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Review

Ulva and *Gracilaria* in finfish aquafeeds: standardisation, health effects and evidence for bacterial disease resistance

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Abstract
Bacterial diseases remain a major constraint in intensive aquaculture and have increased interest in functional feed ingredients that can support fish health while reducing dependence on antimicrobials. The present review critically examined *Ulva* and *Gracilaria* derived meals, extracts and enriched fractions as functional aquafeed ingredients, with focus on the links between biomass origin, cultivation, processing, chemical composition and disease-related outcomes in finfish. Both genera provided nutritional components together with sulfated polysaccharides, phenolics, pigments, fatty acids and sterols that may act through direct antibacterial effects, redox regulation, innate immune modulation, maintenance of mucosal and intestinal barriers, and changes in the gut microbiota. However, the available evidence showed that efficacy was strongly product specific. Responses varied with seaweed species, production environment, extraction method, dietary dose, fish host and pathogen model. Studies in commercially important fishes, including seabass and tilapia, indicated that improved growth, immune responses or gut microbiota profiles did not necessarily demonstrate resistance to infection. Studies combining a chemically characterised product with dietary exposure and a controlled bacterial challenge provided stronger evidence. Major drawbacks include incomplete taxonomic authentication, poor reporting of extraction and feed-manufacturing conditions, limited chemical standardisation, short feeding periods and uneven pathogen coverage, particularly for gram-positive bacteria. Controlled cultivation and tissue culture may improve traceability and biomass consistency, but their functional advantages remain to be demonstrated. Progress therefore depends on converting variable seaweed preparations into safe, reproducible and chemically defined products whose efficacy is validated in the intended fish-pathogen system.

Keywords: aquaculture; bacterial disease resistance; functional aquafeeds; *Gracilaria*; seaweed extracts; *Ulva*

1 | INTRODUCTION

Aquaculture is now a major source of aquatic food, employment and income, yet its intensification often creates conditions that favour infectious disease (Siddik *et al.* 2023). High stocking densities, repeated handling, variable water quality and frequent pathogen exposure can disturb physiological homeostasis and weaken mucosal and systemic defences, leading to poor growth, mortality and economic loss (Bondad-Reantaso *et al.* 2005; Stentiford *et al.* 2017; Thépot *et al.* 2021). Bacterial pathogens belonging to *Vibrio*, *Aeromonas*, *Streptococcus*, *Edwardsiella*, *Photobacterium*, *Tenacibaculum* and *Flavobacterium* are particularly important in cultured marine and freshwater fishes (Reverter *et al.* 2014; Vatsos and Rebours 2015).

Antimicrobials remain valuable for treating bacterial infections, but repeated or inappropriate use can select resistant populations and introduce residues and resistance determinants into aquatic environments (Rico *et al.* 2013; Reverter *et al.* 2014). Medicated feed may also be unreliable during acute disease because infected fish commonly reduce feed intake. These limitations have encouraged preventive strategies, including vaccines, probiotics, prebiotics, immunostimulants and functional aquafeeds intended to strengthen host resistance before pathogen exposure (Reverter *et al.* 2014; Thépot *et al.* 2021).

Functional aquafeeds provide benefits beyond basic nutrition by supporting antioxidant defence, innate immunity, intestinal integrity, microbial balance and stress tolerance. Their effectiveness depends on ingredient composition, dose, stability, palatability, digestibility and bioavailability, together with the fish species, life stage, basal diet and rearing conditions (Siddik *et al.* 2023). Marine macroalgae are relevant candidates because they provide proteins, minerals, vitamins and dietary fibre together with pigments, phenolics, peptides, fatty acids, sterols and sulfated polysaccharides (Lomartire and Gonçalves 2022; Siddik *et al.* 2023). These constituents may contribute through direct bacterial inhibition, redox and inflammatory regulation, innate immune stimulation, reinforcement of mucosal barriers and modulation of intestinal microbial communities (Vatsos and Rebours 2015; Siddik *et al.* 2023; Petit *et al.* 2024).

Among the seaweeds investigated in aquaculture, the green genus *Ulva* and the red genus *Gracilaria* are particularly relevant because both are cultivable and biochemically diverse. *Ulva* contains ulvan, a sulfated heteropolysaccharide composed mainly of sulfated rhamnose, uronic acids and xylose, together with proteins, minerals, chlorophylls, carotenoids, phenolics, fatty acids and sterols (Lahaye and Robic 2007; Siddik *et al.* 2023). *Gracilaria* contains agarans and related sulfated galactans, proteins, pigments, phenolics, fatty acids, sterols and other secondary metabolites (de Almeida *et al.* 2011; Capillo *et al.* 2018). These compound groups have been associated with antioxidant, antibacterial, immunomodulatory and gut-related effects, although their abundance and structure vary among species, biomass sources and processed products (García-Poza *et al.* 2022; Siddik *et al.* 2023).

This product specificity is evident experimentally. Sulfated-polysaccharide-rich *Ulva* extracts inhibited selected fish-pathogenic bacteria and stimulated reactive-oxygen-species production and cytokine-related responses in fish leukocytes, but responses differed between Nile tilapia and rainbow trout (Petit *et al.* 2024). Antibacterial activity also varied among solvent extracts of cultivated *Gracilaria gracilis*, demonstrating the influence of extraction on the recovered functional fractions (Capillo *et al.* 2018). In feeding trials, a *Gracilaria*-supplemented diet reduced lipid peroxidation, increased lysozyme activity and delayed mortality in European seabass challenged with *Photobacterium damselae* subsp. *piscicida* (Peixoto *et al.* 2019). A hot-water extract of *Ulva rigida* enhanced selected immune and intestinal-microbiota responses and increased survival of Asian seabass challenged with *Vibrio vulnificus* (Klongklaew *et al.* 2025). These outcomes should not be generalised across seaweed species, product forms, doses, fish hosts or pathogens.

Reproducible application is further constrained by variation in the starting biomass. Seaweed composition changes with species, genotype, geographical origin, season, nutrient availability, salinity, temperature, irradiance, developmental condition and harvest stage, while washing, drying, storage and extraction introduce further variation (García-Poza *et al.* 2022; Siddik *et al.* 2023). Wild biomass may also carry sediments, epiphytes, microorganisms or accumulated metal(oid)s. Controlled cultivation can improve traceability, and tissue-culture techniques may support clonal propagation of selected material; however, neither approach alone guarantees chemical uniformity, contaminant-free biomass or superior aquafeed efficacy (Baweja *et al.* 2009; Roleda *et al.* 2021; Vargas-Murga *et al.* 2025).

2 | METHODOLOGY

This critical review was based on literature identified through Google Scholar, PubMed, Scopus and Wiley Online Library, with relevant publications available up to July 2026 considered. Search terms were used individually or in combination and included "*Ulva*", "*Gracilaria*", "seaweed", "macroalgae", "aquafeed", "fish feed", "functional feed", "seaweed extract", "bioactive compounds", "sulfated polysaccharides", "antibacterial activity", "fish immunity", "immunomodulation", "antioxidant response", "gut microbiota", "mucosal health", "bacterial disease", "bacterial challenge", "*Vibrio*", "*Aeromonas*",

"*Streptococcus*", "Asian seabass", "European seabass", "tilapia", "tissue culture", "controlled cultivation", "extraction" and "standardisation".

Peer-reviewed studies were included when they provided information relevant to the use of *Ulva* or *Gracilaria* in finfish aquafeeds, including composition, extraction, dietary application, immune and antioxidant responses, microbiota, safety and bacterial-challenge outcomes. Studies reporting only *in-vitro* activity or physiological and immune responses were considered supporting evidence, whereas greater emphasis was given to feeding studies that included controlled bacterial challenge. Studies on other seaweed genera were used only when they provided relevant methodological or mechanistic context.

3 | FUNCTIONAL SIGNIFICANCE OF *Ulva* AND *Gracilaria*

The functional value of *Ulva* and *Gracilaria* in aquafeeds depends on both their nutritional composition and bioactive constituents that remain available after processing and ingestion. Both genera provide proteins, minerals, pigments, fatty acids, sterols, phenolics and structurally distinct polysaccharides, but their composition varies with species, cultivation conditions and post-harvest treatment (García-Poza *et al.* 2022; Siddik *et al.* 2023). Findings from one biomass source or processed product therefore should not be generalised to another.

