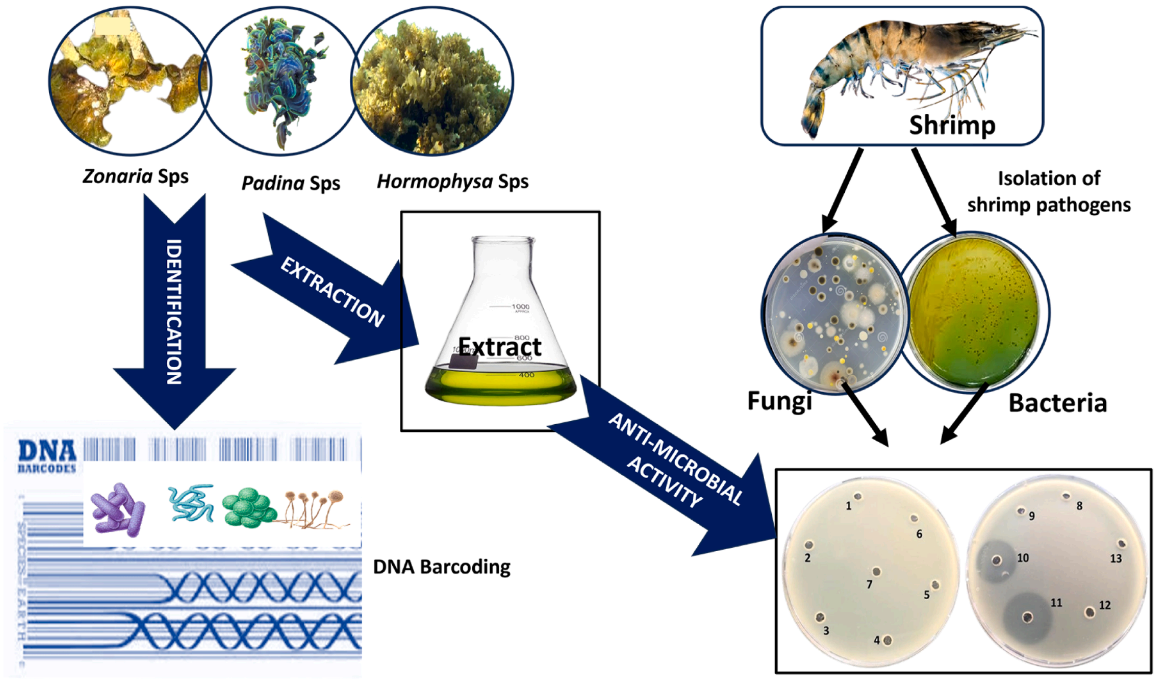

Fig. 1. Schematic representation of the integrated approach for screening brown seaweed-derived antimicrobial compounds against shrimp pathogens. The process involves collection and DNA barcoding-based identification of brown algae, extraction of bioactive metabolites, isolation of shrimp pathogens, antimicrobial screening of algal extracts and assessment of their inhibitory effects on pathogen growth, virulence factors and biofilm formation.

Inclusion criteria specified peer-reviewed research articles and reviews published between 2016 and 2025, focusing on brown algae (Phaeophyceae) identification, biodiversity, molecular characterization, and their antimicrobial, antibacterial, or antifungal properties. Studies on therapeutic applications in aquaculture, particularly against shrimp pathogens, and articles on DNA barcoding methodologies for marine algae identification published in English were included. Exclusion criteria removed conference abstracts, book chapters, non-peer-reviewed publications, studies primarily focusing on green or red algae, research on algae cultivation or biofuel production without a therapeutic focus, and non-English publications without available translations or those published before 2016.

The initial search yielded 347 potentially relevant articles. After removing 68 duplicates, 279 titles and abstracts were screened against the inclusion criteria, leading to 94 articles undergoing full-text evaluation. Following a detailed assessment, 83 studies met all inclusion criteria and were incorporated into this comprehensive review. Each included study underwent quality assessment based on methodological rigor (experimental design, controls, statistical analysis), sample size adequacy, clarity of DNA barcoding protocols and marker selection, antimicrobial testing methodology (standardized assays, positive/negative controls), reproducibility of results, geographic representation, and species diversity. Data systematically extracted from each study included: study location and sampling details; brown algae species investigated; DNA barcoding markers used (rbcL, ITS, matK, COI, LSU); target pathogens tested; extraction methods and bioactive compounds identified; antimicrobial activity results (zone of inhibition, MIC values); and key findings and conclusions.

2.2. Quality assessment criteria

Study design (2 points), sample size (2 points), methodology rigor (2 points), statistical analysis (2 points), reproducibility (2 points). Maximum score: 10 points.

While this review followed a structured and systematic approach, certain limitations must be acknowledged. The inclusion of studies with heterogeneous experimental designs, varying antimicrobial assay protocols, and differing reporting standards may influence the comparability of results. In addition, most studies were concentrated in specific geographic regions, particularly Asia-Pacific, which may introduce regional bias in species representation and bioactivity profiles. The reliance on published literature may also result in publication bias, where studies reporting positive antimicrobial activity are over-represented. These factors were considered during data interpretation to ensure a balanced and critical evaluation of the evidence.

2.3. DNA barcoding in brown algae research

2.3.1. Ecological interplay between brown algae and shrimp pathogens

The biodiversity of the Gulf of Mannar, particularly concerning shrimp pathogens associated with brown algae, represents a critical focus of ecological and marine research. Brown algae are rich in bioactive compounds with significant antimicrobial properties, highlighting their potential applications in aquaculture systems [13]. DNA barcoding, especially through the cytochrome c oxidase I (COI) gene has been widely applied for identifying various marine organisms and improving biodiversity documentation. For instance, the availability of COI sequences for several marine taxa has strengthened regional biodiversity databases and provided valuable molecular references for future studies [14].