3.1 Nutritional basis and limitations of whole biomass

Whole *Ulva* and *Gracilaria* meals contain carbohydrates, soluble and insoluble fibre, proteins, minerals, vitamins and pigments, although their total lipid content is generally low. At moderate inclusion levels, these components may contribute nutritional value while also supplying functional metabolites. At higher inclusion, however, structural carbohydrates and ash can dilute dietary protein and energy or reduce nutrient accessibility, particularly in carnivorous fishes with limited capacity to utilise complex algal carbohydrates (Siddik *et al.* 2023).

The function of seaweed in a formulated diet consequently changes with the inclusion level. At low inclusion, it may act mainly as a source of bioactive constituents. As the inclusion level increases, its contribution to dietary fibre, ash, protein, energy and mineral balance becomes more important. Whole seaweed biomass should therefore be incorporated through appropriate diet reformulation rather than added to an otherwise unchanged feed.

Product form also affects interpretation. Whole meals retain a broad range of nutritional and bioactive constituents, whereas crude extracts, enriched fractions and purified compounds provide more selective chemical exposures and can be used at lower inclusion levels.

3.2 Functional constituents of *Ulva*

Ulvan is the principal sulfated cell-wall polysaccharide associated with *Ulva*. It consists mainly of sulfated rhamnose, glucuronic and iduronic acids, and xylose. Its physicochemical and biological properties are influenced by molecular weight, branching, monosaccharide composition and degree of sulfation (Lahaye and Robic 2007). Ulvan preparations obtained from different species or extraction procedures should therefore not be regarded as chemically equivalent.

Ulvan-rich fractions have been associated with antioxidant, immunomodulatory, antimicrobial and microbiota-related effects. Their value in fish may arise less from strong direct bacterial killing than from interactions with leukocytes, mucosal tissues and intestinal microorganisms. Petit *et al.* (2024), for example, found that sulfated-polysaccharide-rich *Ulva* extracts inhibited selected fish-pathogenic bacteria and stimulated reactive-oxygen-species production and cytokine-related responses in fish leukocytes. The response differed between Nile tilapia and rainbow trout, showing that the same preparation may be recognised differently by different hosts.

Ulva also contains chlorophylls, carotenoids, phenolics, fatty acids and sterols, whose recovery depends strongly on processing (Pappou *et al.* 2022). Accordingly, an "*Ulva* extract" should be described by its extraction conditions and chemical characteristics.

3.3 Functional constituents of *Gracilaria*

The cell walls of *Gracilaria* are dominated by agarans and related sulfated galactans. These polysaccharides mainly contain alternating galactose residues, with variable proportions of 3,6-anhydrogalactose and sulfate groups. Molecular weight, linkage pattern and sulfation affect their solubility and possible interactions with bacterial surfaces, intestinal microorganisms and host immune systems (de Almeida *et al.* 2011).

Gracilaria also contains phycobiliproteins, phenolics, fatty acids, sterols and other metabolites whose contribution depends on the fraction recovered. Solvent-dependent activity in *G. gracilis* and active lipid-associated fractions in *G. edulis* indicate that multiple compound classes may contribute to antibacterial effects (Capillo *et al.* 2018; Kasanah *et al.* 2019).

3.4 Comparative suitability as aquafeed ingredients

Ulva and *Gracilaria* share several practical advantages, including cultivable biomass, dietary fibre, minerals and chemically diverse bioactive fractions. Their principal functional profiles are nevertheless different. *Ulva* is characterised by ulvan, chlorophylls and green-seaweed carotenoids, whereas *Gracilaria* contains agarans, sulfated galactans and phycobiliproteins. These compositional differences affect extraction behaviour, digestibility, chemical standardisation and the biological pathways through which each product may act (de Almeida *et al.* 2011; Lahaye and Robic 2007).

Neither genus can therefore be described as univer-

sally superior. Practical suitability depends on the authenticated species, biomass origin, product form, chemical composition, dietary dose, target fish and intended health outcome. The appropriate comparison is not simply *Ulva* versus *Gracilaria*, but rather one defined product against another under comparable dietary and biological conditions. This product-based approach is essential for interpreting the effects of cultivation, processing and extraction discussed in the following sections.

4 | BIOMASS ORIGIN, CONTROLLED CULTIVATION AND TISSUE CULTURE

The characteristics of a seaweed-derived feed ingredient are shaped before extraction or feed manufacture begins. Biomass identified as the same genus, or even the same species, may differ in proteins, carbohydrates, fatty acids, pigments, minerals and other metabolites when produced under different environmental and cultivation conditions. Temperature, salinity, nutrient supply, irradiance, water movement, physiological condition and harvest stage can all influence biomass composition (García-Poza *et al.* 2022). Biomass origin and production history should therefore be treated as defining features of the final ingredient rather than as secondary collection details.

4.1 Biomass origin and cultivation-dependent composition

Wild harvesting provides ready access to naturally occurring biomass, but the production environment is largely uncontrolled. Material collected from the field may vary among sites and seasons and may contain sediments, epiphytes, associated microorganisms or accumulated contaminants. Cultivation offers greater control over species selection, stocking density, nutrient supply, harvesting and post-harvest handling, thereby improving traceability. It does not, however, guarantee that all production batches will be chemically identical (García-Poza *et al.* 2022).

García-Poza *et al.* (2022) compared fatty-acid and monosaccharide profiles in wild, controlled and semi-controlled *Ulva* biomass. Controlled cultivation produced higher contents of several essential fatty acids and monosaccharides than the semi-controlled systems, although some values were similar to those observed in wild material. These findings show that the production environment can modify selected nutritional traits, but they do not establish that cultivated biomass is superior in every chemical or functional characteristic.

The physiological condition of cultivated seaweed may also be reflected in its appearance. Robertson-Andersson *et al.* (2009) quantified the relationship between thallus colour and tissue nitrogen in cultivated *U. lactuca* and *G. gracilis*. In *U. lactuca*, the transition between green and yellow-green thalli occurred at approximately 1.5–1.7 mg N g^{-1} tissue, whereas in *G. gracilis* the transition between green-yellow and yellow-brown thalli occurred at approximately 0.8–1.3 mg N g^{-1} tissue. Thallus colour may therefore provide a rapid indication of nitrogen and nutritional status, but it cannot replace direct measurement of proteins, pigments or functional metabolites.

Cultivation conditions also affect biomass yield and the material available for further processing. Capillo *et al.* (2018) compared two cultivation approaches for *Gracilaria gracilis* and reported differences in daily growth rate and biomass production. The cultivated material was subsequently extracted using solvents of different polarity, and antibacterial activity varied markedly among the resulting extracts. Ethanol and methanol extracts produced stronger bacterial inhibition than the less-polar fractions (Capillo *et al.* 2018).

Because wild biomass was not included in that experiment, the antibacterial response cannot be attributed to cultivation alone. Instead, the study illustrates that cultivation and extraction are linked stages: cultivation determines the physiological and biochemical condition of the starting biomass, whereas extraction determines which constituents are recovered in the final preparation. For functional-aquafeed development, cultivation records should therefore include the authenticated species or strain, production location, cultivation system, nutrient regime, environmental conditions, harvest stage and post-harvest handling. These records should be accompanied by chemical analysis of each production batch. Traceable cultivation can reduce uncontrolled variation, but compositional testing is still required before the biomass can be considered a reproducible feed ingredient.

4.2 Tissue culture as a biomass-production route

Seaweed tissue culture involves the propagation of explants, isolated cells, callus-like tissues or protoplasts under controlled laboratory conditions. It has been investigated for rapid multiplication, germplasm conservation, strain selection, genetic improvement and year-round production of planting material (Baweja *et al.* 2009). In functional-aquafeed development, its main value lies in repeatedly propagating selected donor material rather than depending entirely on seasonal wild collection or continuously recycled farm stocks.

Direct regeneration, callus-mediated regeneration and protoplast culture are the principal approaches. Direct regeneration produces branches or thalli from an explant without an intervening callus phase and is generally preferred where true-to-type planting material is required. Callus culture can generate new plantlets and potentially useful variation, but regeneration is often slow or inconsistent. Protoplast systems offer opportunities for cell fusion, mutant selection and genetic manipulation, although cell-wall removal, survival and whole-thallus

regeneration remain technically demanding and highly species dependent (Baweja *et al.* 2009; Jiksing *et al.* 2022).

Establishing clean cultures is itself a major constraint. Seaweed surfaces commonly carry epiphytes and microorganisms, while their tissues lack the thick protective structures found in many terrestrial plants. Sterilisation that is too mild may allow contamination to persist, whereas harsh treatment can bleach or damage the explant. Culture success also depends on donor condition, explant type, medium composition, salinity, temperature, irradiance and photoperiod (Baweja *et al.* 2009). Moreover, some seaweed-associated bacteria contribute to normal morphogenesis, so complete removal of the natural microbiota may not always support healthy development (Baweja *et al.* 2009). A protocol developed for one species should therefore not be transferred to another without validation.

These studies demonstrate that controlled or clonal propagation is technically feasible within both genera. They do not, however, show that tissue-culture-derived biomass necessarily contains more ulvan, agarans, phenolics, pigments or antibacterial compounds than wild or conventionally cultivated material.

4.3 Evidence within *Ulva* and *Gracilaria*

Regeneration from explants, isolated cells or protoplasts has been reported in several *Ulva* species, demonstrating substantial regenerative capacity within the genus (Baweja *et al.* 2009). Culture requirements nevertheless differ among species, and a universally validated protocol for *U. lactuca* is not yet available. Few studies have directly compared wild, conventionally cultivated and tissue-culture-derived *U. lactuca* for chemical composition or aquafeed-related biological activity.

Callus induction, adventitious branching and thallus regeneration have been reported in *G. chilensis*, *G. corticata*, *G. tenuistipitata*, *G. tenuifrons* and *G. verrucosa* (Baweja *et al.* 2009). These reports establish the technical feasibility of clonal propagation within *Gracilaria*, but they do not show that all species regenerate equally well or produce comparable chemical profiles.

Field performance is not uniformly improved by tissue-culture origin. Rejeki *et al.* (2018) found that tissue-cultured *G. verrucosa* seedlings grew more slowly than farm-propagated seedlings under the tested conditions, although cultivation method also influenced agar content and other production traits. This finding shows that micropropagation cannot be assumed to improve growth in every species, because genotype, laboratory conditions, acclimatisation and the subsequent grow-out environment may all influence field performance.

Evidence for tissue-culture-derived *G. edulis* remains more limited and mainly concerns regeneration or seedling production rather than direct comparisons of polysaccharide composition, phenolics, pigments, antibacterial activity, antioxidant capacity or dietary efficacy. There is currently no firm basis for claiming that tissue-cultured *G. edulis* is functionally superior to wild or conventionally cultivated material.

4.4 Comparative field evidence from other commercial seaweeds

Direct field comparisons of tissue-cultured and conventionally propagated *Ulva* or *Gracilaria* remain limited. Evidence from *Kappaphycus alvarezii* is therefore discussed here only as supporting context for understanding the potential and limitations of tissue-culture-based seaweed production and is not considered direct evidence for *Ulva* or *Gracilaria*. Faisan *et al.* (2024) compared tissue-cultured plantlets with farm-sourced seedlings over three 60-day cultivation periods. After day 15, specific growth rates of tissue-cultured plantlets were approximately 1.5–6.5 times higher, depending on season and sampling point. Carrageenan yield and gel strength were generally comparable between seedling sources, while viscosity differed significantly at only one mean sampling point.

Ice-ice disease occurred during the later stages of the May-June and August–October trials. Reported incidence ranged from 31.2–86.2% in farm-sourced seedlings and 12.8–60.6% in tissue-cultured plantlets, but tissue-cultured material did not perform better at every observation. Epiphytic filamentous algae appeared later in tissue-cultured plantlets, although both groups were eventually affected. The study therefore supports improved growth and broadly maintained carrageenan quality in the tested cultivar, but not universal or season-independent disease resistance (Faisan *et al.* 2024). These findings cannot be transferred directly to *Ulva* or *Gracilaria*, but they show why biomass growth, product quality and disease susceptibility should be evaluated separately.

4.5 Implications for functional-aquafeed standardisation

Tissue culture can improve control over donor identity, propagation and planting-material availability, but it represents only the first stage of biomass production. Cell and tissue cultures may also permit manipulation of light, nutrients, aeration or precursor supply to influence metabolite production. However, the available bioreactor evidence largely concerns non-target seaweeds and pharmaceutical metabolites rather than functional aquafeeds (Rorrer *et al.* 1999). Regenerated plantlets must still be acclimatised and grown under nursery or field conditions, where light, nutrients, temperature, salinity and microbial exposure may alter their biochemical composition. Successful regeneration or rapid growth should therefore be considered a production outcome rather than proof of superior functional activity.

The studies examined in this review do not provide a direct comparison of wild, conventionally cultivated and tissue-culture-derived *Ulva* or *Gracilaria* under a common aquafeed-development framework. A meaningful comparison would require molecularly authenticated material from the same species and, where possible, the same genotype. Biomass from each production route should be assessed for yield, proximate composition, polysaccharide characteristics, phenolics, pigments, minerals, contaminants, extraction yield and product stability. The resulting meals or extracts should then be tested at defined dietary doses, followed by measurements of fish health and a controlled bacterial challenge.

Controlled cultivation and tissue culture should therefore be viewed as tools for improving biomass traceability and production consistency rather than as evidence of functional superiority. Their contribution to aquafeed development can be established only when propagation history is connected with the chemical characteristics of the final product and with reproducible biological responses in the intended fish species.

5 | EXTRACTION AND CHEMICAL STANDARDISATION

Extraction converts variable seaweed biomass into a product with a defined composition and intended function. Solvent polarity, temperature, extraction time, biomass-to-solvent ratio and subsequent concentration or fractionation determine which polysaccharides, phenolics, pigments, fatty acids, sterols and other metabolites are recovered. Extracts prepared from the same species may therefore differ substantially in composition and activity. The term “seaweed extract” is not sufficiently informative unless the product form, extraction procedure and representative chemical characteristics are reported (Matos *et al.* 2021; Quitério *et al.* 2022).

5.1 Product form and extraction selectivity

Ulva- and *Gracilaria*-derived ingredients may be used as whole meals, crude extracts, enriched fractions or purified compounds. Whole meals retain the broad nutritional profile of the biomass but also contribute fibre and ash and generally require higher dietary inclusion. Crude extracts concentrate solvent-soluble constituents and can be used at lower doses, although their complexity makes batch comparison difficult. Enriched fractions and purified compounds provide better chemical definition, but additional processing increases cost and may remove interactions among compounds present in the original preparation (Matos *et al.* 2021; Siddik *et al.* 2023).

Water mainly recovers polar constituents, including ulvan, agarans and sulfated galactans. Ethanol and aqueous-ethanol mixtures can recover phenolics, pigments and moderately polar metabolites and are more suitable for feed-oriented products than methanol or chloroform. Less-polar solvents may recover fatty acids and sterols, but their use requires complete solvent removal and safety verification (Matos *et al.* 2021; Quitério *et al.* 2022).

Extraction yield and biological activity are not equivalent. A high yield may reflect abundant carbohydrates, salts or inactive material, whereas a smaller fraction may contain more functionally relevant compounds. Extraction should therefore be evaluated using chemical markers together with the biological endpoint for which the product is intended.

5.2 Extraction-dependent evidence from *Ulva* and *Gracilaria*

Pappou *et al.* (2022) compared water, ethanol, ethyl acetate and a 70:30 ethanol–water mixture for extracting compounds from cultivated *Ulva lactuca*. The aqueous-ethanol mixture provided effective combined recovery of phenolic and carotenoid fractions, with yields of approximately 10–15% under the tested conditions. Extraction at 60°C for 3 h and a 1:10 biomass-to-solvent ratio produced the strongest antioxidant response among the evaluated treatments. These conditions are specific to the biomass and target compounds examined and should not be treated as a universal protocol for *Ulva*.

Afonso *et al.* (2021) used sequential water and ethanol extraction of *Gracilaria gracilis* at room temperature, 40°C and 70°C. A 30-min treatment at 40°C gave favourable overall recovery, while aqueous extracts had higher mean phenolic content and slightly greater DPPH activity than ethanolic extracts. Increasing the temperature to 70°C did not improve the overall antioxidant response, showing that harsher extraction does not necessarily produce a more active preparation.

Solvent choice also influenced antibacterial activity. Among five extracts of cultivated *G. gracilis*, ethanol produced the strongest inhibition, followed by methanol, whereas acetone, chloroform and diethyl ether were generally less active (Capillo *et al.* 2018). The more active extracts contained comparatively high concentrations of carbohydrates and polyphenols, but no single compound was shown to be independently responsible. These studies demonstrate that conditions favouring antioxidant recovery may not maximise antibacterial activity and that the largest extract yield may not represent the most useful functional fraction.

5.3 Fractionation and structure–activity relationships

Crude extracts can demonstrate activity but rarely identify the responsible compounds. Kasanah *et al.* (2019) fractionated an ethyl-acetate extract of *Gracilaria edulis*. The crude extract and separated fractions showed pathogen-dependent inhibition of fish-derived Vibrio isolates and *Aeromonas hydrophila*. GC–MS detected saturated and unsaturated fatty acids and a sterol-related compound in active fractions, indicating that antibacterial activity was

not restricted to sulfated polysaccharides or phenolics.