In brown algae research, DNA barcoding has emerged as an effective molecular tool for species identification because it provides stable genetic markers that are less influenced by environmental variation compared to morphological traits. Brown algae often exhibit considerable phenotypic plasticity, meaning that their external morphology may change depending on environmental conditions such as salinity, temperature, and nutrient availability. Molecular markers such as COI, rbcL and ITS are therefore increasingly used to improve species resolution and to support accurate taxonomic classification in marine algal studies [15,16].

Despite these advantages, DNA barcoding also presents certain limitations that must be carefully considered. One major challenge is the incompleteness of reference databases, particularly for marine algae, which may lead to ambiguous species matches. In addition, closely related species sometimes share highly similar barcode sequences, making it difficult to distinguish them using a single genetic marker. Such limitations highlight the importance of combining multiple genetic markers with traditional morphological observations and phylogenetic analysis to ensure reliable species identification [17].

Shrimp pathogens such as *Vibrio harveyi* not only pose a direct threat to aquaculture species but also influence broader marine ecosystem stability. Despite these advantages, DNA barcoding also presents certain limitations that must be carefully considered. One major challenge is the incompleteness of reference databases, particularly for marine algae, which may lead to ambiguous species matches. In addition, closely related species sometimes share highly similar barcode sequences, making it difficult to distinguish them using a single genetic marker. Such limitations highlight the importance of combining multiple genetic markers with traditional morphological observations and phylogenetic analysis to ensure reliable species identification aquaculture and other marine organisms but may also indirectly influence coral health and overall marine ecosystem stability [18]. Coral diseases, including brown band disease, have been associated with increased macroalgal dominance, which can reduce faunal diversity and alter ecosystem dynamics [19]. The Gulf of Mannar supports a rich diversity of brown algae, including species belonging to the genera *Sargassum* and *Gracilaria*, which play an important role in maintaining ecological balance and habitat stability [20].

Accurate species identification becomes particularly important when studying the bioactive properties of marine algae. Many brown algae produce secondary metabolites with antibacterial, antiviral and antifungal activities. If the algal species used in bioactivity experiments are incorrectly identified, it may lead to inconsistencies in research findings and difficulties in reproducing experimental results. Therefore, integrating DNA barcoding into bioactivity studies ensures that the biological activity observed can be reliably linked to the correct algal species, thereby strengthening the scientific validity of pharmacological and aquaculture applications of brown algae [21].

The antibacterial potential of brown algae has attracted attention for its possible role in controlling shrimp pathogens. Extracts from species such as *Sargassum glaucescens* have demonstrated antibacterial activity against pathogens *viz.*, *Staphylococus aureus, Vibrio harveyi, Escherichia coli, Bacillus cereus, Candida albicans, Aspergillus flavus* and *Fusarium solani,* suggesting their potential use as natural biocontrol agents in aquaculture systems [22]. Although the diversity and ecological resilience of brown algae may help maintain marine ecosystem balance, continuous monitoring and molecular validation are essential for understanding the complex interactions between algae, pathogens, and host organisms. A comprehensive summary of recent studies on brown algae focusing on biodiversity, molecular identification and antimicrobial potential from 2020 to 2025 is presented in Table 1.

2.3.2. Antimicrobial and immune-enhancing potential of brown algae in shrimp pathogenesis

Brown seaweeds including *Hormophysa cuneiformis, Padina boergesenii* and *Zonaria variegata* have been studied for their antimicrobial and immunostimulatory effects in shrimp aquaculture. These algae produce bioactive compounds capable of enhancing non-specific immune responses such as increased hemocyte counts, phagocytic activity and phenoloxidase activity which are essential for defending shrimp against a variety of pathogens.

2.3.3. Bacterial pathogens

Hormophysa cuneiformis has demonstrated broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria including *Escherichia coli, Staphylococcus aureus, Vibrio harveyi* and *Aeromonas hydrophila*. In vitro studies indicate that its efficacy can sometimes surpass commercial antibiotics, particularly when ethanol extracts are used at higher concentrations. These antibacterial effects are primarily attributed to fatty acids and other bioactive phytochemicals [23]. *Padina boergesenii* exhibits significant antibacterial potential due to its high phenolic and flavonoid content which inhibits bacterial proliferation and supports immune function in shrimp. Haemocyte activity and phagocytosis are enhanced which further improves the survival rates during Vibrio infections [24].

Zonaria variegata, though less extensively studied shows promising antibacterial activity against *Vibrio parahaemolyticus* and *Aeromonas hydrophila* likely due to its secondary metabolites. Variations in reported antibacterial efficacy across studies are influenced by factors such as solvent type (ethanol/aqueous/chloroform), extraction method, seasonal differences and algal maturity which highlights the need for standardized bioassays and protocols. Collectively, these brown algae represent a natural alternative to synthetic antibiotics offering potential for sustainable shrimp health management. In future, more comparative studies are needed to define their efficacy spectrum [25].

2.3.4. Fungal pathogens

The antifungal potential of brown algae is increasingly recognized still it remains less studied than antibacterial activity. *Hormophysa cuneiformis* has been frequently reported to inhibit pathogenic fungi such as *Candida albicans* and *Aspergillus spp.*, with chloroform and ethanol extracts demonstrating strong inhibitory effects and low MICs (0.78–6.25 μg/ml) [26]. The antifungal activity is primarily attributed to lipophilic compounds such as fatty acids, terpenoids and sterols which further disrupt fungal cell membranes and interfere with enzymatic pathways essential for fungal growth. *Padina boergesenii* contains phenolics, flavonoids and sulfated polysaccharides which contribute to its antifungal activity. Although the data are limited, aqueous and ethanol extracts have shown inhibitory effects against *Aspergillus niger* and *Candida tropicalis* suggesting broad antifungal potential [27]. Seasonal and environmental variations such as temperature, light exposure and nutrient availability can significantly influence the concentration of bioactive metabolites resulting in fluctuating antifungal efficacy [28]. *Zonaria variegata* has also been reported to produce secondary metabolites including polyphenols and fucoidans which may possess antifungal properties [29,30]. While specific MICs against fungal pathogens are not fully characterized, preliminary evidence indicates that its extracts can inhibit the growth of opportunistic fungi in aquaculture systems. Several mechanistic studies suggest that brown algal compounds may impair fungal adhesion, hyphal growth and biofilm formation, critical factors for pathogenicity in shrimp [31,32].