Polysaccharide structure can be equally important. Kamble *et al.* (2022) compared native high-molecular-weight sulfated galactans from *Gracilaria fisheri* with lower-molecular-weight fractions produced by controlled depolymerisation. The modified fractions were more active against *Vibrio parahaemolyticus* and *V. harveyi* and caused leakage of intracellular material and membrane damage. This links activity with molecular size and sulfation characteristics, although the finding remains specific to the modified *G. fisheri* fractions.

An extract may also alter bacterial susceptibility without showing strong direct inhibition. Lu *et al.* (2022) found that an ethanolic *Gracilaria* extract had weak independent activity against *V. parahaemolyticus* and *V. cholerae*, but enhanced carbenicillin activity and reduced VarG-mediated degradation of β-lactam substrates. Conventional inhibition-zone or minimum-inhibitory-concentration assays may therefore overlook resistance-modifying effects. Such activity remains preliminary until dietary safety, stability and efficacy in fish are established.

5.4 Requirements for chemical standardisation

Standardisation should clearly identify the material as a meal, crude extract, enriched fraction or purified compound. Extraction yield should be reported relative to dry biomass, together with solvent composition, biomass-to-solvent ratio, temperature, duration, number of cycles, concentration method and storage conditions (Matos *et al.* 2021; Quitério *et al.* 2022).

Analytical markers should match the product. Ulvan- or galactan-rich preparations require total carbohydrate and sulfate measurements together with molecular-weight distribution and monosaccharide composition. Phenolic- or pigment-oriented extracts require total-content assays supported by chromatographic identification, while fatty-acid- and sterol-rich fractions require lipid profiling. Complex crude extracts may need markers from more than one compound class.

FTIR is useful for comparing broad functional-group patterns but cannot identify individual compounds. HPLC or LC–MS can resolve phenolics, pigments and other non-volatile metabolites, whereas GC–MS is useful for fatty acids, sterols and volatile or semi-volatile constituents (Kasanah *et al.* 2019; Cotas *et al.* 2020; Pappou *et al.* 2022). Antioxidant assays also require careful interpretation because DPPH, ABTS and FRAP measure different reaction mechanisms and are often reported using different standards and units. Complementary assays and quantitative antibacterial measurements, preferably MIC or MBC values, provide stronger evidence than single percentage-inhibition or diffusion results.

Biological testing should use the same chemically characterised batch. Representative markers should also be measured after feed manufacture to confirm that the intended dose remains present after mixing, pelleting, coating and storage.

5.5 Translation into standardised aquafeed products

Ultrasound-, microwave-, enzyme- and pressurised-liquid-assisted extraction may reduce extraction time or solvent use and improve recovery of selected compounds. Their performance remains dependent on the seaweed matrix and operating conditions, and any technical advantage must be assessed against cost, scalability and retention of biological activity (Quitério *et al.* 2022).

For aquafeed development, the preferred method is not necessarily the one producing the highest yield. It should balance chemical recovery, biological activity, solvent safety, stability and manufacturing feasibility. Extract stability should be examined during storage and feed processing, while water-soluble compounds should be assessed for leaching before ingestion. The final feed, rather than the freshly prepared extract alone, is the product administered to fish.

A remaining question is whether wild, conventionally cultivated and tissue-culture-derived biomass yield functionally different extracts under identical processing conditions. Addressing this requires authenticated biomass, a common extraction protocol and the same analytical-marker panel, followed by biological testing with the same product batches. This would connect biomass history, extraction-dependent composition, dietary delivery and bacterial-challenge outcomes within a single validation pathway. Key extraction and chemical-standardisation evidence is summarised in Table 1.

6 | MECHANISMS SUPPORTING FISH HEALTH AND BACTERIAL DISEASE RESISTANCE

Ulva- and *Gracilaria*-derived products may influence bacterial disease resistance through several connected pathways. Some preparations act directly on bacterial cells, whereas others mainly affect the fish through redox regulation, innate immunity, epithelial protection or changes in the intestinal microbiota. Their relative contribution depends on product composition and stability, dietary dose and bioavailability, and the fish–pathogen system in which the product is tested (Vatsos and Rebours 2015; Thépot *et al.* 2021; Siddik *et al.* 2023). No single antibacterial, antioxidant or immune measurement can therefore establish disease protection on its own.

6.1 Direct antibacterial and resistance-modifying effects

Direct antibacterial activity varies with seaweed identity, extraction method, chemical fraction and bacterial strain. Even preparations from the same genus may differ because they contain different proportions of polysaccharides, phenolics, pigments and lipid-associated compounds (Vatsos and Rebours 2015; Capillo *et al.* 2018).

Sulfated-polysaccharide-rich extracts prepared from *Ulva* and the red seaweed *Solieria* inhibited several fish-pathogenic bacteria *in-vitro*, although activity differed among extracts and isolates. The strongest inhibition was mainly associated with selected *Solieria* preparations; the study therefore provides evidence for the tested *Ulva* extracts but not for *Gracilaria*, which was not included (Petit *et al.* 2024).

More specific evidence has been obtained from *Gracilaria fisheri*. Lower-molecular-weight galactan fractions produced by controlled depolymerisation inhibited *Vibrio parahaemolyticus* and *V. harveyi* more strongly than the native material and caused leakage of intracellular contents and visible membrane damage (Kamble *et al.* 2022). This mechanism applies to the modified *G. fisheri* fractions and should not be generalised to all *Gracilaria* polysaccharides.

Other constituents may contribute through different routes. Active *G. edulis* fractions contained fatty acids and a sterol-related compound and inhibited fish-pathogenic Vibrio isolates and *Aeromonas hydrophila* (Kasanah *et al.* 2019). An ethanolic *Gracilaria* extract also enhanced carbenicillin activity and reduced VarG-mediated β-lactam degradation despite weak independent inhibition (Lu *et al.* 2022). Direct growth-inhibition assays may therefore overlook resistance-modifying effects.

These findings remain preliminary for aquafeed use. Active compounds may be altered during feed manufacture, degraded during digestion or delivered at concentrations below those used in-vitro. Antibacterial activity must therefore be followed by dietary safety, feed-stability and challenge evaluation.

TABLE 1 Key extraction and chemical-standardisation evidence for *Ulva*- and *Gracilaria*-derived aquaproducts.

Product or analytical approach	Main evidence	Standardisation implication	Reference
Aqueous-ethanol extraction of *U. lactuca*	A 70:30 ethanol–water mixture recovered phenolic and carotenoid fractions, with a yield of approximately 10–15%	Report solvent ratio, temperature, time and biomass-to-solvent ratio	Pappou *et al.* (2022)
Sequential water and ethanol extraction of *G. gracilis*	Aqueous extracts had higher mean phenolic content and slightly stronger DPPH activity; 40°C performed better overall than room temperature or 70°C	Greater extraction intensity does not necessarily increase functional recovery	Afonso *et al.* (2021)
Solvent-dependent *G. gracilis* extraction	Ethanol showed the strongest antibacterial activity among five solvents, followed by methanol	Evaluate extraction yield and biological activity separately	Capillo *et al.* (2018)
Bioassay-guided *G. edulis* fractionation	Active ethyl-acetate fractions contained fatty acids and a sterol and showed pathogen-dependent inhibition	Do not assign activity to a compound class without fraction-level evidence	Kasanah *et al.* (2019)
Galactan depolymerisation	Lower-molecular-weight *G. fisheri* galactans showed stronger anti-*Vibrio* activity and membrane damage than native galactan	Include molecular weight and sulfation among polysaccharide markers	Kamble *et al.* (2022)
Antibiotic-potentiating extract	Ethanolic *Gracilaria* extract weakly inhibited bacteria directly but enhanced carbenicillin and reduced VarG-mediated β-lactam degradation	Direct inhibition assays may overlook resistance-modifying activity	Lu *et al.* (2022)
Instrumental characterisation	FTIR, HPLC/LC–MS and GC–MS provide complementary structural and compositional information	Select analytical methods according to the targeted compound classes	Pappou *et al.* (2022); Kasanah *et al.* (2019); Kamble *et al.* (2022)
Advanced extraction	UAE, MAE, EAE and pressurised methods remain dependent on operating conditions and the seaweed matrix	Evaluate scalability, cost and retention of activity	Quitério *et al.* (2022)

6.2 Redox regulation and innate immune responses

Reactive oxygen species have a dual role in fish defence. Controlled production contributes to the respiratory burst of phagocytic cells, whereas excessive or prolonged production damages cellular lipids, proteins and nucleic acids. A useful functional ingredient should therefore support balanced redox regulation rather than completely suppress or continuously stimulate oxidative activity

(Vatsos and Rebours 2015; Siddik *et al.* 2023).

Sulfated-polysaccharide-rich *Ulva* extracts stimulated reactive-oxygen-species production in a concentration-dependent manner in Nile tilapia head-kidney leukocytes, whereas rainbow-trout leukocytes showed a different response and comparatively high basal oxidative activity. The same preparations altered pro- and anti-inflammatory cytokine genes and genes associated with intracellular signalling. These findings indicate immunological recognition of the extracts, but they do not demonstrate direct binding of an *Ulva* constituent to a specific receptor (Petit *et al.* 2024).

Dietary studies provide a stronger connection between redox regulation, immunity and infection outcome. European seabass receiving an aqueous *Gracilaria* extract showed lower lipid peroxidation and higher lysozyme activity after challenge with *Photobacterium damselae* subsp. *piscicida*. Mortality was delayed rather than prevented, indicating partial protection rather than complete resistance (Peixoto *et al.* 2019).

In Asian seabass, a hot-water *U. rigida* extract reduced malondialdehyde, increased serum bactericidal activity and IgM, and altered genes associated with complement, lysozyme and cytokine responses. These changes occurred together with microbiota shifts and improved survival after *Vibrio vulnificus* challenge (Klongklaew *et al.* 2025). Because product characterisation, feeding, host responses and challenge outcome were assessed in the same study, the evidence is stronger than isolated *in-vitro* or immune-marker observations.

Even so, immune indices must be interpreted cautiously. Dietary seaweed supplementation generally improved innate immune responses and challenge survival in the meta-analysis of Thépot *et al.* (2021), but measured immune variables explained only part of the variation in protection. Increased lysozyme, respiratory burst or cytokine expression demonstrates immune modulation, not guaranteed resistance to infection.

6.3 Mucosal barriers and intestinal microbiota

The skin, gills and gastrointestinal tract are the principal interfaces between fish and their microbial environment. Mucus contains lysozyme, immunoglobulins, complement components, lectins and antimicrobial peptides, while epithelial junctions restrict the movement of microorganisms and luminal material into internal tissues (Vatsos and Rebours 2015; Siddik *et al.* 2023).

Dietary *G. tenuistipitata* extract increased expression of the tight-junction-associated genes occludin, claudin-1 and ZO-1 in heat-stressed Nile tilapia. The treatment also increased nrf2 expression, reduced hsp70 expression and lowered malondialdehyde and erythrocyte abnormalities (Siddik *et al.* 2024). These findings support antioxidant and barrier-associated regulation. However, intestinal permeability was not measured, and the experimental stressor was high temperature rather than bacterial infection. The study therefore demonstrates improved stress resilience and barrier-related signalling, not confirmed protection against a bacterial pathogen.

European seabass fed *G. gracilis* biomass or extract showed changes in selected anterior-intestinal bacterial groups, including reduced *Photobacterium*, without significant changes in overall richness or Shannon diversity. Because no pathogen challenge was conducted, the study demonstrates microbiota modulation but not a causal improvement in disease resistance (Gonçalves *et al.* 2022).

In Asian seabass, dietary *U. rigida* extract increased selected Bacillus taxa, including *B. amyloliquefaciens*, while immune responses and survival after *Vibrio vulnificus* challenge also improved (Klongklaew *et al.* 2025). This association is biologically relevant, but it does not establish that the microbiota shift caused the improved protection, because the extract may also have acted directly on host tissues, immune pathways or the pathogen. Changes in the abundance of a microbial taxon should not automatically be interpreted as beneficial or harmful, since their significance depends on strain identity, metabolic activity and interactions with the host and wider microbial community. Demonstrating causality would require microbial-metabolite analysis, isolation of responsive strains, controlled colonisation or microbiota-transfer experiments.

6.4 Integration of direct and host-mediated mechanisms

Direct antibacterial and host-mediated actions are likely to operate together. Partial inhibition of bacterial growth may slow infection sufficiently for innate responses to restrict pathogen proliferation. Antioxidant protection may preserve phagocyte and epithelial function, while intact mucosal surfaces and microbial competition may reduce attachment, colonisation or translocation (Peixoto *et al.* 2019; Gonçalves *et al.* 2022; Siddik *et al.* 2023).

The opposite outcome is also possible. A product that strongly inhibits bacteria in-vitro may perform poorly after oral administration because its active constituents are unstable, poorly available or supplied at an ineffective dose. Excessive or prolonged immune stimulation may also impose metabolic costs and contribute to oxidative or inflammatory injury (Thépot *et al.* 2021; Siddik *et al.* 2023).

Evidence should therefore be judged according to the level of validation. Chemical characterisation and in-vitro assays establish preliminary activity; cellular assays indicate possible immune recognition; and feeding trials demonstrate dietary exposure and physiological compatibility. Controlled pathogen challenge, preferably supported by bacterial-burden, tissue-condition and survival measurements, provides the strongest evidence of disease resistance.

6.5 Current mechanistic limitations

Several proposed pathways remain unresolved. Direct receptor binding has not been demonstrated for most Ulva- or *Gracilaria*-derived compounds in fish, and altered signalling-gene expression cannot identify the responsible receptor by itself (Petit et al. 2024). Functional intestinal permeability, mucus composition and mucosal immunity have also been studied less often than systemic blood indices or gene expression. Most microbiota studies continue to show association rather than causality (Gonçalves *et al.* 2022; Siddik *et al.* 2024; Klongklaew *et al.* 2025).

Pathogen coverage is uneven and is concentrated mainly in *Vibrio*, *Aeromonas* and *Photobacterium* models. Dietary studies using chemically defined *Ulva* or *Gracilaria* products against fish-derived Streptococcus remain scarce (Thépot *et al.* 2021; Siddik *et al.* 2023). Protection against gram-negative bacteria should not be assumed to predict protection against gram-positive streptococci because their surface structures, virulence mechanisms and interactions with the fish immune system differ.

Future studies should follow the same chemically characterised product batch from in-vitro testing through feed manufacture, dietary exposure and bacterial challenge. Product retention in feed, intake, bacterial burden, oxidative and immune responses, epithelial condition and survival should be assessed together. This would connect the proposed mechanisms with biologically meaningful protection rather than isolated laboratory measurements.

7 | COMPARATIVE EVIDENCE ACROSS COMMERCIALLY IMPORTANT FINFISH

Evidence for *Ulva*- and *Gracilaria*-derived aquafeed products is concentrated mainly in seabass and tilapia, with fewer studies in trout, rabbitfish and other cultured finfish. Direct comparison is difficult because whole biomass, fermented meals, crude extracts and enriched polysaccharide fractions represent chemically different exposures. A diet containing 10% seaweed meal, for example, cannot be compared directly with one containing a few grams or milligrams of extract per kilogram unless the concentrations of relevant bioactive markers are known.

The strength of the evidence also depends on the endpoint measured. Improvements in growth, antioxidant status, immune indices or intestinal microbiota demonstrate biological activity, but they do not by themselves establish protection against bacterial disease. Controlled challenge trials provide stronger evidence, particularly when survival is interpreted together with bacterial burden, tissue condition and host responses (Thépot *et al.* 2021; Siddik *et al.* 2023). The principal dietary studies and their limitations are summarised in Table 2.

7.1 Evidence from Asian and European seabass

Asian seabass provides one of the clearest examples of product-specific efficacy. Diets containing a 1:1 mixture of *Gracilaria pulvinata* and *Sargassum ilicifolium* powders produced several serum, mucus, antioxidant and immune-gene responses, mainly at 9–12% inclusion, without improving weight gain (Seyedalhosseini *et al.* 2024). Because the formulation contained two seaweed genera and no bacterial challenge was conducted, the independent contribution of *Gracilaria* and its effect on disease resistance could not be determined.

Stronger evidence was obtained with a chemically described hot-water extract of *Ulva rigida* fed to Asian seabass at 0.5–5 g kg^{-1} diet for four weeks. The extract reduced malondialdehyde, increased serum bactericidal activity and IgM, altered immune-related gene expression and shifted the intestinal microbiota. Following *Vibrio vulnificus* challenge, fish receiving the *U. rigida* extract showed higher survival than the control group (Klongklaew *et al.* 2025). Blood-biochemical and histological assessments indicated no major adverse effects at the tested doses. This study provides a relatively complete evidence chain, but its conclusions remain specific to *U. rigida*, the hot-water product, the four-week feeding period and the *V. vulnificus* model.