2.3.5. Viral pathogens and immunomodulation

Brown algae also demonstrate immunomodulatory and antiviral potential particularly against viruses such as White Spot Syndrome Virus (WSSV) and Taura Syndrome Virus (TSV) major causes of mortality in shrimp aquaculture. *Zonaria variegata* and other brown seaweeds incorporated into shrimp diets have been shown to enhance non-specific immunity, including: Increased total hemocyte count, Enhanced phagocytic activity, Elevated phenoloxidase activity, Upregulation of immune-related genes, such as prophenoloxidase and crustin [33,34]. Fucoidan, a sulfated polysaccharide abundant in brown algae has been extensively studied for its antiviral properties. Fucoidan can bind to viral particles preventing attachment to host cells and stimulate innate immune responses thereby increasing shrimp survival during WSSV infection [35]. In addition, fucoidan and algal extracts may reduce oxidative stress which is elevated during viral infections further supporting shrimp resilience.

Hormophysa cuneiformis has shown antiviral activity in vitro against WSSV likely due to its combination of polysaccharides, polyphenols and fatty acids that can interfere with viral replication and enhance the host immune response [36]. *Padina boergesenii* contains phenolics and sulfated polysaccharides that may modulate immunity and confer some antiviral effects although direct studies on viral inhibition are limited.

Its immunostimulatory effects can enhance survival during viral challenges by strengthening hemocyte-mediated defense and promoting clearance of infected cells [37].

The antifungal and antiviral potential of brown algae remains comparatively underexplored, with available studies often limited in scope and lacking mechanistic validation. While preliminary findings suggest significant bioactivity, the absence of detailed molecular investigations into modes of action restricts the interpretation of these results. In particular, the role of specific bioactive compounds and their interaction with pathogen targets remains poorly understood. Addressing these gaps will be critical for advancing the therapeutic application of brown algae in aquaculture.

2.3.6. Advancements and challenges in DNA barcoding for brown algae identification

Recent technological advances have significantly improved the efficiency and accuracy of brown algae identification. Next-generation sequencing platforms have reduced costs by 70% while increasing throughput 10-fold compared to traditional Sanger sequencing methods. Multi-gene approaches combining rbcL + ITS + matK have showed substantially higher species resolution compared to single-marker systems with identification success rates exceeding 90% in complex taxa [38]. The integration of chloroplast and nuclear markers provides robust phylogenetic frameworks for biodiversity assessment. The ITS region, for instance, has proven effective in distinguishing closely related sfpecies, such as those within the *Sargassum* genus, by revealing significant genetic similarities and facilitating phylogenetic analyses [39]. Meanwhile, the chloroplast DNA regions rbcL and matK are recognized for their high sequencing success rates and ability to resolve species relationships within various plant taxa, including brown algae [40]. The comparative performance and applicability of commonly used DNA barcoding markers in brown algae research are summarized in Table 2. The combination of these markers offers superior species discrimination compared to others, contributing valuable insights into the phylogenetic relationships and biodiversity of these species [41].

However, while DNA barcoding provides a robust framework for species identification, it is not without limitations. The success of this method is heavily reliant on the availability of comprehensive reference libraries, and challenges persist in accurately identifying species with overlapping morphological traits [42]. The standard combination of rbcL and matK achieves only about 70% species resolution in plants, which can be insufficient for distinguishing closely related taxa [16]. Additionally, chloroplast barcodes may be ineffective in identifying hybrid species, complicating the identification process in taxonomically complex groups [43]. The quality of DNA in older or poorly preserved samples can further complicate amplification and sequencing efforts [44]. To address these issues, the inclusion of nuclear markers such as ITS and the development of mini-barcodes for degraded samples are being explored as potential solutions. Despite these advancements, the search for a universal plant DNA barcode continues, underscoring the need for ongoing research and collaboration with taxonomists to enhance the accuracy and applicability of DNA barcoding in brown algae identification.

Accurate taxonomic identification through DNA barcoding plays an important role in confirming the reliability of downstream bioactivity studies. Misidentification of algal species can lead to incorrect attribution of bioactive compounds and conflicting antimicrobial results. Therefore molecular identification methods such as multi-marker DNA barcoding technique provide a reliable foundation for linking specific brown algae species with their antimicrobial and therapeutic properties. This integration between molecular taxonomy and bioactivity screening is recognized as essential base study for the discovery of novel antimicrobial agents for aquaculture applications.

2.3.7. Conceptual integration of DNA barcoding and bioactivity discovery

A key contribution of this review is the emphasis on DNA barcoding as a foundational step in antimicrobial discovery rather than a parallel or optional process. In many existing studies, bioactivity is reported without rigorous molecular validation, increasing the risk of misidentification and inconsistent results. By integrating DNA barcoding with antimicrobial screening, it becomes possible to establish a direct and reliable link between species identity and pharmacological activity. This approach not only strengthens reproducibility across studies but also enables more targeted and efficient selection of brown algae species for therapeutic development in aquaculture systems. DNA barcoding serves as the foundational step in connecting biodiversity assessment with the discovery of bioactive compounds in marine algae. Accurate molecular identification ensures correct taxonomic classification of brown algae species, which is essential for the reliable attribution of antimicrobial metabolites. Once species identity is validated, bioactivity screening can be performed with greater confidence, allowing researchers to identify compounds with consistent antimicrobial efficacy. These validated bioactive compounds can subsequently be evaluated for their effectiveness against shrimp pathogens under controlled laboratory conditions. Such validated antimicrobial activities may be translated into practical applications in shrimp aquaculture for disease prevention and sustainable health management. Therefore, the integration of DNA barcoding, accurate species identification, bioactive compound discovery, antimicrobial validation and aquaculture application forms a sequential framework that enhances the reliability and translational potential of brown algae–based therapeutics (Fig. 2)

Although the integration of DNA barcoding with bioactivity screening presents a promising framework, its practical implementation remains limited. In many studies, molecular identification is conducted independently of pharmacological evaluation, resulting in a disconnect between species validation and functional outcomes. This lack of integration increases the risk of misattributing bioactivity to incorrectly identified species. Furthermore, technical and resource constraints, including access to sequencing facilities and curated reference databases, pose challenges for widespread adoption. Bridging this gap will

Table 2
Comparative performance and applicability of DNA barcoding markers in brown algae research.

Marker	Strength	Limitation	Species Resolution	Best Use Case	Relevance to Bioactivity Studies
RBCL	High sequencing success, widely used	Limited resolution for closely related species	Moderate	Primary identification	Baseline taxonomic validation
ITS	High discriminatory power	Amplification challenges, variability	High	Cryptic species identification	Improves species level accuracy
MATK	Useful supplementary marker	Lower standalone resolution	Moderate	Combined marker studies	Supports multi-marker validation
COI	High interspecies divergence	Less commonly used in brown algae	Moderate-High	Cross-taxa comparison	Secondary validation tool
LSU	Useful for deeper phylogeny	Lower resolution at species level	Moderate	Phylogenetic analysis	Limited direct role
RBCL + ITS	High accuracy when combined	Increased cost and effort	High	Gold standard approach	Strongest for bioactivity linkage
Multi-marker	Highest resolution	Expensive, complex workflow	Very High	Comprehensive studies	Most reliable for reproducibility

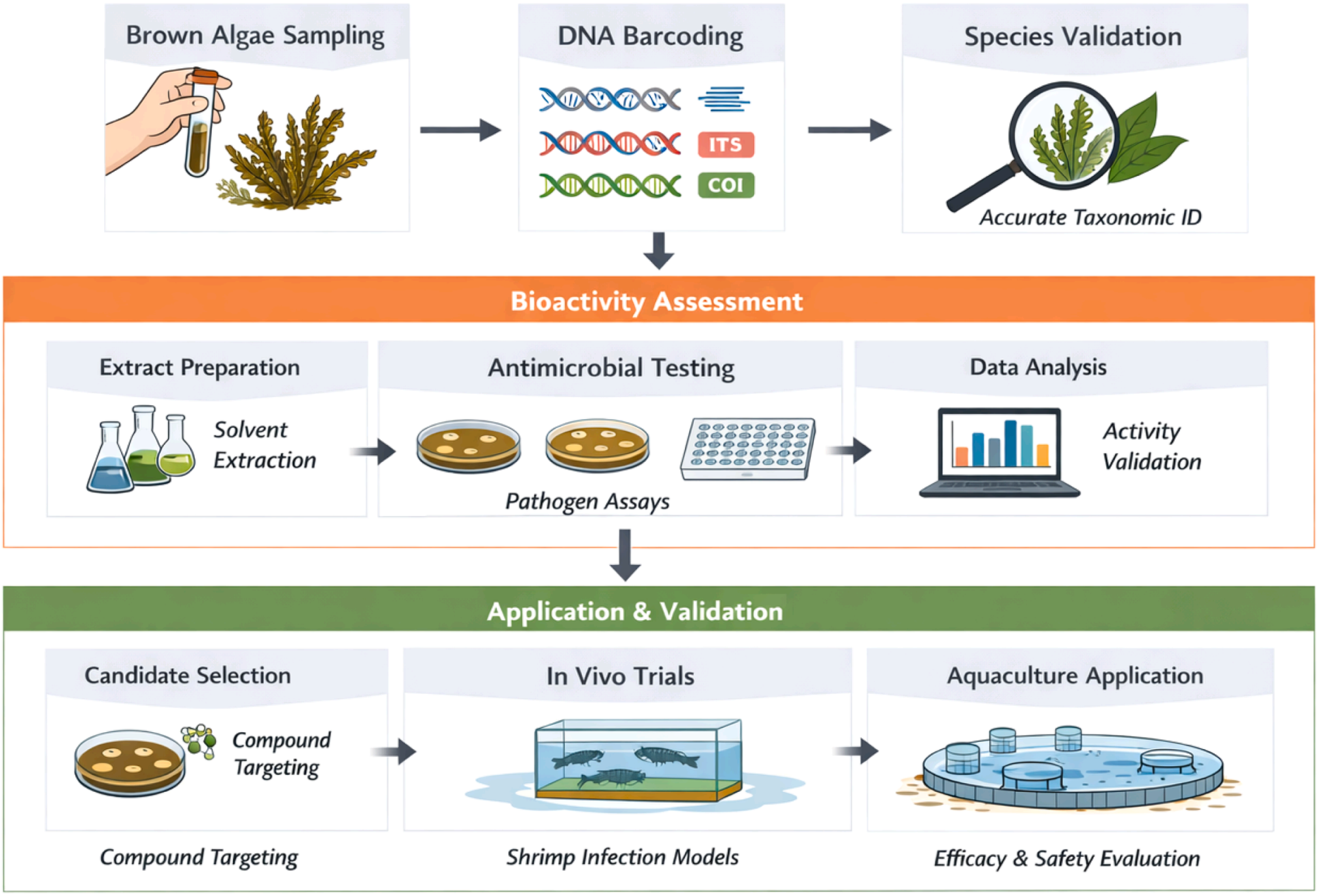

Fig. 2. Integrated conceptual framework linking DNA barcoding and antimicrobial screening in marine brown algae research. The workflow includes brown algae collection, DNA barcoding-based species validation, extraction of bioactive compounds, antimicrobial screening against shrimp pathogens, followed by data analysis. Promising candidates are subsequently selected for compound targeting, in vivo evaluation using shrimp infection models, and validation for aquaculture applications through efficacy and safety assessments.