European seabass studies provide complementary evidence for *Gracilaria*. Dietary *G. gracilis* biomass or extract altered selected anterior-intestinal bacterial groups without significant effects on growth, feed conversion or mortality, demonstrating microbiota modulation but not disease protection (Gonçalves *et al.* 2022). In a separate trial, a diet containing 5% aqueous *Gracilaria* extract increased lysozyme activity, reduced lipid peroxidation and altered stress-, antioxidant- and immune-related gene expression during *Photobacterium damselae* subsp. *piscicida* infection. Mortality was delayed rather than prevented, indicating partial protection (Peixoto *et al.* 2019). Together, the seabass evidence shows that immune or microbial responses may occur without growth improvement and that challenge outcomes range from partial to stronger protection according to the tested product and pathogen.

7.2 Evidence from tilapia

Tilapia studies cover a wider range of product forms, including raw and fermented *Ulva* meals, low-dose *Ulva* extracts and *Gracilaria*-containing preparations. Priyatharshni *et al.* (2024) compared raw and fermented Ulva meals at 5, 10 and 15% inclusion in GIFT tilapia over 60 days. Fish receiving 10% fermented *Ulva* showed the highest growth, lowest feed-conversion ratio and strongest overall responses in several immune, antioxidant and stress-related indices. Performance was weaker in several raw-meal and 15% treatments, indicating that both processing and inclusion level influenced the outcome. However, the study did not include a bacterial challenge and therefore supports physiological and immunological activity rather than bacterial-disease resistance.

An *Ulva reticulata* extract administered to red tilapia at up to 200 mg kg^{-1} diet improved feed utilisation, haematological indices, phagocytic activity and growth-related gene expression, but no bacterial challenge was conducted (Sudrajat *et al.* 2025). *Gracilaria tenuistipitata* extract reduced oxidative damage and influenced barrier-associated genes and intestinal microbial characteristics in heat-stressed Nile tilapia (Siddik *et al.* 2024). Since this was a thermal-stress model, the findings should be interpreted as evidence of stress resilience rather than antibacterial protection.

Challenge-level evidence has been reported for a microencapsulated formulation containing extracts of *Gracilaria foliifera* and *Sargassum longifolium* in Mozambique tilapia challenged with *Aeromonas salmonicida* (Thanigaivel *et al.* 2019). The combined seaweed formulation and encapsulation system form a single tested product, so the response cannot be attributed independently to *Gracilaria*. Overall, tilapia studies show that fermentation, extraction and delivery method can strongly influence the response, but the diversity of products prevents identification of a universal inclusion level.

TABLE 2 Selected dietary evidence for *Ulva*- and *Gracilaria*-related products in commercially important finfish.

Fish species and product	Dose and duration	Main response	Challenge evidence	Principal limitation
Asian seabass; hot-water *U. rigida* extract	0.5, 1 or 5 g kg^{-1} diet; 28 days	Reduced MDA; increased bactericidal activity, IgM and immune-gene expression; altered microbiota	Higher survival after *V. vulnificus* challenge	Specific to the tested extract, duration and pathogen (Klongklaew *et al.* 2025)
Asian seabass; 1:1 *G. pulvinata–S. ilicifolium* powders	3–15% of diet; 56 days	No growth benefit; strongest immune, mucus and antioxidant responses mainly at 9–12%	No challenge	Effect cannot be assigned independently to *Gracilaria* (Seyedalhosseini *et al.* 2024)
European seabass; *G. gracilis* biomass or extract	2.5 or 5% biomass, or 0.35% extract; 47 days	Changed selected intestinal bacterial groups without altering growth or feed conversion	No challenge	Microbiota association did not establish disease protection (Gonçalves *et al.* 2022)
European seabass; aqueous *Gracilaria* extract	5% of diet; 80 days	Increased lysozyme, reduced lipid peroxidation and altered immune- and stress-related genes	Delayed mortality after *P. damselae* subsp. *piscicida* challenge	Partial rather than complete protection (Peixoto *et al.* 2019)
GIFT tilapia (Oreochromis niloticus); raw or fermented Ulva meal	5, 10 or 15% of diet; 60 days	The 10% fermented meal produced the highest growth, lowest FCR and strongest responses in several immune, antioxidant and stress-related indices	No bacterial challenge reported	Supports physiological and immunological activity, not bacterial-disease resistance; effects responsible for the fermentation response were not identified (Priyatharshni *et al.* 2024)
Red tilapia; *U. reticulata* extract	50, 100, 150 or 200 mg kg^{-1} diet; 30 days	Improved feed use, haematology, phagocytosis and growth-gene expression	No challenge	Growth and immune effects only (Sudrajat *et al.* 2025)
Nile tilapia; *G. tenuistipitata* extract	1% of diet; 70 days	Reduced oxidative damage and influenced barrier genes and microbiota	No bacterial challenge	Thermal-stress model, not infection (Siddik *et al.* 2024)
Mozambique tilapia; microencapsulated *G. foliifera–S. longifolium* extracts	Dose not reported; 15 days	Improved health and infection outcome	Improved survival after *A. salmonicida* challenge	Mixed genera and delivery system prevent attribution to *Gracilaria* (Thanigaivel *et al.* 2019)
Rainbow trout; *U. intestinalis* and *Gracilariopsis persica* polysaccharides	0.5 or 1.5 g kg^{-1} diet; 56 days	Influenced growth, fatty-acid composition and immunity	No defined challenge	*Gracilariopsis* is not *Gracilaria* (Safavi *et al.* 2019)

7.3 Supporting evidence from trout and other finfish

Evidence from other finfish species further shows that the effects of seaweed-based feed ingredients depend on the seaweed type, inclusion level and fish species. In rainbow trout (*Oncorhynchus mykiss*), dietary inclusion of *Gracilaria pygmaea* at 3, 6, 9 and 12% for seven weeks improved growth and feed utilisation at moderate inclusion levels, with the best growth response observed at 6%, whereas the 12% inclusion level reduced lipid retention. Hematological responses were generally within the normal range, although some changes in red blood cell and lymphocyte values were observed. These findings indicate that intact *Gracilaria* biomass can provide nutritional benefits, but higher inclusion may reduce nutrient utilisation (Sotoudeh and Jafari 2017).

Similar dose-dependent responses have been reported with *Ulva* in dusky kob (*Argyrosomus japonicus*). Dietary inclusion of *Ulva* at increasing levels affected growth and feed utilisation, with the lower inclusion level providing better performance than higher inclusion levels (Madibana *et al.* 2017). In juvenile Senegalese sole (*Solea senegalensis*), dietary *Ulva ohnoi* also produced changes in intestinal morphology and gene expression, but these responses were accompanied by reduced growth and digestive enzyme activity under the tested conditions (Vizcaíno *et al.* 2019). Together, these studies show that seaweed supplementation may influence intestinal and physiological functions without necessarily producing an overall improvement in growth or health.

Evidence from these species therefore provides useful supporting context for *Ulva* and *Gracilaria* as functional feed ingredients, but it should not be interpreted as direct evidence of bacterial disease resistance. Most of these studies evaluated growth, feed utilisation, haematological responses, intestinal condition or other physiological endpoints rather than controlled bacterial challenge outcomes. Their main value is in showing the importance of fish species, seaweed source and dietary inclusion level when evaluating the functional effects of macroalgal ingredients.

7.4 Cross-study interpretation

Across fish species, efficacy belongs to the tested product rather than to the genus in general. Whole meals retain nutrients and multiple bioactive fractions but usually require percentage-level inclusion. Extracts can be used at lower doses but are more dependent on extraction selectivity, concentration and stability. Dose responses are often non-linear, as shown by the stronger responses at intermediate rather than highest inclusion levels in Asian seabass and tilapia.

The reviewed studies provide different levels of evidence and should not be interpreted as equivalent. Changes in growth, haematology, antioxidant status, immune indices, gene expression or intestinal microbiota demonstrate physiological or immunological activity, but do not by themselves establish resistance to bacterial infection. This level of evidence is represented by Gonçalves *et al.* (2022), Priyatharshni *et al.* (2024), Siddik *et al.* (2024), Seyedalhosseini *et al.* (2024), and Sudrajat *et al.* (2025), where biological responses were measured without a bacterial challenge. Stronger evidence for disease resistance comes from feeding studies followed by a controlled bacterial challenge and assessment of infection-related outcomes. These include the *Gracilaria*-supplemented European seabass study of Peixoto *et al.* (2019), the *U. rigida* Asian seabass study of Klongklaew *et al.* (2025), and the mixed microencapsulated formulation evaluated by Thanigaivel *et al.* (2019). Even among these challenge studies, outcomes ranged from delayed mortality to improved survival and remain specific to the tested product, fish host and pathogen.