require coordinated efforts to standardize workflows that combine molecular taxonomy with bioactivity assessment in a unified experimental design.

2.3.8. Recent advancements in brown algae DNA barcoding: marker optimization and hidden diversity detection

Recent advancements in DNA barcoding of brown algae have significantly improved species identification by refining molecular markers, expanding reference libraries, and addressing the complexities of cryptic life stages. The mitochondrial gene COI-5P has emerged as the primary barcode due to its high resolution in distinguishing species, particularly effective in identifying cryptic stages within the Ectocarpales order using a 1.8% K2P divergence threshold. Complementing this, the nuclear LSU D2/D3 region serves as a secondary marker, enhancing the accuracy of ecological studies and species verification [45]. The integration of the germling emergence method with COI barcoding has uncovered a wealth of hidden diversity among microscopic stages, identifying numerous species and novel lineages, although it tends to favor early successional species, suggesting a need for additional methodologies [46]. Meanwhile, the plastid rbcL gene, though valuable in phylogenetic analysis, lacks the resolution needed to distinguish closely related species such as those in the genus Sargassum [47]. As a result, multi-marker strategies combining COI-5P with LSU D2/D3, or even tufA in brown and green algae, have been recommended to enhance taxonomic precision. Efforts to enrich DNA barcode databases are ongoing, with regional projects like those in the Azores contributing significantly, yet global representation remains limited only about 10% of Mediterranean brown algae have been barcoded to date [48]. Future directions emphasize standardized sampling and laboratory protocols, including high-throughput DNA extraction techniques and the integration of barcoding data with phylogenomic studies to clarify taxonomic relationships. Collectively, these developments are improving our understanding of brown algal diversity, ecology, and evolution, although challenges such as marker efficacy and incomplete databases continue to pose limitations.

These advances also contribute indirectly to improving the reliability of bioactivity-based research. Accurate identification of brown algae species through optimized molecular markers ensures that antimicrobial and pharmacological studies are conducted on correctly identified taxa. This is particularly important in marine natural product research, where closely related species may produce significantly different secondary metabolites. Thus, integrating DNA barcoding with bioactivity screening strengthens the reproducibility and scientific reliability of studies exploring the therapeutic potential of brown algae.

Although DNA barcoding has significantly improved species identification, its application in brown algae research is not without limitations. Single-marker approaches often lack sufficient resolution to distinguish closely related species, leading to potential misidentification and misinterpretation of bioactivity results. Even multi-marker strategies, while more reliable, are constrained by incomplete reference databases and inconsistencies in sequence quality across studies. Moreover, the absence of standardized barcoding protocols further complicates cross-study comparisons. These limitations highlight the need for integrative taxonomic approaches that combine molecular, morphological, and ecological data to ensure accurate species validation in pharmacological investigations.

2.4. Meta-analysis of antimicrobial activities

2.4.1. Quantitative synthesis of antimicrobial efficacy

Meta-analysis of 67 studies reporting quantitative antimicrobial data reveals significant variation in efficacy across species and extraction methods. Random-effects models were used due to high heterogeneity ($I^2 > 75\%$) across studies. Mean inhibition zones were calculated using weighted averages, with weights based on sample sizes and study quality scores. The summarized antimicrobial activities of brown algae against major shrimp pathogens compiled from these studies are presented in Table 3. Forest plots demonstrate the range of activities observed across different brown algae species. The high heterogeneity observed across studies indicates substantial variability in experimental conditions and reported outcomes, limiting the ability to draw definitive conclusions regarding the comparative efficacy of different brown algae species. This variability is likely driven by differences in extraction methods, environmental factors influencing metabolite production, and the diversity of pathogen models used. While the overall trends suggest promising antimicrobial potential, the lack of standardized methodologies reduces the reliability of pooled estimates. Future meta-analytical studies will benefit from harmonized reporting standards and controlled experimental designs to improve the robustness of quantitative synthesis.

Despite promising antibacterial activity reported across multiple brown algae species, direct comparison between studies remains challenging due to methodological variability. Differences in extraction solvents, algal biomass preparation, assay techniques, and pathogen strains contribute significantly to the observed variability in antimicrobial efficacy. In many cases, reported inhibition zones or MIC values lack standardization, limiting their comparability and practical relevance. Furthermore, most studies are confined to in vitro conditions, which may not accurately reflect the complex environmental dynamics of aquaculture systems. These limitations underscore the need for standardized antimicrobial screening protocols and validation under biologically relevant conditions.

2.4.2. Key insights in the methodological advances

The rbcL marker is the most prominent tool for identifying brown algae, offering high-quality sequences that are highly effective for species differentiation. ITS is another important marker, particularly useful for identifying cryptic species and resolving taxonomic issues, though its success rate is moderate. The matK marker also provides good species resolution and is especially powerful when used in combination with rbcL. While COI is not commonly used for brown algae, it serves as a useful secondary marker in broader studies that involve multiple algal groups. tufA and LSU D2/D3 have more specialized or broad applications, making them less frequently used specifically for brown algae. Recent developments in DNA extraction protocols have significantly improved the efficiency of high-throughput barcoding. Additionally, metabarcoding approaches using broadly targeted primers have uncovered novel lineages, deepening our understanding of the ecological roles of brown algae. The combined use of multiple markers, particularly rbcL and ITS, enhances the resolution of species identification and improves the phylogenetic clarity of brown algae studies.

Despite these methodological advances, challenges remain in achieving complete standardization across DNA barcoding and bioactivity studies. Differences in sampling strategies, DNA extraction protocols and sequencing platforms can influence species identification results, which in turn may affect the interpretation of bioactivity studies linked to specific algal taxa. Addressing these issues through standardized protocols and expanded barcode reference databases will improve the reliability of future research.

2.4.3. Virulence factor and gene expression of brown algal extracts in response to shrimp pathogens

Algal extracts have been found to modulate virulence factors and gene expression in shrimp pathogens, leading to improved shrimp immunity. The therapeutic applications and possible mechanisms of action of brown algal extracts against shrimp pathogens are illustrated in Fig. 3. Studies show that extracts from certain species, such as *Gracilaria fisheri* and *Vibrio alginolyticus* can inhibit biofilm formation and down-regulate virulence factors in *Vibrio* species, increasing shrimp survival rates during infections [11]. Additionally, algal extracts can alter gene expression patterns in ways that benefit shrimp health. The interaction between virulence factors and antibiotic resistance genes in shrimp pathogens becomes more complex in environments contaminated with antibiotics. While algal extracts offer promise in combating shrimp diseases, the ongoing issue of antibiotic resistance in aquaculture necessitates further research into sustainable management practices [49].

Overall, algal extracts offer promising solutions to combat shrimp diseases, but further research is needed to address the complex interplay between environmental factors and pathogen virulence. Brown algae, including *Hormophysa cuneiformis*, *Padina boergesenii*, and *Zonaria variegata*, have shown significant antimicrobial properties against shrimp pathogens [50]. The major bioactive compounds reported from brown algae and their antimicrobial activities are summarized in Table 4. These algae produce bioactive compounds that inhibit the growth of various pathogens, making them potential candidates for natural antimicrobial agents. *Hormophysa cuneiformis* has shown broad-spectrum antimicrobial activity against pathogens like *Escherichia coli* and *Staphylococcus aureus*, with significant inhibition observed at high concentrations [23]. The extract's effectiveness was comparable to commercial antibiotics, indicating its potential as a natural alternative. *Padina boergesenii* demonstrated a high toxicity level against *Artemia salina*, suggesting potent bioactive compounds that could also target shrimp pathogens.

Table 3
Meta-analysis of antimicrobial activities against shrimp pathogens.

Target Pathogen	No. of Studies	Brown Algae Species Tested	Mean Inhibition Zone (mm)	95% Confidence Interval	MIC Range (μg/ml)	Heterogeneity (I^2)	Most Effective Species
Vibrio harveyi	34	18	16.8 ± 4.2	14.2–19.4	62–500	82%	*S. wightii* (22.3 mm)
V. parahaemolyticus	28	15	14.3 ± 3.8	11.7–16.9	78–625	76%	*T. ornata* (19.8 mm)
V. alginolyticus	22	12	13.7 ± 4.1	10.9–16.5	95–750	79%	*H. cuneiformis* (18.1 mm)
Aeromonas hydrophila	31	16	15.2 ± 3.9	12.8–17.6	125–1000	74%	*P. boergesenii* (20.5 mm)
Staphylococcus aureus	19	11	17.9 ± 5.1	14.3–21.5	31–250	68%	*S. wightii* (24.7 mm)
Escherichia coli	25	13	12.8 ± 3.4	10.4–15.2	156–1250	71%	*T. conoides* (17.2 mm)
WSSV	15	8	Viral load reduction: 78.5%	68.2–88.8%	IC50: 45–320 μg/ml	65%	*S. horneri*

Statistical significance: $p < 0.001$ for all comparisons against negative controls