Future studies should compare chemically characterised meals and extracts at marker-equivalent doses, verify retention of relevant compounds after feed manufacture, measure feed intake and bacterial burden, and combine physiological assessment with standardised survival analysis. This would allow products to be compared on the basis of delivered chemistry and demonstrated protection rather than seaweed name alone.

8 | SAFETY AND PRACTICAL CONSIDERATIONS

The natural origin of *Ulva* and *Gracilaria* does not ensure that every derived meal, extract or enriched fraction is safe or suitable for aquafeeds. Products differ in fibre, ash, mineral content, bioactive-compound concentration and possible solvent residues. Safety must therefore be assessed for the exact product, dietary dose, fish species and feeding duration, preferably using the final formulated diet rather than the source biomass alone (Vatsos and Rebours 2015; Siddik *et al.* 2023).

8.1 Dose and nutritional compatibility

Dietary responses to seaweed products are often non-linear, but reported inclusion levels are not directly comparable across different product forms. Whole or fermented seaweed meals, crude extracts and enriched fractions differ substantially in the concentration and composition of their bioactive constituents. Therefore, percentage-level inclusion of a seaweed meal cannot be considered equivalent to g kg^{-1} or mg kg^{-1} doses of an extract or fraction unless relevant chemical markers are quantified. In Asian seabass fed a 1:1 mixture of *Gracilaria pulvinata* and *Sargassum ilicifolium* at 3–15% for 56 days, several immune and antioxidant responses were strongest at approximately 9–12%, while the highest inclusion did not produce a proportional improvement (Seyedalhosseini *et al.* 2024). Likewise, GIFT tilapia receiving 10% fermented *Ulva* meal showed the strongest overall combination of

growth, immune, antioxidant and stress-related responses, whereas several raw-meal and 15% treatments were less favourable (Priyatharshni *et al.* 2024). These findings therefore indicate product-specific dose responses rather than equivalent bioactive doses or universally optimal inclusion levels.

Whole biomass can provide proteins, minerals, pigments and functional polysaccharides, but high inclusion may also increase structural carbohydrate and ash, dilute digestible protein and energy, or restrict nutrient availability, particularly in carnivorous fishes (Siddik *et al.* 2023). Diets containing substantial amounts of seaweed should therefore be reformulated to remain nutritionally comparable with the control rather than prepared by adding biomass to an unchanged basal feed.

Extracts permit lower inclusion, but lower dietary mass should not be confused with broader safety. Asian seabass fed a hot-water *U. rigida* extract at 0.5–5 g kg^{-1} diet for four weeks showed no impaired growth or major adverse blood-biochemical changes. Histological examination found no severe lesions, although slight hepatic vacuolisation occurred at 1 and 5 g kg^{-1}, and a slight increase in melanomacrophage centres was observed in the head kidney at 5 g kg^{-1}; intestinal tissues remained unchanged (Klongklaew *et al.* 2025). These findings support short-term compatibility within the tested range, not unrestricted safety at higher doses, during longer feeding periods or for chemically different *Ulva* products.

8.2 Elemental and microbiological safety

Seaweeds can accumulate essential minerals together with potentially harmful metal(oid)s from the surrounding environment. A critical assessment of 176 *Ulva* studies found substantial variation associated with location, environmental exposure, biomass form, cultivation status and analytical method (Vargas-Murga *et al.* 2025). Information on element speciation was also limited. Total arsenic does not distinguish inorganic from less-toxic organic forms, while total mercury does not indicate methylmercury exposure. Taxonomic confirmation, standardised analytical procedures and batch-specific testing are therefore required before safety findings are generalised.

Controlled cultivation improves traceability but does not remove the need for contaminant analysis. Four molecularly identified Ulva strains cultivated under land-based conditions showed relatively limited variation in several chemical constituents and low concentrations of the heavy metals measured under those conditions (Roleda *et al*. 2021). The result applies only to the tested strains, water source and cultivation system.

Cultivated *Gracilaria* also shows location-dependent variation. Biomass from different Indonesian cultivation areas differed in protein, iodine, selenium, carotene and antioxidant characteristics (Purwaningsih *et al.* 2024). Material assigned to the same genus should therefore not be assumed to provide identical nutritional or elemental exposure.

Mineral-rich biomass may be useful at low inclusion but could supply excessive iodine, iron, selenium or other elements when used at higher levels or combined with a mineral premix. Elemental analysis should consequently be performed on the seaweed product and, where inclusion is substantial, on the complete diet. Biomass from uncontrolled environments should also be checked for sediments, epiphytes and undesirable microorganisms. Wild biomass is not necessarily unsafe, just as cultivated biomass is not automatically contaminant-free.

8.3 Processing, feed stability and delivery

Drying, extraction, concentration, pelleting, extrusion and storage can alter polysaccharides, phenolics, pigments and lipid-associated compounds (Matos *et al.* 2021; Quitério *et al.* 2022). Water, ethanol and aqueous ethanol are practical starting solvents for feed-oriented products, but solvent removal must be completed and verified. Product identity cannot be defined by solvent alone because extraction temperature, duration, biomass-to-solvent ratio and concentration procedure also influence composition.

The composition of the original extract may not represent the amount ultimately consumed by fish. Active constituents can degrade during processing, bind to other feed ingredients, become unevenly distributed or leach from pellets after immersion. Representative markers should therefore be measured after feed manufacture and, where relevant, after water exposure.

Delivery method also forms part of the tested product. An extract incorporated into the pellet, applied by post-pellet coating or protected by encapsulation may differ in retention and availability even when the nominal dose is identical. The microencapsulated formulation containing *G. foliifera* and *S. longifolium* illustrates the possible value of protected delivery, although the effects of the two seaweeds and the carrier could not be separated (Thanigaivel *et al.* 2019).

8.4 Biological safety and commercial relevance

Absence of acute toxicity or short-term histological damage does not establish long-term safety. Feeding trials should assess feed intake and growth together with haematology, serum biochemistry, oxidative condition and histology of the intestine, liver and kidney. Persistent immune stimulation also requires attention because prolonged inflammatory or respiratory-burst activity may impose metabolic and oxidative costs rather than provide continued protection (Thépot *et al.* 2021; Siddik *et al.* 2023).

Most available trials lasted about four to ten weeks and involved juveniles under controlled conditions. Evidence remains limited for full production-cycle feeding,

accumulation in edible tissues, withdrawal after supplementation, broodstock use and commercial feed-manufacturing conditions. Short-term absence of adverse effects should therefore be described as compatibility within the tested conditions, not as proof of unrestricted safety.

Commercial adoption will depend on the balance among efficacy, effective dose, biomass availability and processing cost. At minimum, a candidate ingredient should have confirmed species identity, documented origin, proximate and elemental composition, acceptable microbiological quality, relevant chemical markers, demonstrated feed stability and a safe, effective dose for the target fish.

9 | RESEARCH GAPS AND STANDARDISATION ROADMAP

The literature supports the functional potential of *Ulva*- and *Gracilaria*-derived products, but comparison among studies remains difficult because seaweed identity, biomass origin, processing, composition, dose, fish host and pathogen model often differ. The priority is therefore not simply to report further antioxidant or immune responses, but to connect a defined product, its dietary delivery and protection against a specified bacterial pathogen within one reproducible framework (Thépot *et al.* 2021; Siddik *et al.* 2023).

9.1 Biomass identity and traceability

Product development should begin with authenticated and traceable biomass. Genus-level or morphology-based identification is inadequate when closely related species may differ in polysaccharides, minerals, pigments, phenolics and lipid-associated metabolites. Voucher material and molecular confirmation should be used wherever possible, while geographical origin, season, cultivation system, harvest stage and post-harvest handling should be documented (García-Poza *et al.* 2022; Vargas-Murga *et al.* 2025).

Direct comparisons among wild, cultivated and tissue-culture-derived biomass remain scarce. Controlled cultivation and tissue culture may improve traceability and preserve selected genotypes, but neither has been shown consistently to increase aquafeed-related activity. Future comparisons should use the same species and, where feasible, the same genotype under common environmental, harvesting, processing and analytical conditions (Baweja *et al.* 2009).

9.2 Product definition and chemical markers

"Seaweed meal" and "seaweed extract" are not reproducible treatment descriptions. Whole biomass should be reported with its proximate composition, fibre, ash, minerals and relevant functional constituents. Extracts additionally require details of pretreatment, solvent composition, biomass-to-solvent ratio, temperature, duration, yield, concentration and storage (Matos *et al.* 2021; Quitério *et al.* 2022).