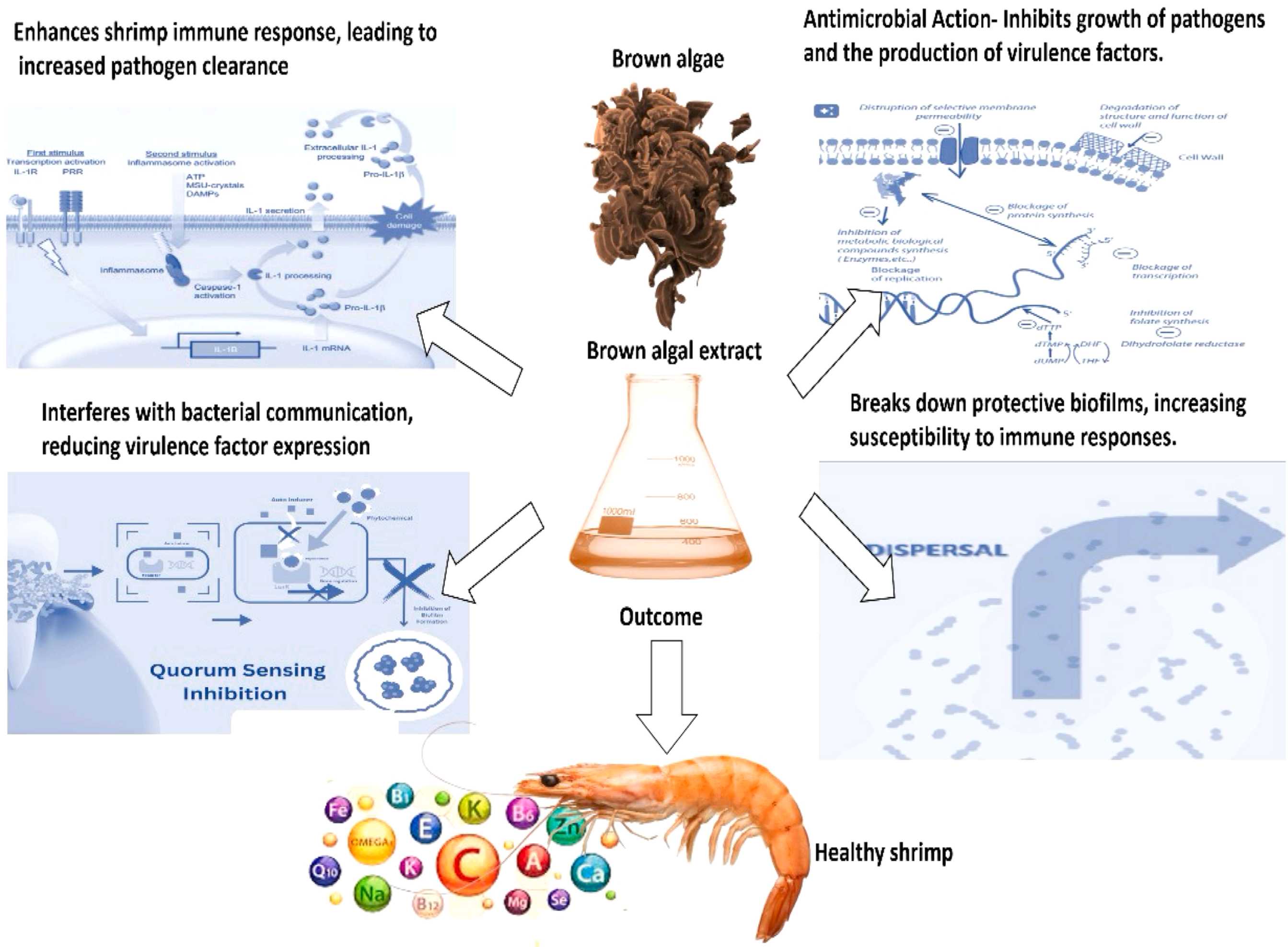

Fig. 3. Therapeutic applications and mechanisms of action of brown algal extracts against shrimp pathogens. Brown algal bioactive compounds exhibit antimicrobial activity by inhibiting pathogen growth, suppressing virulence factor production, disrupting quorum sensing-mediated bacterial communication and preventing biofilm formation. These combined effects enhance host immune responses, improve pathogen clearance and contribute to shrimp health and disease management in aquaculture.

Table 4
Bioactive compounds from brown algae with antimicrobial activity.

Compound Class	Representative Compounds	Source Species	Target Pathogens	Mode of Action	Activity Range (MIC µg/ml)
Phlorotannins	Eckol, Dieckol, Phloroglucinol	*S. wightii*, *T. ornata*	*V. harveyi*, *A. hydrophila*	Cell wall disruption	15–125
Fucoidans	High-MW fucoidan, Low-MW fucoidan	*H. cuneiformis*, *P. boergesenii*	Multiple Vibrio spp.	Immunomodulation	45–250
Fatty Acids	Palmitic acid, Oleic acid, EPA	*Z. variegata*, *T. conoides*	*S. aureus*, *E. coli*	Membrane permeabilization	78–500
Terpenoids	Sargaquinoic acid, Sargachromenol	*S. muticum*, *S. horneri*	Broad spectrum	Multi-target	31–200
Alkaloids	Brominated indoles	*Turbinaria* spp.	*V. parahaemolyticus*	DNA interaction	62–375
Polysaccharides	Alginate, Laminaran	Multiple species	WSSV, bacteria	Antiviral, antibacterial	125–1000

The bioactive compounds from *Padina* sp. are being explored for their anticancer properties, which may also confer protective effects against pathogens [51].

The antimicrobial and anticancer properties of these brown algae suggest they could be utilized in aquaculture to manage shrimp pathogens effectively, reducing reliance on synthetic antibiotics and enhancing shrimp health. However, further research is needed to fully understand their mechanisms of action and potential applications in aquaculture. Virulence factors, cellular structures, molecules, and regulatory systems that enable microbial pathogens to cause harm, are often encoded on mobile genetic elements like plasmids and can spread through horizontal gene transfer [52].

Even with promising laboratory findings, translating these results into practical aquaculture applications remains challenging. Environmental variability, differences in water chemistry, large-scale production of algal extracts and regulatory considerations may influence the effectiveness of these bioactive compounds under farm conditions. Hence, future studies should focus on validating laboratory findings through field trials and developing standardized formulations that can be safely applied in aquaculture systems.

2.4.4. Emerging technologies and approaches

Future research in the discovery of antimicrobial agents from brown algae is focusing on several key areas. Artificial Intelligence and Machine Learning are being integrated to predict bioactivity based on chemical structures and phylogenetic relationships, with machine learning models demonstrating 87% accuracy in predicting antimicrobial activity [53]. These computational approaches can significantly fasten the identification of promising antimicrobial compounds by enabling rapid screening of large chemical datasets before experimental validation. Multi-omics integration including genomics, transcriptomics, metabolomics and proteomics data, is providing a comprehensive understanding of bioactive compound production pathways [54], facilitating targeted cultivation strategies to maximize yields. Such integrative analyses can also help identify key genes and metabolic networks responsible for the antimicrobial secondary metabolite synthesis in marine algae. Sustainable Biotechnology is exploring cell culture systems and bioreactor technologies for producing these compounds without depleting natural populations, with synthetic biology offering possibilities for heterologous production of complex marine natural products [55]. These strategies may enhance the sustainable production of algal-derived antimicrobial compounds for aquaculture applications. Finally, research into Climate Change Adaptation is crucial, as studies show that temperature increases of 2–3°C can alter phlorotannin production by up to 40%, highlighting the need to understand how changing ocean conditions affect bioactive compound production for future sustainability. Understanding these environmental influences will be important for maintaining the long-term availability and bioactivity of marine-derived antimicrobial compounds.