Chemical markers should reflect the product. Polysaccharide-rich preparations require carbohydrate and sulfate measurements, supported where possible by molecular-weight and monosaccharide information. Phenolic-, pigment- and lipid-oriented products require appropriate chromatographic or compositional profiling. FTIR fingerprints and total-content assays can support batch comparison but cannot identify the compound responsible for an observed response. The same characterised batch should therefore be followed from screening through feed preparation, feeding and challenge experiments (Cotas *et al.* 2020; Kamble *et al.* 2022).

9.3 Harmonisation of dietary trials

Whole meals, crude extracts and purified compounds should not be compared only by inclusion percentage or mass. Studies should report both total product inclusion and the concentration of representative markers in the final feed. Experimental diets must remain nutritionally comparable, with changes in protein, lipid, energy, fibre, ash and minerals accounted for.

Feed intake, palatability, marker retention after manufacture and leaching during water exposure should also be measured because the formulated dose may differ from the amount consumed. Dose-response trials should include levels below and above the expected effective range, and neutral or adverse findings should be reported alongside beneficial effects. Moderate inclusion has sometimes outperformed the highest dose, showing that efficacy cannot be assumed to increase linearly (Priyatharshni *et al.* 2024; Seyedalhosseini *et al.* 2024).

9.4 Mechanistic and challenge validation

In-vitro antioxidant and antibacterial assays are useful for screening, but they do not demonstrate dietary protection. Conversely, improved survival from a poorly characterised product gives limited information about the active constituents or mechanism. Stronger studies should follow the same batch through chemical characterisation, dietary safety testing, physiological assessment and bacterial challenge.

Mechanistic endpoints should match the product. Polysaccharide-rich preparations may require greater attention to immune signalling, whereas phenolic- or pigment-rich extracts may warrant closer examination of oxidative and inflammatory regulation. Challenge studies should report the bacterial strain, dose, route, timing, environmental conditions and confirmation of infection. Injection, immersion and cohabitation models should not be treated as equivalent because they engage mucosal barriers differently. Relative percentage survival should be accompanied by raw survival or mortality, and bacteri-

al burden should be measured where possible to distinguish reduced infection from delayed mortality or improved tolerance.

Current evidence is concentrated in *Vibrio*, *Aeromonas* and *Photobacterium* models. Defined *Ulva* or *Gracilaria* products should also be tested against fish-derived gram-positive pathogens, particularly Streptococcus agalactiae, within clearly specified fish–pathogen systems (Thepot *et al.* 2021a; Siddik *et al.* 2023). Protection demonstrated against gram-negative bacteria should not be assumed to predict protection against streptococcal infection.

9.5 Translation to commercial feeds

Laboratory efficacy is only one stage of development. Candidate ingredients must also be assessed for batch consistency, shelf life, compatibility with pelleting or extrusion, storage stability and retention after immersion. Contaminants, microbiological quality and residual solvents should be evaluated in both the ingredient and final feed (Roleda *et al.* 2021; Vargas-Murga *et al.* 2025).

Longer trials are needed to examine full-production-cycle safety, persistence of responses and accumulation in edible tissues. Farm-scale studies should then consider growth, feed conversion, disease occurrence, treatment use, survival benefit and economic return. A biologically active product may still be impractical if biomass supply is inconsistent, processing is costly or the effective dose substantially increases feed price.

The proposed pathway begins with authenticated biomass and documented production, followed by controlled processing, chemical characterisation, feed incorporation, safety and dose-response testing, standardised challenge and farm-scale validation. Biological and economic findings should then guide further adjustment of product composition and dietary dose.

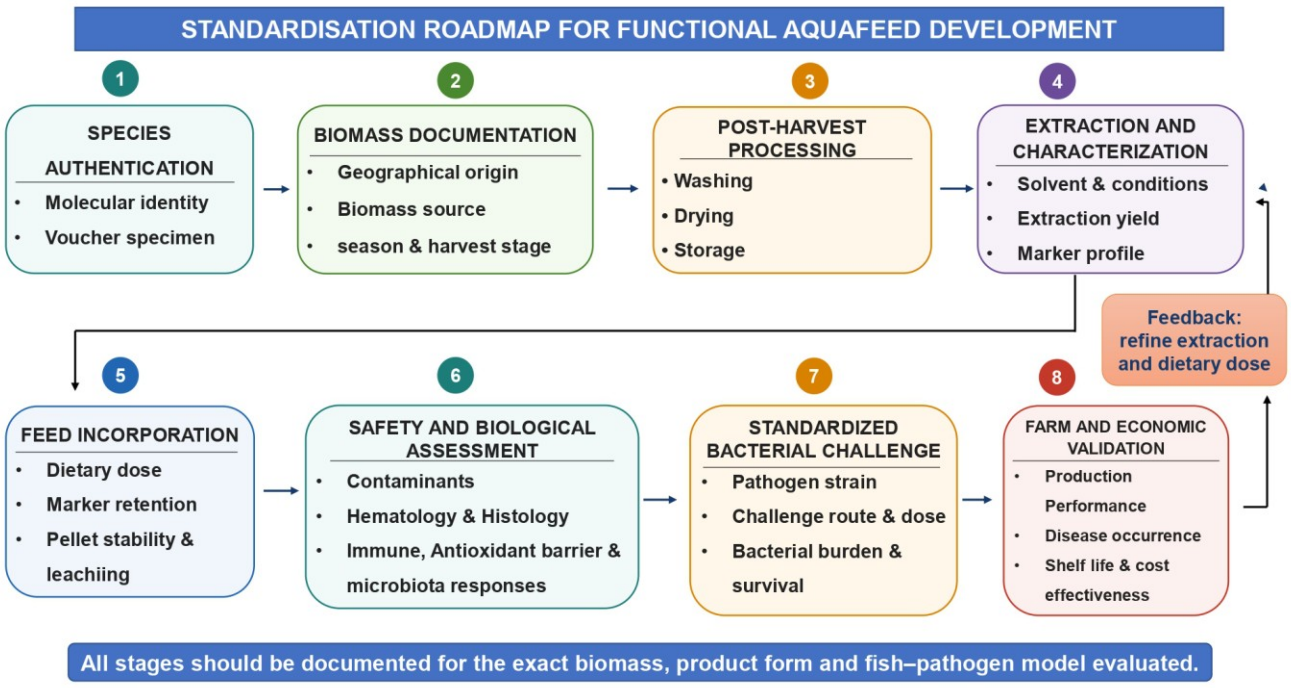

FIGURE 1 Proposed standardisation pathway for *Ulva*- and *Gracilaria*-derived functional aquafeed ingredients, linking authenticated biomass, controlled processing, chemical characterisation, feed incorporation, safety and dose-response assessment, bacterial challenge and farm-scale validation.

10 | CONCLUSIONS

Ulva- and *Gracilaria*-derived meals, extracts and enriched fractions can influence antioxidant status, innate immunity, intestinal condition and microbiota in finfish, but these effects vary with seaweed species, biomass origin, processing, dietary dose and host. They should therefore be interpreted as properties of the tested product rather than of the genus as a whole.

Evidence for bacterial disease resistance is more limited than evidence for physiological or immune effects. The strongest studies combined dietary exposure with controlled bacterial challenge, with *Gracilaria*- and *Ulva*-related products either delaying mortality or improving survival in European and Asian seabass (Peixoto *et al.*

2019; Klongklaew *et al.* 2025). In contrast, in vitro activity, immune markers or microbiota changes without challenge should be considered supporting evidence rather than proof of disease resistance.

Overall, *Ulva* and *Gracilaria* should be viewed as promising development platforms rather than universally effective disease-control agents. Current evidence supports short-term physiological, immune and, in a limited number of challenge studies, disease-resistance benefits. However, long-term safety, full-production-cycle performance and commercial-scale efficacy remain insufficiently demonstrated. Future development should therefore focus on safe, chemically defined and reproducible products whose efficacy and economic feasibility are validated in the intended fish–pathogen system under longer-term and farm-relevant conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

NKD, SS: Conceptualization, Data curation, Writing – original draft. DK, VE, TSK: Supervision, Writing – review & editing. PS, SV, DP: Writing – review & editing. All authors read and approved the final manuscript.

AI-USE DECLARATION

Generative artificial intelligence tools were used during manuscript development for language editing, organisation and drafting assistance. All scientific statements, interpretations, citations and references were independently checked and approved by the authors, who take full responsibility for the final manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new datasets were generated or analysed in this review.

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