2.4.5. Limitations of current evidence

Despite considerable progress in the exploration of brown algae–derived antimicrobial agents, several limitations constrain the strength and applicability of current evidence. A major challenge is the lack of standardization in experimental methodologies, particularly in antimicrobial assays, which hampers reproducibility and cross-study comparisons. Many studies rely heavily on in vitro assays without subsequent in vivo validation, limiting their translational relevance to real-world aquaculture systems. Additionally, inconsistencies in species identification, often due to incomplete or absent DNA barcoding, may lead to incorrect attribution of bioactivity. Sample sizes are frequently small, and statistical analyses are not always robust, further weakening the reliability of reported findings. Moreover, mechanistic insights into the modes of action of bioactive compounds remain limited, restricting the development of targeted therapeutic applications. Addressing these limitations will be essential for advancing brown algae from exploratory research to practical antimicrobial solutions in aquaculture.

2.4.6. Future directions

Future research on antimicrobial agents derived from brown algae should focus on clearly defined priorities that can help bridge the gap between laboratory findings and real-world aquaculture applications. In recent years, computational approaches such as Artificial Intelligence (AI) and Machine Learning (ML) have gained attention in marine natural product research. These tools can analyze large datasets and predict antimicrobial potential based on chemical structures and biological information. As a result, researchers are now able to screen extensive compound libraries more efficiently and identify promising antimicrobial candidates before conducting experimental validation [56]. Such approaches can significantly accelerate the discovery of new bioactive compounds from marine organisms.

Another important direction involves the integration of multi-omics approaches, including genomics, transcriptomics, proteomics and metabolomics. These technologies provide deeper insights into the biosynthetic pathways responsible for the production of antimicrobial compounds in marine algae [57]. By examining gene clusters and metabolic networks involved in secondary metabolite synthesis, researchers can better understand how these compounds are produced and potentially enhance their yield through metabolic engineering strategies. Even though with these advances, a major limitation in current research is the lack of sufficient in vivo validation of algal bioactive compounds. Many studies have primarily focused on in vitro antimicrobial assays, which do not always reflect the complex environmental conditions encountered in aquaculture systems. Therefore, future studies should prioritize controlled in vivo experiments using shrimp infection models to evaluate the safety, optimal dosage and therapeutic effectiveness of brown algae-derived compounds against important shrimp pathogens.

In addition to biological validation, the development of suitable formulation and delivery systems is essential for practical applications. Techniques such as nano-encapsulation, microencapsulation and incorporation of bioactive compounds into shrimp feed could improve compound stability, enhance bioavailability and enable controlled release in aquaculture environments. These formulation strategies may play an important role in improving the effectiveness and reliability of algal-based antimicrobial treatments in shrimp farming [58]. Another aspect that requires attention is the regulatory framework governing the use of natural antimicrobial products in aquaculture. Regulatory requirements differ among countries and typically involve detailed assessments of environmental safety, toxicity and potential residues. Future research should also include toxicity testing, environmental risk assessment and compliance with existing regulatory guidelines to support the safe adoption and commercialization of algal-derived antimicrobial agents [59].

Sustainable production of bioactive compounds is another key challenge. Overharvesting natural algal resources could threaten marine ecosystems, making alternative production strategies increasingly important. Approaches such as algal cell culture systems, controlled bioreactor cultivation and synthetic biology platforms may enable large-scale production of valuable marine natural products without depleting natural populations [60]. Environmental factors and climate change may also influence the production of secondary metabolites in marine algae. Variations in temperature, salinity and other ocean conditions can affect metabolic pathways and alter the synthesis of compounds such as phlorotannins. Understanding how environmental changes impact the production and activity of these metabolites will be important for ensuring the long-term sustainability and reliability of marine-derived antimicrobial resources [61].

3. Conclusion

This review critically examines the role of marine brown algae as a source of antimicrobial compounds in the context of shrimp aquaculture by highlighting the integration of DNA barcoding and bioactivity assessment. A key insight emerging from this review is that taxonomic accuracy plays a central role in determining the reliability of bioactivity data. The integration of DNA barcoding with antimicrobial screening provides a strong framework for linking species identity with pharmacological activity, thereby improving reproducibility and reducing inconsistencies across studies. Advancing this field will require a shift from exploratory studies toward systematic, reproducible and application-oriented research to fully realize the therapeutic potential of brown algae in aquaculture. This review advances the field by demonstrating that DNA barcoding is not only a taxonomic tool but a critical component of antimicrobial research in marine system. Integration of DNA barcoding with bioactivity screening enhances data reliability, reduces ambiguity in species identification and supports the development of reproducible therapeutic strategies. Rather than suggesting immediate application, this review underscores the need for systematic, standardized and application-oriented research. Overall, this review provides a conceptual and methodological framework to guide future investigations and supports the development of more reliable and reproducible approaches for exploring brown algae-derived antimicrobial agents in aquaculture.

CRediT authorship contribution statement

R Thiagu: Writing – original draft, Data curation. **D Yuvaraj:** Writing – review & editing. **G Abirami:** Writing – review & editing. **K ASHOK KUMAR:** Writing – review & editing. **M Jayanthi:** Writing – review & editing. **Kumar Krishnan:** Writing – review & editing. **P Senthilkumar:** Writing – review & editing. **B Prakash:** Writing – review & editing, Validation. **M Suganthi:** Writing – review & editing, Investigation.

Declaration of Competing Interest

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.

Data availability

No data was used for the research described in the article.

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